ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2014 October 15; 5(5): 577-729

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

A peer-reviewed, online, open-access journal of diabetes

Editorial Board

2011-2015

The *World Journal of Diabetes* Editorial Board now consists of 710 members, representing a team of worldwide experts in diabetes mellitus. They are from 56 countries, including Argentina (2), Australia (27), Austria (11), Belgium (5), Brazil (13), Canada (24), Chile (3), China (40), Cuba (1), Czech Republic (3), Denmark (15), Egypt (3), Finland (5), France (12), Germany (27), Greece (17), Hungary (4), India (28), Iran (8), Iraq (2), Ireland (3), Israel (10), Italy (56), Japan (31), Jordan (1), Kuwait (3), Lebanon (1), Malaysia (1), Malta (1), Mexico (4), Netherlands (8), New Zealand (3), Nigeria (2), Norway (2), Oman (3), Pakistan (2), Poland (7), Portugal (1), Qatar (1), Romania (2), Saudi Arabia (1), Singapore (4), Slovakia (1), South Africa (1), South Korea (15), Spain (24), Sweden (5), Switzerland (4), Thailand (4), Tunisia (1), Turkey (13), United Arab Emirates (3), United Kingdom (27), United States (213), Venezuela (1), and Yemen (1).

Subrata Chakrabarti, *London*
David Cherney, *Ontario*
Mervyn Deitel, *Toronto*
Jean-Pierre Després, *Quebec*
David Joseph Hill, *London*
Tian-Ru Jin, *Toronto*
Arulmozhi D Kandasamy, *Edmonton*
Jennifer L Kuk, *Toronto*
Ismail Laher, *Vancouver*
Roger S McIntyre, *Toronto*
Joseph Fomusi Ndisang, *Saskatoon*
Raj Padwal, *Alberta*
Ciriaco A Piccirillo, *Montreal*
Remi Rabasa-Lhoret, *Montreal*
AM James Shapiro, *Edmonton*
Guang Sun, *St. John's*
Valerie Taylor, *Hamilton*
Cory Toth, *Calgary*
André Tremblay, *Montréal*
Vincent C Woo, *Winnipeg*
James Roscoe Wright, *Calgary*
Xi-Long Zheng, *Calgary*

Chile

Sebastian San Martin, *Valparaiso*
Armando Rojas-Rubio, *Talca*
Luis Sobrevia, *Santiago*

China

Pang-Zeng Chang, *Qingdao*
Jie Chen, *Nanjing*
Bernard Man Yung Cheung, *Hong Kong*
William Chi-shing Cho, *Hong Kong*
Tian-Pei Hong, *Beijing*
Qin Huang, *Shanghai*
Po Sing Leung, *Hong Kong*
Chao Liu, *Nanjing*
Jian-Kang Liu, *Xi'an*
Lie-Gang Liu, *Wuhan*
Ronald Ching Wan Ma, *Hong Kong*
Jin-Sheng Qi, *Shijiazhuang*
Wing Yee So, *Hong Kong*
Cheuk Chun Szeto, *Hong Kong*
Kathryn Tan, *Hong Kong*
Cheng-Ming Wang, *Yangzhou*
Cong-Yi Wang, *Wuhan*
Yu Wang, *Hong Kong*
Guang-Da Xiang, *Wuhan*
Bao-Feng Yang, *Harbin*
Shu-Yu Yang, *Fujian*
Xi-Lin Yang, *Hong Kong*
Zai-Qing Yang, *Wuhan*
Shan-Dong Ye, *Hefei*
Shi-Sheng Zhou, *Dalian*
Zhi-Guang Zhou, *Changsha*

Cuba

Luis Sarmiento-Pérez, *Havana*

Czech Republic

Martin Haluzik, *Prague*
Michal Krcma, *Plzen*
Terezie Pelikanova, *Prague*

Denmark

Charlotte Brøns, *Gentofte*
Jens Sandahl Christiansen, *Arhus*
Flemming Dela, *Copenhagen*
Kristine Færch, *Gentofte*
Erik L Grove, *Aarhus*
Louise Groth Grunnet, *Gentofte*
R Scott Heller, *Gentofte*
Kurt Højlund, *Odense C*
Filip K Knop, *Hellerup*
Helle Markholst, *Måløv*
Jens D Mikkelsen, *Copenhagen*
Ole Hartvig Mortensen, *Copenhagen*
Oluf Pedersen, *Copenhagen*
Esben Thyssen Vestergaard, *Aarhus*
Milan Zdravkovic, *Søborg*

Egypt

Mamdouh Moawad Ali Hssan, *Cairo*
Moshira Abdel Hakim Rateb, *Cairo*
Mona Farag Schaalan, *Cairo*

Finland

Siamak Bidel, *Helsinki*
Gang Hu, *Helsinki*
Thomas Kietzmann, *Oulu*
Qing Qiao, *Helsinki*
Karoliina Wehkalampi, *Helsinki*

France

Jean Claude Ansquer, *Dijon*
Bertrand Cariou, *Nantes*
Sylvie Dejager, *Rueil-Malmaison*
Naim Akhtar Khan, *Dijon*
Jean-Philippe Lavigne, *Nîmes*
Michel Marre, *Paris*
Marie-Claude Morice, *Massy*
Riccardo Perfetti, *Paris*
Gérard Said, *Paris*
Sophie Visvikis Siest, *Nancy*
Dominique Simon, *Paris*
Didier Vieau, *Villeneuve d'Ascq*

Germany

Ioanna Gouni Berthold, *Cologne*
Christa Buechler, *Regensburg*
Roland Büttner, *Heidelberg*
Michael Froehner, *Dresden*
Hammes Hans-Peter, *Mannheim*
Nadj Herbach, *Munich*
Andrea Icks, *Düsseldorf*
Thomas Jax, *Neuss*
Ulrich Arthur Julius, *Dresden*
Michael Kluge, *Munich*
Florian Lang, *Tuebingen*
Matthias Laudes, *Köln*
Ralf Lobmann, *Stuttgart*
Rafael T Mikolajczyk, *Bremen*
Andreas Stefan Mueller, *Halle (Saale)*
Karsten Müssig, *Tübingen*
Nahid Parvizi, *Neustadt am Rübenberge*
Thomas Peter Reinehr, *Datteln*
Michael Ristow, *Jena*
Sven Schinner, *Duesseldorf*
Peter Egbert Hermann Schwarz, *Dresden*
Konstantinos Stellos, *Tubingen*
Ovidiu Alin Stirban, *Bad Oeynhausen*
Diego J Walther, *Berlin*
Silvia Anette Wein, *Kiel*
Christian Wrede, *Berlin*
Dan Ziegler, *Düsseldorf*

Greece

George P Chrousos, *Athens*
Moses S Elisaf, *Ioannina*
Panagiotis Georgoulias, *Larissa*
Nikolaos Kadoglou, *Thessaloniki*
Gerasimos E Krassas, *Krini*
Spilios Manolakopoulos, *Attiki*
Nikolaos Papanas, *Alexandroupolis*
Dimitrios Papazoglou, *Alexandroupolis*
Sokratis Pastromas, *Athens*
Melpomeni Peppa, *Athens*
Christina Piperi, *Goudi*
Nicholas K Tentolouris, *Athens*
Konstantinos A Toulis, *Salonika*
Apostolos Tsapas, *Thessaloniki*
Konstantinos Tziomalos, *Thessaloniki*
Elias Zintzaras, *Thessaly*

Hungary

Mária Bagyánszki, *Szeged*
György Jermendy, *Budapest*
Karoly Racz, *Budapest*
Gyula Soltesz, *Pécs*

India

Deepak Narayan Amrapurkar, *Mumbai*
C V Anuradha, *Tamil Nadu*
Sarika Arora, *New Delhi*
Pitchai Balakumar, *Sivakasi*
Muthuswamy Balasubramanyam, *Chennai*
Subhabrata Chakrabarti, *Hyderabad*
Abhay Sankar Chakraborti, *Kolkata*
Tapan K Chaudhuri, *New Delhi*
Kanwaljit Chopra, *Chandigarh*
Malabika Datta, *Delhi*
Debidas Ghosh, *West Bengal*
Ravinder Goswami, *New Delhi*
Pappachan M Joseph, *Kerala*
Jothydev Kesavadev, *Kerala*
KVS Hari Kumar, *Lucknow*
Anoop Misra, *New Delhi*
Analava Mitra, *Kharagpur*
Viswanathan Mohan, *Chennai*
S P Murthy, *Bangalore*
Pallavi Panchu, *Guntur*
Usharani Pingali, *Hyderabad*
Ambady Ramachandran, *Egmore Chennai*
Vadde Ramakrishna, *Kadapa*
Geetha Vani Rayasam, *Haryana*

Rajat Sandhir, *Chandigarh*
Manju Sharma, *New Delhi*
Suman Bala Sharma, *Delhi*
Tarun Sharma, *Chennai*

Iran

Mohammad Abdollahi, *Tehran*
Mohammad Kazemi Arababadi, *Rafsanjan*
Leila Azadbakht, *Isfahan*
Hamid Baradaran, *Tehran*
Behrooz Broumand, *Tehran*
Ahmad Esmaillzadeh, *Isfahan*
Majid Ghayour-Mobarhan, *Mashhad*
Mohsen Janghorbani, *Isfahan*

Iraq

Saad Abdul-Rahman Hussain, *Baghdad*
Abbas Ali Mansour, *Basrah*

Ireland

Amar Agha, *Dublin*
Mark Philip Hehir, *Dublin*
Gerald H Tomkin, *Dublin*

Israel

Michael Aviram, *Haifa*
Gal Dubnov-Raz, *Tel Hashomer*
Shimon Efrat, *Tel Aviv*
Raymond Elias Farah, *Safed*
Oren Froy, *Rehovot*
Saher Hamed, *Haifa*
Arid Nakhoul, *Haifa*
Orit Pinhas-Hamiel, *Tel Hashomer*
Haim Werner, *Tel Aviv*
Marina Shargorodsky Zimlichman, *Holon*

Italy

Luigi Angrisani, *Napoli*
Moschetta Antonio, *Bari*
Antonio Aversa, *Rome*
Roberto Baldelli, *Rome*
Giuseppe Barbaro, *Rome*
Alessandro Bartolomucci, *Parma*
Giuseppina Basta, *Pisa*
Simona Bertoli, *Milano*
Federico Bilotta, *Rome*
Fabio Broglio, *Torino*
Francesco G Chiarelli, *Chieti*
Sergio Coccheri, *Bologna*
Massimo Collino, *Torino*
Marco Aristide Comaschi, *Genoa*
Renzo Cordera, *Genova*
Francesco Dotta, *Siena*
Gagliardini Elena, *Bergamo*
Stefano Fiorucci, *Perugia*
Maurizio Galderisi, *Naples*
Amalia Gastaldelli, *Pisa*
Ezio Ghigo, *Turin*
Carla Giordano, *Palermo*
Paolo Gisondi, *Verona*
Riccarda Granata, *Turin*
Giorgio Iervasi, *Pisa*
Claudia Kusmic, *Pisa*
Carmelo La Rosa, *Catania*
Francesco Landi, *Rome*
Monica Rosa Loizzo, *Arcavacata Rende*
Paolo Magni, *Milano*
Mariano Malaguarnera, *Catania*
Melania Manco, *Rome*
Piero Marchetti, *Pisa*
Massimo Massi-Benedetti, *Perugia*
Antonio Nicolucci, *Imbaro*
Lucia Pacifico, *Rome*
Stefano Palomba, *Catanzaro*
Giampaolo Papi, *Carpi*
Renato Pasquali, *Bologna*
Piermarco Piatti, *Milano*
Dario Pitocco, *Rome*
Antonio E Pontiroli, *Milano*
Giulio Marchesini Reggiani, *Bologna*
Giuseppe Remuzzi, *Bergamo*
Manfredi Rizzo, *Palermo*
Raffaella Rosso, *Genoa*
Giuseppe Schillaci, *Perugia*
Leonardo A Sechi, *Sassari*
Imad Sheiban, *Torino*
Cesare R Sirtori, *Milano*
Giovanni Tarantino, *Naples*
Giovanni Targher, *Verona*
Donadon Valter, *Pordenone*
Alberto Verrotti, *Chieti*
Andrea Viggiano, *Napoli*
Gianvincenzo Zuccotti, *Milan*

Japan

Masato Asahina, *Chiba*
Takuya Awata, *Saitama*
Yuichiro Eguchi, *Saga*
Goji Hasegawa, *Kyoto*
Satoshi Inoue, *Tokyo*
Eiji Ishimura, *Osaka*
Masayuki Iwano, *Nara*
Takashi Kadowaki, *Tokyo*
Eisuke Kagawa, *Hiroshima*
Masahito Katahira, *Aichi*
Eiji Kawasaki, *Nagasaki*
Noriyuki Koibuchi, *Gunma*
Kazuhiko Kotani, *Tochigi*
Daisuke Koya, *Ishikawa*
Norikazu Maeda, *Osaka*
Takayuki Masaki, *Oita*
Yuji Matsuzawa, *Osaka*
Kazuaki Nishio, *Tokyo*
Kenji Okumura, *Nagoya*
Motoaki Saito, *Yonago*
Toshiyasu Sasaoka, *Toyama*
Michio Shimabukuro, *Okinawa*
Kohzo Takebayashi, *Saitama*
Hiroyuki Tamemoto, *Tochigi*
Takashi Togo, *Yokohama*
Jun Udagawa, *Izumo*
Yoshinari Uehara, *Fukuoka*
Takuya Watanabe, *Tokyo*
Toshihiko Yada, *Tochigi*
Daisuke Yasuhara, *Kagoshima*
Tohru Yorifuji, *Osaka*

Jordan

Yousef S Khader, *Irbid*

Kuwait

Kamal AA Sulaiman Al-Shoumer, *Kuwait*
Ibrahim Fadil Benter, *Safat*
Abu Salim Mustafa, *Kuwait*

Lebanon

Ramzi F Sabra, *Beirut*

Malaysia

Mafauzy Mohamed, *Kota Bharu*

Malta

Charles Savona-Ventura, *Msida*

Mexico

Manuel González-Ortiz, *Guadalajara*
Fernando Guerrero-Romero, *Durango*
Jesus Alberto Olivares-Reyes, *Mexico City*
Rocío Salceda, *Mexico City*

Netherlands

Sander Kersten, *Wageningen*
Nanne Kleefstra, *Zwolle*
Edwin Mariman, *Maastricht*
Don Poldermans, *Rotterdam*
Han Roelofsen, *Groningen*
Hendrik-Jan Schuurman, *Utrecht*
Suat Simsek, *Alkmaar*
Marcel Twickler, *Bergen op Zoom*

New Zealand

Paul Hofman, *Auckland*
Peter E Lobie, *Auckland*
Elaine Rush, *Auckland*

Nigeria

Adejuwon A Adeneye, *Lagos*
Anthonia Okeoghene Ogbera, *Lagos*

Norway

Akhtar Hussain, *Oslo*

Odd Erik Johansen, *Hovik*

Oman

Mohammed Al Shafaee, *Muscat*
Jumana S Saleh, *Muscat*
Radha Shenoy, *Muscat*

Pakistan

Shahid Hameed, *Islamabad*
Jamil A Malik, *Islamabad*

Poland

Marcin Baranowski, *Bialystok*
Jerzy Beltowski, *Lublin*
Alicia Hubalewska Dydejczyk, *Krakow*
Maciej Owecki, *Poznań*
Ewa Pankowska, *Warsaw*
Agnieszka Piwowar, *Wroclaw*
Dorota Anna Zieba, *Krakow*

Portugal

M Graça Pereira, *Braga*

Qatar

Hong Ding, *Doha*

Romania

Elena Ganea, *Bucharest*
Adriana Georgescu, *Bucharest*

Saudi Arabia

J Fernando Arevalo, *Caracas*

Singapore

S Thameem Dheen, *Singapore*
Yung Seng Lee, *Singapore*
Daniel Ng, *Singapore*
Rob Martinus van Dam, *Singapore*

Slovakia

Katarína Šebeková, *Bratislava*

South Africa

Md Shahidul Islam, *Durban*

South Korea

Huneg-Sik Choi, *Gwangju*
Kyung Mook Choi, *Seoul*
Won Mi Hwang, *Seoul*
Eui-Bae Jeung, *Chungbuk*
Ju-Hee Kang, *Incheon*
Sin Gon Kim, *Seongbuk-Gu*
Sung-Jin Kim, *Seoul*
Young-Gyu Ko, *Seoul*
Kang-Beom Kwon, *Chonbuk*
Myung Gull Lee, *Bucheon*
Soo Lim, *Seongnam*
Byung-Hyun Park, *Jeonbuk*
Seungjoon Park, *Seoul*
Kun-Ho Yoon, *Seoul*
Jeesuk Yu, *Cheonan*

Spain

Vivencio Barrios, *Madrid*
M Lusia Bonet, *Palma de Mallorca*
Manuel Vazquez Carrera, *Barcelona*
Maria Luz Martinez Chantar, *Derio*
Manuel Aguilar Diosdado, *Cádiz*
Javier Espino, *Badajoz*
Ricardo V García-Mayor, *Vigo*
José Manuel Gómez-Sáez, *Barcelona*
Oreste Gualillo, *Santiago de Compostela*
J Alfredo Martínez Hernández, *Pamplona*
Emilio Herrera, *Madrid*
Amelia Marti, *Pamplona*
Merce Miranda, *Tarragona*
JF Navarro-González, *Santa Cruz de Tenerife*
Alberto Ortiz, *Madrid*
Maria Javier Ramirez, *Pamplona*
Eugenia Resmini, *Barcelona*
Pedro Romero-Aroca, *Reus*
Jordi Salas-Salvadó, *Reus*
Gines M Salido, *Caceres*
Victor Sanchez-Margalet, *Seville*
Helmut Schröder, *Barcelona*
Carmen Segundo, *Cádiz*
Rafael Simó, *Barcelona*

Sweden

Joanna Hlebowicz, *Malmö*
Kaj S Stenlöf, *Göteborg*
Ann-Britt Wiréhn, *Linköping*
Weili Xu, *Stockholm*
Shao-Nian Yang, *Stockholm*

Switzerland

Kaspar Berneis, *Zurich*
Pascal Bovet, *Lausanne*
Luc Tappy, *Lausanne*
Christian Toso, *Geneva*

Thailand

Narattaphol Charoenphandhu, *Bangkok*
Arthorn Riewpaiboon, *Bangkok*
Rawee Teanpaisan, *Hat-Yai*
Viroj Wiwanitkit, *Bangkok*

Tunisia

Khaled Hamden, *Sfax*

Turkey

Ugur Cavlak, *Denizli*
Teoman Dogru, *Ankara*
Ersin Fadillioglu, *Ankara*
Abdurrahman Fatih Fidan, *Afyonkarahisar*
Muammer Karadeniz, *Bornova-Izmir*
Cevdet Kaya, *Istanbul*
Fahrettin Kelestimur, *Kayseri*
Altan Onat, *Istanbul*
Semir Ozdemir, *Antalya*
Mustafa Şahin, *Ankara*
Ilker Tasci, *Ankara*
Belma Turan, *Ankara*
Serap Yalın, *Mersin*

United Arab Emirates

Ernest Akingunola Adeghate, *Al Ain*
Mukesh M Agarwal, *Al Ain*
Samir M Awadallah, *Sharjah*

United Kingdom

Nisreen Alwan, *Leeds*
Ambika P Ashraf, *Birmingham*
Chen Bing, *Liverpool*
Fay Crawford, *Edinburgh*
Tim M Curtis, *Belfast*
Umesh Dashora, *Hastings*
Gareth Davison, *Belfast*
Peter Raymond Flatt, *Coleraine*
Kathleen M Gillespie, *Bristol*
Peter John Grant, *Leeds*
Lorna W Harries, *Exeter*
Nigel Hoggard, *Aberdeen*
Nigel Irwin, *Coleraine*
Edward Jude, *Lancashire*
Andreas F Kolb, *Aberdeen*
Stefan Marciniak, *Cambridge*
Moffat Joha Nyirenda, *Edinburgh*
Jeetesh Patel, *Birmingham*
Snorri Bjorn Rafnsson, *Edinburgh*
Thozhukat Sathyapalan, *Yorkshire*
Latika Sibal, *Newcastle*
Rajagopalan Sriraman, *Lincoln*
Ramasamyiyer Swaminathan, *London*
Abd A Tahrani, *Birmingham*
G Neil Thomas, *Birmingham*
Cecil Thompson, *London*
Paul Henry Whiting, *Leicester*

United States

Varun Agrawal, *Springfield*
Mohamed Al-Shabrawey, *Augusta*
Pascale Alard, *Louisville*
Omar Ali, *Milwaukee*

Judith Aponte, *New York*
Balamurugan N Appakalai, *Minneapolis*
Hwyda A Arafat, *Philadelphia*
Carl V Asche, *Salt Lake*
Sanford A Asher, *Pittsburgh*
Anthony Atala, *Winston-Salem*
Sami Toufic Azar, *Beirut*
George Louis Bakris, *Chicago*
Alistair J Barber, *Hershey*
Daniel C Batlle, *Chicago*
David SH Bell, *Birmingham*
Rita Bortell, *Worcester*
Sebastien G Bouret, *Los Angeles*
David Lloyd Brown, *Stony Brook*
Lu Cai, *Louisville*
Jack D Caldwell, *Erie*
Anna C Calkin, *Los Angeles*
Roberto A Calle, *Groton*
R Keith Campbell, *Pullman*
Carlos Campos, *New Braunfels*
Heping Cao, *New Orleans*
Krista Casazza, *Birmingham*
Aaron Brandon Caughey, *Portland*
Eileen R Chasens, *Pittsburgh*
Munmun Chattopadhyay, *Ann Arbor*
Xiao-Li Chen, *St Paul*
Sheri Renee Colberg, *Norfolk*
Craig Ian Coleman, *Hartford*
Robert Russell Conley, *Indianapolis*
Colleen M Croniger, *Cleveland*
Doyle M Cummings, *Greenville*
William C Cushman, *Memphis*
Patricia Ann D'Amore, *Boston*
Patricia Darbishire, *West Lafayette*
Guillaume Darrasse-Jèze, *New York*
Ravi M Dasu, *Sacramento*
Michael Harvey Davidson, *Chicago*
Prakash Deedwania, *SanFrancisco*
Hong-Wen Deng, *Kansas City*
Teresa P DiLorenzo, *Bronx*
Scot E Dowd, *Lubbock*
Samuel C Durso, *Baltimore*
Krystal L Edwards, *Dallas*
Alexander M Efanov, *Indianapolis*
Azza B El-Remessy, *Augusta*
Amy Zhihong Fan, *Atlanta*
Melissa Spezia Faulkner, *Tucson*
George S Ferzli, *Staten Island*
Paolo Fiorina, *Boston*
James Edward Foley, *East Hanover*
Samuel N Forjuoh, *Temple*
Alessia Fornoni, *Miami*
Martha M Funnell, *Ann Arbor*
Trudy Gaillard, *Columbus*
Pietro Galassetti, *Irvine*
Claudia Gragnoli, *Hershey*
Jennifer B Green, *Durham*
Gary J Grover, *Piscataway*
Alok Kumar Gupta, *Baton Rouge*
Werner K Gurr, *New Haven*
Samy L Habib, *San Antonio*
Abdel Rahim Hamad, *Baltimore*
Daniel M Herron, *New York*
Tiffany Hilton, *Rochester*
Raimund Hirschberg, *Torrance*
Michael Francis Holick, *Boston*
Philip Hooper, *Aurora*
Zhaoyong Hu, *Houston*
Rachel Mary Hudacko, *New Brunswick*
Yasuo Ido, *Boston*
Brian K Irons, *Lubbock*
Pamela Itkin-Ansari, *La Jolla*
Hieronim Jakubowski, *Newark*
Hong-Lin Jiang, *Blacksburg*
Ping Jiao, *Providence*
Shengkan Jin, *Piscataway*
Arpita Kalla, *St Louis*
Richard Evers Katholi, *Springfield*
Melina Rae Kibbe, *Chicago*
Bhumsoo Kim, *Ann Arbor*
Tomoshige Kino, *Bethesda*
Julienne K Kirk, *Winston-Salem*
Renu A Kowluru, *Detroit*
Lewis H Kuller, *Pittsburgh*
Rajesh Kumar, *Temple*
Blandine Laferrère, *New York*
Sang Yeoup Lee, *Mayo*
Cong-Jun Li, *Beltsville*
Ching-Shwun Lin, *San Francisco*
Julie Lin, *Boston*
Shuo Lin, *Los Angeles*
Peter Lindgren, *San Diego*
James F List, *Princeton*
Dong-Min Liu, *Blacksburg*
Zhen-Qi Liu, *Charlottesville*
George William Lyerly, *Conway*
Jian-Xing Ma, *Oklahoma City*
Rong Ma, *Fort Worth*
Xin-Laing Ma, *Philadelphia*
David Maggs, *San Diego*
Kenneth Maiese, *Detroit*
Kevin C Maki, *Glen Ellyn*
Sridhar Mani, *Bronx*
Suresh Mathews, *Auburn*
Lauraar McCabe, *East Lansing*
Sarah E Messiah, *Miami*
Thomas O Metz, *Richland*
Shannon A Miller, *Orlando*
Murielle Mimeault, *Omaha*
Raghavendra G Mirmira, *Indianapolis*
Prasun J Mishra, *Bethesda*
Reema Mody, *Grayslake*
Arshag D Mooradian, *Jacksonville*
Mohammad Reza Movahed, *Tucson*
James Mu, *Rahway*
Muraleedharan G Nair, *East Lansing*
Manuel F Navedo, *Seattle*
Charles B Nemeroff, *Atlanta*
Joshua J Neumiller, *Spokane*
Steven Nissen, *Cleveland*
Hirofumi Noguchi, *Fort Worth*
Craig Nunemake, *Charlottesville*
Patrick J O'Connor, *Minneapolis*
Erin St Onge, *Apopka*
Wei-Hong Pan, *Baton Rouge*
Naushira Pandya, *Fort Lauderdale*
Michael R Peluso, *Corvallis*
Inga Peter, *New York*
Axel Pflueger, *Rochester*
Gretchen A Piatt, *Pittsburgh*
John D Piette, *Ann Arbor*
Leonid Poretsky, *New York*
Walter J Pories, *Greenville*
Parviz M Pour, *Omaha*
Wei Qiao Qiu, *Boston*
Teresa Quattrin, *Buffalo*
Cristina Rabadán-Diehl, *Bethesda*
Rajendra S Raghow, *Memphis*
Swapnil Rajpathak, *Bronx*
Armin Rashidi, *Norfolk*
Mohammed S Razzaque, *Boston*
Beverly A S Reyes, *Philadelphia*
David Rodbard, *Potomac*
Helena W Rodbard, *Rockville*
June Hart Romeo, *Cleveland*
Raul J Rosenthal, *Fort Lauderdale*
Juan M Saavedra, *Bethesda*
Stephen W Schaffer, *Mobile*
Frank AJL Scheer, *Boston*
Richard E Scranton, *Tiverton*
Vallabh (Raj) O Shah, *Albuquerque*
Aziz Shaibani, *Houston*
Jin-Xiong She, *Augusta*
Guo-Ping Shi, *Boston*
Carol Ann Shively, *Winston-Salem*
Anders AF Sima, *Detroit*
Pramil N Singh, *Loma Linda*
Rajan Singh, *Los Angeles*
Jay S Skyler, *Miami*
Dawn Smiley, *Atlanta*
Matthew D Solomon, *Stanford*
Mark A Sperling, *Pittsburgh*
Rakesh K Srivastava, *Tyler*
Bangyan Stiles, *Los Angeles*
Yu-Xiang Sun, *Houston*
Salim Surani, *Corpus Christi*
Arthur L M Swislocki, *Martinez*
Ya-Xiong Tao, *Auburn*
John A Tayek, *Torrance*
John Gaylord Teeter, *New Haven*
Carlos Marcelo Telleria, *Vermillion*
Christopher Gordon Thanos, *Providence*
Ronald G Tilton, *Galveston*
Serena Tonstad, *Loma Linda*
Michael Lawrence Traub, *Staten Island*
Guillermo E Umpierrez, *Atlanta*
Margrit Urbanek, *Chicago*
Gabriel I Uwaifo, *Baton Rouge*
Volker Vallon, *San Diego*
Shambhu D Varma, *Baltimore*
Maria Virella, *Charleston*
Hong-Jun Wang, *Boston*
Mark E Williams, *Boston*
Nathan D Wong, *Irvine*
Guangyu Wu, *New Orleans*
Zhong-Jian Xie, *San Francisoco*
Ming-Zhao Xing, *Baltimore*
Hariom Yadav, *Bethesda*
Lijun Yang, *Gainesville*
Ruojing Yang, *Rahway*
Subhashini Yaturu, *Albany*
Joseph Yeboah, *Charlottesville*
Dengping Yin, *Nashville*
Yisang Yoon, *Rochester*
Yi-Hao Yu, *New York*
Kevin CJ Yuen, *Portland*
Ian Stuart Zagon, *Hershey*
Robert Yuk-Lung Zee, *Boston*
Cui-Lin Zhang, *Rockville*
James Xuejie Zhang, *Richmond*
Sarah Zhang, *Oklahoma*
Guixiang Zhao, *Atlanta*
Yang Zhao, *Indianapolis*
Ming-Hui Zou, *Oklahoma City*

Venezuela

Fuad Lechin, *Caracas*

Yemen

Khaled Abdul-Aziz Ahmed, *Ibb*

World Journal of Diabetes

Contents

Bimonthly Volume 5 Number 5 October 15, 2014

TOPIC HIGHLIGHT

577 Peripheral arterial disease, type 2 diabetes and postprandial lipidaemia: Is there a link?
Valdivielso P, Ramírez-Bollero J, Pérez-López C

586 Hepatitis C virus infection and type 1 and type 2 diabetes mellitus
Antonelli A, Ferrari SM, Giuggioli D, Di Domenicantonio A, Ruffilli I, Corrado A, Fabiani S, Marchi S, Ferri C, Ferrannini E, Fallahi P

601 Prognostic value of endothelial dysfunction in type 1 diabetes mellitus
Ladeia AM, Sampaio RR, Hita MC, Adan LF

REVIEW

606 Counterregulation of insulin by leptin as key component of autonomic regulation of body weight
Borer KT

630 Challenges of emerging adulthood-transition from paediatric to adult diabetes
Gill G, Nayak AU, Wilkins J, Hankey J, Raffeeq P, Varughese GI, Varadhan L

636 Treatment of type 2 diabetes, lifestyle, GLP1 agonists and DPP4 inhibitors
Tomkin GH

651 Diabetes treatment in patients with renal disease: Is the landscape clear enough?
Ioannidis I

659 Acute effects of physical exercise in type 2 diabetes: A review
Asano RY, Sales MM, Browne RAV, Moraes JFVN, Coelho Júnior HJ, Moraes MR, Simões HG

666 Molecular mechanisms of protein induced hyperinsulinaemic hypoglycaemia
Chandran S, Yap F, Hussain K

678 Hepatocyte growth factor, a biomarker of macroangiopathy in diabetes mellitus
Konya H, Miuchi M, Satani K, Matsutani S, Tsunoda T, Yano Y, Katsuno T, Hamaguchi T, Miyagawa JI, Namba M

689 Advances in management of type 1 diabetes mellitus
Aathira R, Jain V

	697	Targeting inflammation in diabetes: Newer therapeutic options *Agrawal NK, Kant S*
EVIDENCE-BASED MEDICINE	711	HLA alleles may serve as a tool to discriminate atypical type 2 diabetic patients *Fernández M, Fabregat M, Javiel G, Mimbacas A*
RETROSPECTIVE STUDY	717	Taste sensitivity, nutritional status and metabolic syndrome: Implication in weight loss dietary interventions *Bertoli S, Laureati M, Battezzati A, Bergamaschi V, Cereda E, Spadafranca A, Vignati L, Pagliarini E*
PROSPECTIVE STUDY	724	Perfluorocarbon in vitreoretinal surgery and preoperative bevacizumab in diabetic tractional retinal detachment *Arevalo JF, Serrano MA, Arias JD*

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Diabetes*, KVS Hari Kumar, MD, Department of Endocrinology, Command Hospital, CH (CC), Lucknow, 226002, India

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α, β, δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ ABSTRACTING

World Journal of Diabetes is now indexed in PubMed Central, PubMed, Digital Object Identifier, and Directory of Open Access Journals.

FLYLEAF I-V Editorial Board

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
April 15, 2010

FREQUENCY
Bimonthly

EDITORIAL OFFICE
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director
World Journal of Diabetes
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
October 15, 2014

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-9358/g_info_20100107165233.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.577

World J Diabetes 2014 October 15; 5(5): 577-585
ISSN 1948-9358 (online)

TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (2): Type 2 diabetes

Peripheral arterial disease, type 2 diabetes and postprandial lipidaemia: Is there a link?

Pedro Valdivielso, José Ramírez-Bollero, Carmen Pérez-López

Pedro Valdivielso, José Ramírez-Bollero, Carmen Pérez-López, UGC de Medicina Interna, Hospital "Virgen de la Victoria" de Málaga y Departamento de Medicina y Dermatología, Universidad de Málaga, 29010 Málaga, Spain
Author contributions: Valdivielso P, Ramírez-Bollero J and Pérez-López C contributed to the paper.
Supported by Grant to Grupo CTS-159 of PAIDI (Plan Andaluz de Investigación, Desarrollo e Innovación) de la Junta de Andalucía
Correspondence to: Dr. Pedro Valdivielso, UGC de Medicina Interna, Hospital "Virgen de la Victoria" de Málaga y Departamento de Medicina y Dermatología, Universidad de Málaga, Campus de Teatinos s/n, 29010 Málaga, Spain. valdivielso@uma.es
Telephone: +34-951-032365 Fax: +34-952-131511
Received: January 24, 2014 Revised: March 19, 2014
Accepted: July 17, 2014
Published online: October 15, 2014

Abstract

Peripheral arterial disease, manifested as intermittent claudication or critical ischaemia, or identified by an ankle/brachial index < 0.9, is present in at least one in every four patients with type 2 diabetes mellitus. Several reasons exist for peripheral arterial disease in diabetes. In addition to hyperglycaemia, smoking and hypertension, the dyslipidaemia that accompanies type 2 diabetes and is characterised by increased triglyceride levels and reduced high-density lipoprotein cholesterol concentrations also seems to contribute to this association. Recent years have witnessed an increased interest in postprandial lipidaemia, as a result of various prospective studies showing that non-fasting triglycerides predict the onset of arteriosclerotic cardiovascular disease better than fasting measurements do. Additionally, the use of certain specific postprandial particle markers, such as apolipoprotein B-48, makes it easier and more simple to approach the postprandial phenomenon. Despite this, only a few studies have evaluated the role of postprandial triglycerides in the development of peripheral arterial disease and type 2 diabetes. The purpose of this review is to examine the epidemiology and risk factors of peripheral arterial disease in type 2 diabetes, focusing on the role of postprandial triglycerides and particles.

Key words: Peripheral arterial disease; Type 2 diabetes; Postprandial lipidaemia; Apolipoprotein B-48; Ankle-brachial index; Non-fasting triglycerides

Core tip: Peripheral arterial disease is highly prevalent in type 2 diabetes; traditional risk factors contribute to the disease. Interestingly, postprandial lipidaemia is increased in both conditions. However, one study showed that only subjects with both type 2 diabetes and peripheral arterial disease had elevation of postprandial lipids; subjects with type 2 diabetes and a normal ankle-brachial index had a normal postprandial response. Because most of the triglycerides of chylomicrons are extracted in muscle and adipose cells in the legs, the authors speculate on whether arteriosclerosis in the legs may contribute to greater postprandial lipidaemia.

Valdivielso P, Ramírez-Bollero J, Pérez-López C. Peripheral arterial disease, type 2 diabetes and postprandial lipidaemia: Is there a link? *World J Diabetes* 2014; 5(5): 577-585 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/577.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.577

EPIDEMIOLOGY OF PERIPHERAL ARTERIAL DISEASE IN TYPE 2 DIABETES MELLITUS

Peripheral arterial disease (PAD) is produced by narrowing of the calibre of the medium-sized arteries and its widest

Table 1 Prevalence of peripheral arterial disease in Spanish cohorts

Study	Number of subjects	Age (yr)	Study population		ABI < 0.9 (%)
HERMEX[17]	2833	51	General	All	3.7
				Without diabetes	2.8
				With Diabetes	6.2
ESTIME[6]	1324	68	General	All	8
				Without diabetes	6.6
				With diabetes	19
MERITO[19]	1519	66	Internal medicine outpatient clinic	SCORE ⩾ 3	26.2
				With Diabetes	26.1
VITAMIN[20]	493	68	Internal medicine outpatient clinic	Without DM2	21
				With DM2	38
ARPTER[18]	3171	63	General	All	6.4
				Without diabetes	5.4
				With diabetes	12.6
REGICOR[21]	6262	56	General	All	4.5
				Without diabetes	4
				With diabetes	8.4
FUENCARRAL Health Center[22]	1360	70	Primary health care centre	Without diabetes	4.3
				With diabetes	11.3
ALBACETE[23]	784	61	General	All	10.5
				Without diabetes	9
				With diabetes	19
RONDA PRIM Health Center[25]	289	65	Primary health centres	Diabetes	21.5
CIUDAD JARDIN Health Center[78]	456	61	Primary health centre	Diabetes	27
PADiD Study[24]	1462	78	Internal medicine outpatient clinics	Diabetes	60
MARINA BAIXA Hospital[89]	360	67	Internal medicine outpatient clinics	Diabetes	27

ABI: Ankle-brachial index.

definition encompasses all extracoronary and extracerebral vascular disease. However, the term PAD is usually restricted to involvement of the lower limbs, particularly in the iliac bifurcation, and the iliofemoral and popliteal arteries[1]. The main cause of arterial stenosis in developed countries is atherosclerosis.

The prevalence of PAD in Europe and the United States is estimated to be 27 million persons[2]. The prevalence of PAD increases progressively with age, with most cases starting after the age of 40 years. It is well known that only a very few PAD patients actually have symptoms, around 10%-20%[3]. The use of a standardized questionnaire in the physician's office can increase the detection of claudicant patients[4,5]. Most patients with PAD are identified from non-invasive tests, such as the ankle-brachial index (ABI). Using this widely extended technique in Spain led to the identification of PAD in 8% of individuals aged 55-85 years[6]. In addition to age, the other cardiovascular risk factors also increase the likelihood of developing PAD. Thus, in persons with a low cardiovascular risk the prevalence of PAD is almost inconsiderable[7], whereas it can reach 27% in persons with type 2 diabetes[8].

The prognosis for patients with PAD, both symptomatic and asymptomatic, is poor[9]. Overall mortality is increased and the risk of death is even greater than that in patients who have angina or acute myocardial infarction[10-13]. Data from Spain confirm these findings. An analysis of the FRENA, REACH and AIRVAG registries showed that patients with PAD have a greater frequency of symptomatic multivessel disease and a worse one-year prognosis than patients with single-vessel involvement or cerebrovascular disease[14].

Diabetes and PAD

Diabetes, together with smoking, is the main risk factor for PAD[15]. Of patients who attended an angiology office in Spain due to intermittent claudication and who underwent arterial surgery or had an ABI ⩽ 0.9, 67% had diabetes mellitus[16]. Population-based studies in Spain, undertaken in either the general population or at various levels of care, showed that the presence of diabetes mellitus doubled or even tripled the possibility of having PAD (Table 1)[6,17-23]. The prevalence of an ABI < 0.9 in series of Spanish patients with diabetes ranges from 21% to 60% (Table 1)[8,24,25]. In the autonomous communities of Andalusia and the Canary Islands, 72% of all lower-limb amputations between 1996 and 2006 involved patients with diabetes[23,26,27]. In patients with diabetes, for every 1% increase in haemoglobin A1c there is a corresponding 26% increased risk of PAD[28]. The presence of PAD also increases the risk of death in patients with diabetes mellitus[29,30]. The prognosis for PAD is worse in patients with diabetes than those without diabetes[31].

Diagnosis of PAD in diabetes

The diagnosis of PAD usually depends on the sum of the symptoms, particularly intermittent claudication, plus the physical examination, especially the lack of pulses and the trophic disorders leading to critical limb ischaemia and distal necrosis[32]. However, patients, particularly diabetic patients, commonly have other processes at

the same time that can alter the traditional symptoms of PAD, making them much less specific[33]. Accordingly, the measurement of the ratio of the systolic blood pressures in the ankle and the arm, the ABI, has been recommended as the screening method for asymptomatic PAD and as a form of confirmation in symptomatic PAD[2,34,35]. A finding in one limb of an ABI < 0.9 with the measurement taken at rest under standard conditions is considered diagnostic of PAD, with an ABI between 0.9 and 1.0 considered borderline[36].

One limitation of the ABI, especially relevant in patients with diabetes, is arterial media calcification, which can lead to non-compressible arteries (ABI > 1.4) or false normal values. A recent study showed that individuals with an ABI > 1.4 have a worse prognosis than those with a normal ABI and even those with an ABI < 0.9. The prevalence of diabetes in the group with an ABI > 1.4 was 58%, compared with 18% and 48% in those with a normal ABI or those with an ABI < 0.9[37]. It has long been known that the sensitivity of the ABI to correctly diagnose PAD is considerably reduced in the presence of arterial media calcification and that, clinically, this calcification is associated with the presence of peripheral neuropathy[38,39]. Accordingly, in the presence of peripheral neuropathy it is recommended to use an alternative method, such as flow wave analysis using Doppler colour ultrasound[40,41]. In our experience this limitation is not negligible. In a series of 456 patients with type 2 diabetes, 35 were found to have intermittent claudication (7.6%); only 22 of these had an ABI < 0.9. Of the other 13, 12 underwent colour Doppler ultrasound and in 3 (25%) we obtained a monophasic wave, diagnostic of PAD. Thus, a normal ABI does not rule out PAD in patients with type 2 diabetes, and these patients should therefore undergo complementary tests if they have symptoms suggestive of PAD[8].

The resting ABI should be used as the diagnostic technique for PAD when lower limb arteriosclerosis is suspected. This should be done in persons with one or more of the following: symptoms in the lower limbs after exercise, wounds with delayed healing, and individuals older than 65 years of age or older than 50 years with a history of smoking or diabetes[34]. Given the high prevalence of PAD in patients with diabetes, the ADA recommends screening with the ABI in patients with diabetes who are older than 50 years and who have another risk factor (smoking, hypertension, hyperlipidaemia, or diabetes for more than 10 years)[42].

LIPIDS, POSTPRANDIAL LIPIDAEMIA AND PAD

Fasting lipids in PAD

Lipid abnormalities in PAD have received less attention than in other areas, as for example, in coronary anomalies. Very few prospective studies have focused on the relation between triglycerides and peripheral vascular disease. The most common feature of PAD is raised levels of triglycerides and lower levels of high-density lipoprotein (HDL) cholesterol as compared with age- and sex-matched controls without vascular disease, with similar levels of cholesterol and low-density lipoprotein (LDL) cholesterol[43-47]. The frequency of a cluster of lipid abnormalities of the type of raised triglycerides and small and dense LDL and reduced HDL was 20% in persons with PAD *vs* 0% in the control group[48]. Several studies have also shown that triglyceride levels are a predictive factor for PAD[49-51], though not all[52].

Postprandial lipidaemia: Atherogenic mechanism

Unlike the carbohydrates, which normally only show transitory increases after a meal, the circulating triglycerides show a pronounced increase (postprandial lipidaemia) one hour after the intake of a fat-rich meal (around 30-60 g), and can remain high for 5-8 h after the meal. As most persons regularly consume fatty meals every 4-5 h, the usual state in humans insofar as their triglyceride metabolism is concerned is clearly a continuous postprandial lipidaemic state[53,54].

The large triglyceride-transporting particles, the chylomicrons and the very low-density lipoprotein (VLDL), are too large to cross the endothelium and they therefore don't contribute to the atherosclerosis, but the same does not occur with the chylomicron remnants and the intermediate-density lipoprotein (IDL), which are much smaller particles[55]. Evidence exists that the cholesterol in the postprandial particles, originating in the intestine, contribute to the phenomenon of atherosclerosis, both in animals and in humans[56-59].

Postprandial lipidaemia and cardiovascular disease: Case-control vs prospective studies

Since the seminal work of Zilversmit, many case-control studies have found an association between the magnitude of the postprandial lipidaemia and the presence and severity of coronary artery disease[60,61]; these studies have been reviewed by Lopez-Miranda *et al*[62]. Prospective studies, however, are few and controversial. Reyes-Soffer *et al*[63] followed 69 patients with type 2 diabetes who were free of coronary disease for a mean of 8.7 years; 33 patients remained disease-free. No differences were found in the postprandial parameters at the initial visit between the groups, and the authors concluded that the postprandial triglycerides do not predict the onset of coronary disease in individuals with diabetes. A more recent study involving 514 survivors of an acute coronary syndrome found that the postprandial triglycerides after the oral intake of 75 g of fat predicted the appearance of new events at 18 mo. In the subgroup of patients without diabetes or oral glucose intolerance the relative increase in postprandial triglycerides was an independent predictor of events[64].

Non-fasting triglycerides

Interest in studying postprandial lipidaemia has increased over recent years as a result of studies showing that serum triglyceride levels measured in a non-fasting state have

proved to be better predictors for the risk of vascular disease than fasting triglyceride concentrations, *i.e.*, when they are quantified after 8-10 h of fasting[65-68]. Two meta-analyses also support the association between fasting and postprandial triglycerides and the vascular risk[69,70]. One of the problems encountered when introducing postprandial triglyceride measurements in the clinical setting is the absence of specific recommendations in the clinical practice guidelines and thus the identification of a threshold level above which postprandial hypertriglyceridaemia is recognised. To date, only the American Association of Clinical Endocrinologists has considered the possibility of evaluating the non-fasting triglyceride concentration[71]. Based on evidence from the above mentioned population-based studies, an expert group estimated non-fasting triglyceride levels < 180 mg/dL as desirable[72]. This means that 38% of the men and 20% of the women in the Copenhagen study who had figures above these levels have postprandial hypertriglyceridaemia[73].

Suggestion for the measurement of postprandial lipidaemia

The study of postprandial (hyper)lipidaemia has several inconveniences. The most important at present is the poor clinical yield and the great complexity of the fat test; its prolonged time is uncomfortable for both the patient and the medical personnel, not to mention the lack of standardization for the test. A few years ago, using data from a meta-analysis of 113 studies in healthy subjects by Mihas *et al*[74], an expert group attempted to standardize the test and recommended a fat tolerance test meal consisting of 75 g fat, 25 g carbohydrates and 10 g protein. Furthermore, the fatty test meal should contain mixtures of saturated and unsaturated fatty acids in a digestible form and be easy to prepare. The candidates for the test should have fasting triglycerides of 90-180 mg/dL and the test can be shortened with the measurement of the serum triglycerides at 4 h, with no need to reach a complete postprandial curve of 8 or 12 h[72].

POSTPRANDIAL LIPIDAEMIA AND PAD IN TYPE 2 DIABETES

Little attention has been given to the study of postprandial lipidaemia in patients with PAD. Only the elegant paper by Lupattelli *et al*[75] showed that the magnitude of postprandial lipidaemia, expressed as "the area under the incremental curve for triglycerides," was higher in 16 non-diabetic normolipidaemic claudicant patients with PAD than in 10 normolipidaemic control subjects, suggesting the relevance of postprandial lipoprotein metabolism in the pathogenesis of peripheral atherosclerosis. However, although normolipidaemic, the patients in Lupattelli's study had slightly higher fasting triglycerides than their controls.

In recent years our group has studied the relation between lipids and postprandial particles, PAD and type 2 diabetes mellitus. Firstly, the postprandial triglycerides were more strongly associated with PAD in individuals with type 2 diabetes mellitus than were the fasting triglycerides. A group of 119 patients with type 2 diabetes mellitus treated with just diet and/or oral glucose lowering agents, with no lipid-lowering treatment, were analyzed at fasting and 4 h after a mixed breakfast containing 50 g of fat and 40 g of carbohydrates. Although the patients with cardiovascular disease, most of them with asymptomatic PAD and identified by an ABI < 0.9, had lower fasting HDL cholesterol levels and higher triglyceride levels, only the triglycerides at 4 h post-breakfast were associated in the multivariate analysis with cardiovascular disease, together with the duration of the disease and smoking[76].

The postprandial triglycerides include not only those contained in chylomicron particles and their remnants, but also those contained in VLDL and IDL. In an attempt to further understand the role of postprandial fat in PAD, we undertook a second experiment to analyze the serum concentration of apolipoprotein B48, a protein that is only associated with chylomicrons and their remnants and is not interchanged with any other circulating particle. This second study involved 101 patients with type 2 diabetes mellitus and 73 controls without diabetes, both groups with no known cardiovascular disease. Asymptomatic vascular disease was identified from the ABI and as a marker of postprandial particles we used the apolipoprotein B48, measured with a commercial enzyme-linked immunosorbent assay. Of the patients with type 2 diabetes mellitus, 21 had PAD as defined by an ABI < 0.9, though no control had PAD. The levels of triglycerides and apolipoprotein B48, both fasting and postprandial, were significantly higher in the group of diabetic patients with PAD than in those without PAD and the controls. Curiously, no differences were found between the controls and the patients with type 2 diabetes mellitus without PAD. Of all the lipid and non-lipid parameters studied, only apolipoprotein B48 and smoking were associated with the presence of PAD in a binary logistic regression analysis. Likewise, the presence of PAD was an independent predictor of the levels of apolipoprotein B48, both fasting and 4 h after a mixed breakfast[77].

As the patients with type 2 diabetes mellitus in the previous studies did not receive any insulin or lipid-lowering therapy, we decided to confirm the findings in a larger population with type 2 diabetes mellitus without these exclusion criteria. Again, using an ABI < 0.9 as a marker of PAD, we found in 456 patients with type 2 diabetes mellitus that fasting apolipoprotein B48 was a marker of PAD, independently of the other lipid factors, statin treatment or insulin therapy[78]. Identical results have also been reported by another group[79].

May PAD delay postprandial lipid catabolism?

Taken together, these studies confirm an association between postprandial particles, measured as triglycerides 4 h after breakfast or as fasting and postprandial apolipoprotein B48, and PAD. In the above-mentioned studies, a diabetic status in itself was not associated with a greater

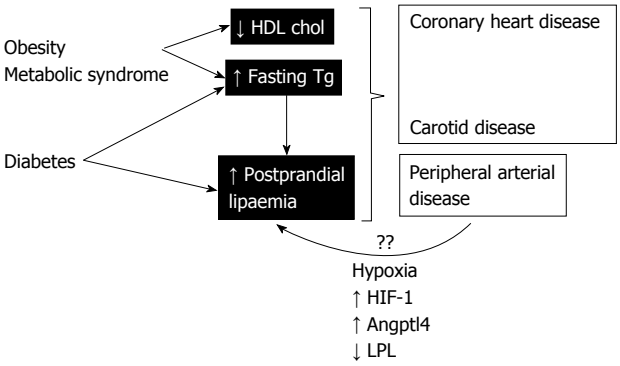

Figure 1 Proposed mechanism linking peripheral arterial disease and worsening postprandial lipaemia. HIF-1: Hypoxia-induced factor 1; Angptl4: Angiopoietin-like protein 4; LPL: Lipoprotein Lipase; HDL: high density lipoproteins.

concentration of postprandial triglycerides or apolipoprotein B48 if there was no PAD. As mentioned earlier, the case-control studies show an association between postprandial lipidaemia and cardiovascular disease, particularly coronary disease.

An explication for this association was provided by Lupattelli *et al*[75]. Somehow, and following the hypothesis of Zilversmit[80], the exposure of the endothelium to greater concentrations of postprandial particles favours the appearance of arteriosclerotic lesions, in our case in the lower limbs. Though this hypothesis is the most plausible, no causality can be deduced from the association studies. Accordingly, it is worth speculating about whether arteriosclerotic disease in the legs could alter chylomicron metabolism, slowing it. With this in mind, consideration should be given to the study by Horton *et al*[81], who showed that men have higher triglyceride concentrations than women because women posses a greater extractive capacity of triglycerides in adipose and muscle tissues in the lower limbs when they undergo a fatty breakfast. For some reason the catabolism of the chylomicrons in the legs is not negligible and an alteration in the circulation in the legs may worsen or slow this metabolism.

The kinetics of lipoproteins are marked by (1) their intestinal production; (2) hydrolysis of their triglycerides by the action of lipoprotein-lipase anchored in the endothelium (but synthesised in adipose and muscle tissue cells); and (3) removal of chylomicron remnants by hepatic receptors. These steps are all modulated by the levels and genetic variants of the apolipoproteins like C-Ⅱ, C-Ⅲ, E, A-5[82,83]. As persons with arteriosclerosis, particularly those with PAD, have a marked endothelial dysfunction[84], it is possible to speculate that the action of an enzyme anchored to the endothelium, as is the case of lipoprotein lipase (LPL), is reduced. Given the great extension of the endothelial surface in the legs (in comparison with coronary arteriosclerosis), established PAD might affect postprandial lipidaemia more intensely than coronary disease.

If this hypothesis were true, what would its mechanism of production be? The consequence of arteriosclerosis is tissue ischaemia. This is usually manifested as intermittent claudication, though the tissues may experience hypoxia in earlier stages. Tissue hypoxia leads to changes in the endothelial cells (where the LPL are anchored) or in the production of LPL (or its associated proteins) by adipose or muscle cells[85]. Cells submitted to hypoxia upregulate the expression of hypoxia-inducible factor 1, a transcription factor that induces changes in innumerable target genes that were reviewed some time ago[86]. Of note among these changes is the raised expression of angiopoietin-like 4 protein (Angptl4) and vascular endothelial growth factor (VEGF). VEGF intervenes in the processes of angiogenesis, much related with chronic ischaemia of the lower limbs and the formation of collateral vessels. Angptl4 is a potent inhibitor of LPL, the enzyme that intervenes critically in the first step of the catabolism of triglyceride-rich particles[87]. A recent experimental animal study showed that mice submitted to cyclic hypoxia experienced inhibition of the catabolism of triglyceride-rich lipoproteins as a consequence of a drastic reduction in adipose tissue LPL activity, coupled with a notable increase in Angptl4[88] (Figure 1).

Taken together, these data suggest that postprandial hyperlipidaemia, a recognised vascular risk factor associated with obesity, the metabolic syndrome and type 2 diabetes, could be aggravated by PAD, further exposing other arterial territories to greater concentrations of postprandial atherogenic particles. Finally, if the hypoxia were an underlying mechanism, it could be improved by percutaneous or surgical revascularization.

ACKNOWLEDGMENTS

Authors would like to thank to Ian Johnstone for the English edition of the manuscript.

REFERENCES

1 **Guijarro C**, Mostaza JM, Hernández-Mijares A. [Lower limb arterial disease and renal artery stenosis]. *Clin Investig Arterioscler* 2013; **25**: 218-223 [PMID: 24238748 DOI: 10.1016/j.arteri.2013.10.002]

2 **Norgren L**, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *J Vasc Surg* 2007; **45** Suppl S: S5-67 [PMID: 17223489 DOI: 10.1016/j.jvs.2006.12.037]

3 **Weitz JI**, Byrne J, Clagett GP, Farkouh ME, Porter JM, Sackett DL, Strandness DE, Taylor LM. Diagnosis and treatment of chronic arterial insufficiency of the lower extremities: a critical review. *Circulation* 1996; **94**: 3026-3049 [PMID: 8941154]

4 **Leng GC**, Fowkes FG. The Edinburgh Claudication Questionnaire: an improved version of the WHO/Rose Questionnaire for use in epidemiological surveys. *J Clin Epidemiol* 1992; **45**: 1101-1109 [PMID: 1474406 DOI: 10.1016/0895-4356(92)90150-L]

5 **Fowkes FG**, Housley E, Cawood EH, Macintyre CC, Ruckley CV, Prescott RJ. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *Int J Epidemiol* 1991; **20**: 384-392 [PMID: 1917239 DOI: 10.1093/ije/20.2.384]

6 **Blanes JI**, Cairols MA, Marrugat J. Prevalence of peripheral artery disease and its associated risk factors in Spain: The ESTIME Study. *Int Angiol* 2009; **28**: 20-25 [PMID: 19190551]

7 **Alonso I**, Valdivielso P, Josefa Zamudio M, Sánchez Chaparro MA, Pérez F, Ramos H, González Santos P. [Usefulness of the ankle-arm index for detection of peripheral arterial disease in a working population of Junta de Andalucía at Málaga]. *Med Clin* (Barc) 2009; **132**: 7-11 [PMID: 19174058 DOI: 10.1016/j.medcli.2008.02.002]

8 **Mancera-Romero J**, Rodríguez-Morata A, Angel Sánchez-Chaparro M, Sánchez-Pérez M, Paniagua-Gómez F, Hidalgo-Conde A, Valdivielso P. Role of an intermittent claudication questionnaire for the diagnosis of PAD in ambulatory patients with type 2 diabetes. *Int Angiol* 2013; **32**: 512-517 [PMID: 23903311]

9 **Diehm C**, Allenberg JR, Pittrow D, Mahn M, Tepohl G, Haberl RL, Darius H, Burghaus I, Trampisch HJ. Mortality and vascular morbidity in older adults with asymptomatic versus symptomatic peripheral artery disease. *Circulation* 2009; **120**: 2053-2061 [PMID: 19901192 DOI: 10.1161/circulationaha.109.865600]

10 **Kieback AG**, Lorbeer R, Wallaschofski H, Ittermann T, Völzke H, Felix S, Dörr M. Claudication, in contrast to angina pectoris, independently predicts mortality risk in the general population. *Vasa* 2012; **41**: 105-113 [PMID: 22403128 DOI: 10.1024/0301-1526/a000172]

11 **Inglis SC**, Lewsey JD, Lowe GD, Jhund P, Gillies M, Stewart S, Capewell S, Macintyre K, McMurray JJ. Angina and intermittent claudication in 7403 participants of the 2003 Scottish Health Survey: impact on general and mental health, quality of life and five-year mortality. *Int J Cardiol* 2013; **167**: 2149-2155 [PMID: 22704868]

12 **Criqui MH**, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ, Browner D. Mortality over a period of 10 years in patients with peripheral arterial disease. *N Engl J Med* 1992; **326**: 381-386 [PMID: 1729621 DOI: 10.1056/NEJM199202063260605]

13 **Allison MA**, Hiatt WR, Hirsch AT, Coll JR, Criqui MH. A high ankle-brachial index is associated with increased cardiovascular disease morbidity and lower quality of life. *J Am Coll Cardiol* 2008; **51**: 1292-1298 [PMID: 18371562 DOI: 10.1016/j.jacc.2007.11.064]

14 **Guijarro C**. Enfermedad arterial oclusiva en los estudios REACH, FRENA y AIRVAG. *Anales de Cirugía Vascular* 2009; **23**: 21-27 [DOI: 10.1016/S1130-2542(09)70813-0]

15 **Criqui MH**. Peripheral arterial disease--epidemiological aspects. *Vasc Med* 2001; **6**: 3-7 [PMID: 11789963 DOI: 10.1177/1358836X0100600i102]

16 **Puras-Mallagray E**, Gutiérrez-Baz M, Cáncer-Pérez S, Alfayate-García JM, de Benito-Fernández L, Perera-Sabio M, Criado-Galán F, Hernández-Mijares A. Estudio de prevalencia de la enfermedad arterial periférica y diabetes en Espa-a. *Angiologia* 2008; **60**: 317-326

17 **Félix-Redondo FJ**, Fernández-Bergés D, Grau M, Baena-Diez JM, Mostaza JM, Vila J. Prevalence and clinical characteristics of peripheral arterial disease in the study population Hermex. *Rev Esp Cardiol* (Engl Ed) 2012; **65**: 726-733 [PMID: 22727799 DOI: 10.1016/j.recesp.2012.03.008]

18 **Baena-Díez JM**, Alzamora MT, Forés R, Pera G, Torán P, Sorribes M. Ankle-brachial index improves the classification of cardiovascular risk: PERART/ARTPER Study. *Rev Esp Cardiol* 2011; **64**: 186-192 [PMID: 21330032 DOI: 10.1016/j.recesp.2010.10.024]

19 **Manzano L**, Mostaza JM, Suárez C, Cairols M, Redondo R, Valdivielso P, Monte R, Blázquez JC, Ferreira EM, Trouillhet I, González-Igual JJ, Sánchez-Zamorano MA. [Value of the ankle-brachial index in cardiovascular risk stratification of patients without known atherotrombotic disease. MERITO study]. *Med Clin* (Barc) 2007; **128**: 241-246 [PMID: 17335735 DOI: 10.1157/13099239]

20 **Manzano L**, García-Díaz Jde D, Gómez-Cerezo J, Mateos J, del Valle FJ, Medina-Asensio J, Viejo LF, Fernández-Ballesteros A, Solís J, Herrero Domingo A, Ferreira E, Sánchez-Fuentes D, Barragán JM, González-Moraleja J, Vargas JA, López-Jiménez M, Zamora J. [Clinical value of the ankle-brachial index in patients at risk of cardiovascular disease but without known atherothrombotic disease: VITAMIN study]. *Rev Esp Cardiol* 2006; **59**: 662-670 [PMID: 16938208 DOI: 10.1157/13091367]

21 **Ramos R**, Quesada M, Solanas P, Subirana I, Sala J, Vila J, Masia R, Cerezo C, Elosua R, Grau M, Cordon F, Juvinya D, Fito M, Isabel Covas M, Clara A, Angel Munoz M, Marrugat J, Investigators obotR. Prevalence of Symptomatic and Asymptomatic Peripheral Arterial Disease and the Value of the Ankle-brachial Index to Stratify Cardiovascular Risk. *Eur J Vasc Endovasc Surg* 2009; **38**: 305-311 [DOI: 10.1016/j.ejvs.2009.04.013]

22 **Vicente I**, Lahoz C, Taboada M, Laguna F, García-Iglesias F, Mostaza Prieto JM. [Ankle-brachial index in patients with diabetes mellitus: prevalence and risk factors]. *Rev Clin Esp* 2006; **206**: 225-229 [PMID: 16750105 DOI: 10.1157/13088561]

23 **Carbayo JA**, Divisón JA, Escribano J, López-Abril J, López de Coca E, Artigao LM, Martínez E, Sanchis C, Massó J, Carrión L. Using ankle-brachial index to detect peripheral arterial disease: prevalence and associated risk factors in a random population sample. *Nutr Metab Cardiovasc Dis* 2007; **17**: 41-49 [PMID: 17174225 DOI: 10.1016/j.numecd.2005.08.009]

24 **González-Clemente JM**, Piniés JA, Calle-Pascual A, Saavedra A, Sánchez C, Bellido D, Martín-Folgueras T, Moraga I, Recasens A, Girbés J, Sánchez-Zamorano MA, Mauricio D. Cardiovascular risk factor management is poorer in diabetic patients with undiagnosed peripheral arterial disease than in those with known coronary heart disease or cerebrovascular disease. Results of a nationwide study in tertiary diabetes centres. *Diabet Med* 2008; **25**: 427-434 [PMID: 18341592 DOI: 10.1111/j.1464-5491.2008.02402.x]

25 **Bundó M**, Aubà J, Vallés R, Torner O, Pérez AM, Massons J. [Peripheral arteriopathy in type 2 diabetes mellitus]. *Aten Primaria* 1998; **22**: 5-11 [PMID: 9741155]

26 **Almaraz MC**, González-Romero S, Bravo M, Caballero FF, Palomo MJ, Vallejo R, Esteva I, Calleja F, Soriguer F. Incidence of lower limb amputations in individuals with and without diabetes mellitus in Andalusia (Spain) from 1998 to 2006. *Diabetes Res Clin Pract* 2012; **95**: 399-405 [PMID: 22133651 DOI: 10.1016/j.diabres.2011.10.035]

27 **Aragón-Sánchez J**, García-Rojas A, Lázaro-Martínez JL, Quintana-Marrero Y, Maynar-Moliner M, Rabellino M, Hernández-Herrero MJ, Cabrera-Galván JJ. Epidemiology of diabetes-related lower extremity amputations in Gran Canaria, Canary Islands (Spain). *Diabetes Res Clin Pract* 2009; **86**: e6-e8 [PMID: 19604593 DOI: 10.1016/j.diabres.2009.06.015]

28 **Selvin E**, Wattanakit K, Steffes MW, Coresh J, Sharrett AR. HbA1c and peripheral arterial disease in diabetes: the Atherosclerosis Risk in Communities study. *Diabetes Care* 2006; **29**: 877-882 [PMID: 16567831 DOI: 10.2337/diacare.29.04.06.dc05-2018]

29 **Leibson CL**, Ransom JE, Olson W, Zimmerman BR, O'fallon WM, Palumbo PJ. Peripheral arterial disease, diabetes, and mortality. *Diabetes Care* 2004; **27**: 2843-2849 [PMID: 15562195 DOI: 10.2337/diacare.27.12.2843]

30 **Norman PE**, Davis WA, Bruce DG, Davis TM. Peripheral arterial disease and risk of cardiac death in type 2 diabetes: the Fremantle Diabetes Study. *Diabetes Care* 2006; **29**: 575-580 [PMID: 16505509 DOI: 10.2337/diacare.29.03.06.dc05-1567]

31 **Jude EB**, Oyibo SO, Chalmers N, Boulton AJ. Peripheral arterial disease in diabetic and nondiabetic patients: a comparison of severity and outcome. *Diabetes Care* 2001; **24**: 1433-1437 [PMID: 11473082 DOI: 10.2337/diacare.24.8.1433]

32 **Layden J**, Michaels J, Bermingham S, Higgins B. Diagnosis and management of lower limb peripheral arterial disease: summary of NICE guidance. *BMJ* 2012; **345**: e4947 [PMID: 22875949 DOI: 10.1136/bmj.e4947]

33 **McDermott MM**, Greenland P, Liu K, Guralnik JM, Criqui

MH, Dolan NC, Chan C, Celic L, Pearce WH, Schneider JR, Sharma L, Clark E, Gibson D, Martin GJ. Leg symptoms in peripheral arterial disease: associated clinical characteristics and functional impairment. *JAMA* 2001; **286**: 1599-1606 [PMID: 11585483 DOI: 10.1001/jama.286.13.1599]

34 **Rooke TW**, Hirsch AT, Misra S, Sidawy AN, Beckman JA, Findeiss LK, Golzarian J, Gornik HL, Halperin JL, Jaff MR, Moneta GL, Olin JW, Stanley JC, White CJ, White JV, Zierler RE. 2011 ACCF/AHA focused update of the guideline for the management of patients with peripheral artery disease (updating the 2005 guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society for Vascular Medicine, and Society for Vascular Surgery. *J Vasc Surg* 2011; **54**: e32-e58 [PMID: 21958560 DOI: 10.1016/j.jvs.2011.09.001]

35 **Tendera M**, Aboyans V, Bartelink ML, Baumgartner I, Clément D, Collet JP, Cremonesi A, De Carlo M, Erbel R, Fowkes FG, Heras M, Kownator S, Minar E, Ostergren J, Poldermans D, Riambau V, Roffi M, Röther J, Sievert H, van Sambeek M, Zeller T. ESC Guidelines on the diagnosis and treatment of peripheral artery diseases: Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteries: the Task Force on the Diagnosis and Treatment of Peripheral Artery Diseases of the European Society of Cardiology (ESC). *Eur Heart J* 2011; **32**: 2851-2906 [PMID: 21873417 DOI: 10.1093/eurheartj/ehr211]

36 **Aboyans V**, Criqui MH, Abraham P, Allison MA, Creager MA, Diehm C, Fowkes FG, Hiatt WR, Jönsson B, Lacroix P, Marin B, McDermott MM, Norgren L, Pande RL, Preux PM, Stoffers HE, Treat-Jacobson D. Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association. *Circulation* 2012; **126**: 2890-2909 [PMID: 23159553 DOI: 10.1161/CIR.0b013e318276fbcb]

37 **Arain FA**, Ye Z, Bailey KR, Chen Q, Liu G, Leibson CL, Kullo IJ. Survival in patients with poorly compressible leg arteries. *J Am Coll Cardiol* 2012; **59**: 400-407 [PMID: 22261162 DOI: 10.1016/j.jacc.2011.09.055]

38 **Williams DT**, Harding KG, Price P. An evaluation of the efficacy of methods used in screening for lower-limb arterial disease in diabetes. *Diabetes Care* 2005; **28**: 2206-2210 [PMID: 16123491 DOI: 10.2337/diacare.28.9.2206]

39 **Potier L**, Halbron M, Bouilloud F, Dadon M, Le Doeuff J, Ha Van G, Grimaldi A, Hartemann-Heurtier A. Ankle-to-brachial ratio index underestimates the prevalence of peripheral occlusive disease in diabetic patients at high risk for arterial disease. *Diabetes Care* 2009; **32**: e44 [PMID: 19336632 DOI: 10.2337/dc08-2015]

40 **Rodriguez-Morata A**, Jiménez-Moleón J, Cuenca-Manteca J, Fernández-Quesada F, Ros-Vidal R, Gómez-Medialdea R, Ros-Díe E. Sensibilidad, especificidad y fiabilidad de la ecografía Doppler arterial en el diagnóstico de la isquemia crítica de los miembros inferiores con relación a la arteriografía. *Angiología* 2007; **59**: 121-127 [DOI: 10.1016/S0003-3170(07)75035-6]

41 **Potier L**, Abi Khalil C, Mohammedi K, Roussel R. Use and utility of ankle brachial index in patients with diabetes. *Eur J Vasc Endovasc Surg* 2011; **41**: 110-116 [PMID: 21095144 DOI: 10.1016/j.ejvs.2010.09.020]

42 **American Diabetes Association**. Executive summary: Standards of medical care in diabetes--2012. *Diabetes Care* 2012; **35** Suppl 1: S4-S10 [PMID: 22187471 DOI: 10.2337/dc12-s004]

43 **O'Neal DN**, Lewicki J, Ansari MZ, Matthews PG, Best JD. Lipid levels and peripheral vascular disease in diabetic and non-diabetic subjects. *Atherosclerosis* 1998; **136**: 1-8 [PMID: 9544725 DOI: 10.1016/s0021-9150(97)00175-5]

44 **Seeger JM**, Silverman SH, Flynn TC, Bailey JC, Klingman NV, Lawson GA, Borgeson MD, Barratt EJ. Lipid risk factors in patients requiring arterial reconstruction. *J Vasc Surg* 1989; **10**: 418-424 [PMID: 2795767 DOI: 10.1016/0741-5214(89)90416-3]

45 **Wang T**, Elam MB, Forbes WP, Zhong J, Nakajima K. Reduction of remnant lipoprotein cholesterol concentrations by cilostazol in patients with intermittent claudication. *Atherosclerosis* 2003; **171**: 337-342 [PMID: 14644405 DOI: 10.1016/j.atherosclerosis.2003.08.017]

46 **Bainton D**, Sweetnam P, Baker I, Elwood P. Peripheral vascular disease: consequence for survival and association with risk factors in the Speedwell prospective heart disease study. *Br Heart J* 1994; **72**: 128-132 [PMID: 7917683 DOI: 10.1136/hrt.72.2.128]

47 **Sentí M**, Nogués X, Pedro-Botet J, Rubiés-Prat J, Vidal-Barraquer F. Lipoprotein profile in men with peripheral vascular disease. Role of intermediate density lipoproteins and apoprotein E phenotypes. *Circulation* 1992; **85**: 30-36 [PMID: 1728461 DOI: 10.1161/01.CIR.85.1.30]

48 **Rizzo M**, Pernice V, Frasheri A, Berneis K. Atherogenic lipoprotein phenotype and LDL size and subclasses in patients with peripheral arterial disease. *Atherosclerosis* 2008; **197**: 237-241 [PMID: 17485096 DOI: 10.1016/j.atherosclerosis.2007.03.034]

49 **Price JF**, Mowbray PI, Lee AJ, Rumley A, Lowe GD, Fowkes FG. Relationship between smoking and cardiovascular risk factors in the development of peripheral arterial disease and coronary artery disease: Edinburgh Artery Study. *Eur Heart J* 1999; **20**: 344-353 [PMID: 10206381]

50 **Uusitupa MI**, Niskanen LK, Siitonen O, Voutilainen E, Pyörälä K. 5-year incidence of atherosclerotic vascular disease in relation to general risk factors, insulin level, and abnormalities in lipoprotein composition in non-insulin-dependent diabetic and nondiabetic subjects. *Circulation* 1990; **82**: 27-36 [PMID: 2194696 DOI: 10.1161/01.cir.82.1.27]

51 **Ridker PM**, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *JAMA* 2001; **285**: 2481-2485 [PMID: 11368701 DOI: 10.1001/jama.285.19.2481]

52 **Wattanakit K**, Folsom AR, Selvin E, Weatherley BD, Pankow JS, Brancati FL, Hirsch AT. Risk factors for peripheral arterial disease incidence in persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Atherosclerosis* 2005; **180**: 389-397 [PMID: 15910867 DOI: 10.1016/j.atherosclerosis.2004.11.024]

53 **Roche HM**, Gibney MJ. Postprandial triacylglycerolaemia--nutritional implications. *Prog Lipid Res* 1995; **34**: 249-266 [PMID: 8685241 DOI: 10.1016/0163-7827(95)00012-O]

54 **Roche HM**, Zampelas A, Jackson KG, Williams CM, Gibney MJ. The effect of test meal monounsaturated fatty acid: saturated fatty acid ratio on postprandial lipid metabolism. *Br J Nutr* 1998; **79**: 419-424 [PMID: 9682660 DOI: 10.1079/BJN19980071]

55 **Goldberg IJ**, Eckel RH, McPherson R. Triglycerides and heart disease: still a hypothesis? *Arterioscler Thromb Vasc Biol* 2011; **31**: 1716-1725 [PMID: 21527746 DOI: 10.1161/atvbaha.111.226100]

56 **Fogelstrand P**, Borén J. Retention of atherogenic lipoproteins in the artery wall and its role in atherogenesis. *Nutr Metab Cardiovasc Dis* 2012; **22**: 1-7 [PMID: 22176921 DOI: 10.1016/j.numecd.2011.09.007]

57 **Stalenhoef AF**, de Graaf J. Association of fasting and nonfasting serum triglycerides with cardiovascular disease and the role of remnant-like lipoproteins and small dense LDL. *Curr Opin Lipidol* 2008; **19**: 355-361 [PMID: 18607182 DOI: 10.1097/MOL.0b013e328304b63c]

58 **Proctor SD**, Mamo JC. Intimal retention of cholesterol de-

rived from apolipoprotein B100- and apolipoprotein B48-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1595-1600 [PMID: 12842838 DOI: 10.1161/01.ATV.0000084638.14534.0A]

59 **Karpe F**, de Faire U, Mercuri M, Bond MG, Hellénius ML, Hamsten A. Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men. *Atherosclerosis* 1998; **141**: 307-314 [PMID: 9862179 DOI: 10.1016/S0021-9150(98)00184-1]

60 **Groot PH**, van Stiphout WA, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofman A, Cresswell SR, Havekes L. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. *Arterioscler Thromb* 1991; **11**: 653-662 [PMID: 2029503 DOI: 10.1161/01.ATV.11.3.653]

61 **Patsch JR**, Miesenböck G, Hopferwieser T, Mühlberger V, Knapp E, Dunn JK, Gotto AM, Patsch W. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arterioscler Thromb* 1992; **12**: 1336-1345 [PMID: 1420093 DOI: 10.1161/01.ATV.12.11.1336]

62 **Lopez-Miranda J**, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. *Br J Nutr* 2007; **98**: 458-473 [PMID: 17705891 DOI: 10.1017/S000711450774268X]

63 **Reyes-Soffer G**, Holleran S, Karmally W, Ngai CI, Chen NT, Torres M, Ramakrishnan R, Blaner WS, Berglund L, Ginsberg HN, Tuck C. Measures of postprandial lipoproteins are not associated with coronary artery disease in patients with type 2 diabetes mellitus. *J Lipid Res* 2009; **50**: 1901-1909 [PMID: 19429886 DOI: 10.1194/jlr.M900092-JLR200]

64 **Werner C**, Filmer A, Fritshc M, Groenewald S, Gräber S, Böhm M, Laufs U. Prospective evaluation of post-prandial triglycerides and cardiovascular events in patients with coronary artery disease. The Homburg Cream and Sugar Study (HCS), 2011

65 **Bansal S**, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007; **298**: 309-316 [PMID: 17635891 DOI: 10.1001/jama.298.3.309]

66 **Nordestgaard BG**, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007; **298**: 299-308 [PMID: 17635890 DOI: 10.1001/jama.298.3.299]

67 **Eberly LE**, Stamler J, Neaton JD. Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease. *Arch Intern Med* 2003; **163**: 1077-1083 [PMID: 12742806 DOI: 10.1001/archinte.163.9.1077]

68 **Stampfer MJ**, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, Hennekens CH. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA* 1996; **276**: 882-888 [PMID: 8782637 DOI: 10.1001/jama.1996.03540110036029]

69 **Sarwar N**, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S, Boekholdt SM, Khaw KT, Gudnason V. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. *Circulation* 2007; **115**: 450-458 [PMID: 17190864 DOI: 10.1161/CIRCULATIONAHA.106.637793]

70 **Di Angelantonio E**, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009; **302**: 1993-2000 [PMID: 19903920 DOI: 10.1001/jama.2009.1619]

71 **Jellinger PS**, Smith DA, Mehta AE, Ganda O, Handelsman Y, Rodbard HW, Shepherd MD, Seibel JA. American Association of Clinical Endocrinologists' Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis. *Endocr Pract* 2012; **18** Suppl 1: 1-78 [PMID: 22522068]

72 **Kolovou GD**, Mikhailidis DP, Kovar J, Lairon D, Nordestgaard BG, Ooi TC, Perez-Martinez P, Bilianou H, Anagnostopoulou K, Panotopoulos G. Assessment and clinical relevance of non-fasting and postprandial triglycerides: an expert panel statement. *Curr Vasc Pharmacol* 2011; **9**: 258-270 [PMID: 21314632 DOI: 10.2174/157016111795495549]

73 **Langsted A**, Nordestgaard BG. Nonfasting lipids, lipoproteins, and apolipoproteins in individuals with and without diabetes: 58 434 individuals from the Copenhagen General Population Study. *Clin Chem* 2011; **57**: 482-489 [PMID: 21189274 DOI: 10.1373/clinchem.2010.157164]

74 **Mihas C**, Kolovou GD, Mikhailidis DP, Kovar J, Lairon D, Nordestgaard BG, Ooi TC, Perez-Martinez P, Bilianou H, Anagnostopoulou K, Panotopoulos G. Diagnostic value of postprandial triglyceride testing in healthy subjects: a meta-analysis. *Curr Vasc Pharmacol* 2011; **9**: 271-280 [PMID: 21314631 DOI: 10.2174/157016111795495530]

75 **Lupattelli G**, Pasqualini L, Siepi D, Marchesi S, Pirro M, Vaudo G, Ciuffetti G, Mannarino E. Increased postprandial lipemia in patients with normolipemic peripheral arterial disease. *Am Heart J* 2002; **143**: 733-738 [PMID: 11923813 DOI: 10.1067/mhj.2002.120302]

76 **Valdivielso P**, Hidalgo A, Rioja J, Aguilar I, Ariza MJ, González-Alegre T, González-Santos P. Smoking and postprandial triglycerides are associated with vascular disease in patients with type 2 diabetes. *Atherosclerosis* 2007; **194**: 391-396 [PMID: 16996523]

77 **Valdivielso P**, Puerta S, Rioja J, Alonso I, Ariza MJ, Sánchez-Chaparro MA, Palacios R, González-Santos P. Postprandial apolipoprotein B48 is associated with asymptomatic peripheral arterial disease: a study in patients with type 2 diabetes and controls. *Clin Chim Acta* 2010; **411**: 433-437 [PMID: 20059992 DOI: 10.1016/j.cca.2009.12.022]

78 **Mancera-Romero J**, Sánchez-Chaparro MA, Rioja J, Ariza MJ, Olivecrona G, González-Santos P, Valdivielso P. Fasting apolipoprotein B48 is a marker for peripheral arterial disease in type 2 diabetes. *Acta Diabetol* 2013; **50**: 383-389 [PMID: 23053881 DOI: 10.1007/s00592-012-0434-x]

79 **Lapice E**, Cipriano P, Patti L, Romano G, Vaccaro O, Rivellese AA. Fasting apolipoprotein B48 is associated with asymptomatic peripheral arterial disease in type 2 diabetic subjects: a case-control study. *Atherosclerosis* 2012; **223**: 504-506 [PMID: 22762727 DOI: 10.1016/j.atherosclerosis.2012.05.038]

80 **Zilversmit DB**. Atherogenesis: a postprandial phenomenon. *Circulation* 1979; **60**: 473-485 [PMID: 222498 DOI: 10.1161/01.CIR.60.3.473]

81 **Horton TJ**, Commerford SR, Pagliassotti MJ, Bessesen DH. Postprandial leg uptake of triglyceride is greater in women than in men. *Am J Physiol Endocrinol Metab* 2002; **283**: E1192-E1202 [PMID: 12424104]

82 **Ariza MJ**, Sánchez-Chaparro MA, Barón FJ, Hornos AM, Calvo-Bonacho E, Rioja J, Valdivielso P, Gelpi JA, González-Santos P. Additive effects of LPL, APOA5 and APOE variant combinations on triglyceride levels and hypertriglyceridemia: results of the ICARIA genetic sub-study. *BMC Med Genet* 2010; **11**: 66 [PMID: 20429872 DOI: 10.1186/1471-2350-11-66]

83 **Coca-Prieto I**, Kroupa O, Gonzalez-Santos P, Magne J, Olivecrona G, Ehrenborg E, Valdivielso P. Childhood-onset chylomicronaemia with reduced plasma lipoprotein lipase activity and mass: identification of a novel GPIHBP1 mutation. *J Intern Med* 2011; **270**: 224-228 [PMID: 21314738 DOI: 10.1111/j.1365-2796.2011.02361.x]

84 **Brevetti G**, Schiano V, Chiariello M. Endothelial dysfunction: a key to the pathophysiology and natural history of peripheral arterial disease? *Atherosclerosis* 2008; **197**: 1-11 [PMID: 18076886]

85 **Martorell L**, Gentile M, Rius J, Rodríguez C, Crespo J, Badimon L, Martínez-González J. The hypoxia-inducible factor 1/NOR-1 axis regulates the survival response of endothelial cells to hypoxia. *Mol Cell Biol* 2009; **29**: 5828-5842 [PMID:

19720740 DOI: 10.1128/mcb.00945-09]
86 **Manalo DJ**, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, Semenza GL. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* 2005; **105**: 659-669 [PMID: 15374877 DOI: 10.1182/blood-2004-07-2958]
87 **Lichtenstein L**, Kersten S. Modulation of plasma TG lipolysis by Angiopoietin-like proteins and GPIHBP1. *Biochim Biophys Acta* 2010; **1801**: 415-420 [PMID: 20056168 DOI: 10.1016/j.bbalip.2009.12.015]
88 **Drager LF**, Li J, Shin MK, Reinke C, Aggarwal NR, Jun JC, Bevans-Fonti S, Sztalryd C, O'Byrne SM, Kroupa O, Olivecrona G, Blaner WS, Polotsky VY. Intermittent hypoxia inhibits clearance of triglyceride-rich lipoproteins and inactivates adipose lipoprotein lipase in a mouse model of sleep apnoea. *Eur Heart J* 2012; **33**: 783-790 [PMID: 21478490 DOI: 10.1093/eurheartj/ehr097]
89 **Ena J,** Argente CR, Molina M, Gonzalez-Sanchez V, Alvarez CE, Lozano T. Infradiagnóstico de enfermedad arterial periférica en pacientes con diabetes mellitus atendidos en consultas de segundo nivel. *Avances en Diabetología* 2013; **29**: 175-181 [DOI: 10.1016/j.avdiab.2013.08.002]

P- Reviewer: Barzilay JI, Neri V, Tarantino G **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.586

World J Diabetes 2014 October 15; 5(5): 586-600
ISSN 1948-9358 (online)

TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (3): Type 1 diabetes

Hepatitis C virus infection and type 1 and type 2 diabetes mellitus

Alessandro Antonelli, Silvia Martina Ferrari, Dilia Giuggioli, Andrea Di Domenicantonio, Ilaria Ruffilli, Alda Corrado, Silvia Fabiani, Santino Marchi, Clodoveo Ferri, Ele Ferrannini, Poupak Fallahi

Alessandro Antonelli, Silvia Martina Ferrari, Andrea Di Domenicantonio, Ilaria Ruffilli, Alda Corrado, Silvia Fabiani, Ele Ferrannini, Poupak Fallahi, Department of Clinical and Experimental Medicine, University of Pisa, I-56126 Pisa, Italy
Dilia Giuggioli, Clodoveo Ferri, Department of Medical, Surgical, Maternal, Pediatric and Adult Sciences, University of Modena and Reggio Emilia, I-41124 Modena, Italy
Santino Marchi, Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, I-56122 Pisa, Italy
Author contributions: Antonelli A and Ferri C designed the research; Ferrari SM, Giuggioli D, Di Domenicantonio A, Ruffilli I, Corrado A, Fabiani S and Fallahi P performed the research; Antonelli A, Marchi S, Ferri C and Ferrannini E analysed the data; Antonelli A, Ferrari SM, Fabiani S, Ferri C and Fallahi P wrote the paper.
Correspondence to: Alessandro Antonelli, MD, Professor, Department of Clinical and Experimental Medicine, University of Pisa, Via Savi, 10, I-56126 Pisa, Italy. alessandro.antonelli@med.unipi.it
Telephone: +39-050-992318 Fax: +39-050-553235
Received: November 29, 2013 Revised: April 10, 2014
Accepted: July 12, 2014
Published online: October 15, 2014

Abstract

Hepatitis C virus (HCV) infection and diabetes mellitus are two major public health problems that cause devastating health and financial burdens worldwide. Diabetes can be classified into two major types: type 1 diabetes mellitus (T1DM) and T2DM. T2DM is a common endocrine disorder that encompasses multifactorial mechanisms, and T1DM is an immunologically mediated disease. Many epidemiological studies have shown an association between T2DM and chronic hepatitis C (CHC) infection. The processes through which CHC is associated with T2DM seem to involve direct viral effects, insulin resistance, proinflammatory cytokines, chemokines, and other immune-mediated mechanisms. Few data have been reported on the association of CHC and T1DM and reports on the potential association between T1DM and acute HCV infection are even rarer. A small number of studies indicate that interferon-α therapy can stimulate pancreatic autoimmunity and in certain cases lead to the development of T1DM. Diabetes and CHC have important interactions. Diabetic CHC patients have an increased risk of developing cirrhosis and hepatocellular carcinoma compared with non-diabetic CHC subjects. However, clinical trials on HCV-positive patients have reported improvements in glucose metabolism after antiviral treatment. Further studies are needed to improve prevention policies and to foster adequate and cost-effective programmes for the surveillance and treatment of diabetic CHC patients.

Key words: Hepatitis C virus infection; Type 1 diabetes mellitus; Type 2 diabetes mellitus; Epidemiology; Pathogenesis; Prevention; Treatment

Core tip: Many studies have shown an association between type 2 diabetes mellitus (T2DM) and chronic hepatitis C (CHC) infection. The processes through which CHC is associated with T2DM seem to involve direct viral effects, insulin resistance, proinflammatory cytokines, and chemokines. Few data have been reported on the association of CHC and T1DM. A small number of studies indicate that interferon-α therapy can induce T1DM. Diabetic CHC patients have an increased risk of developing cirrhosis and hepatocellular carcinoma compared with non-diabetics. Clinical trials on hepatitis C virus-positive patients have reported improvements in glucose metabolism after antiviral treatment.

Antonelli A, Ferrari SM, Giuggioli D, Di Domenicantonio A, Ruffilli I, Corrado A, Fabiani S, Marchi S, Ferri C, Ferrannini E, Fallahi P. Hepatitis C virus infection and type 1 and type 2 diabetes mellitus. *World J Diabetes* 2014; 5(5): 586-600 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/586.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.586

INTRODUCTION

Hepatitis C virus (HCV) infection and diabetes mellitus (DM) are two major public health problems that cause devastating health and financial burdens worldwide[1,2]. Diabetes can be classified into two major types: type 1 (T1DM) and T2DM[3,4]. T2DM is a common endocrine disorder that encompasses multifactorial mechanisms. These mechanisms include resistance to the action of insulin, increased hepatic glucose production, and a defect in insulin secretion, all of which contribute to the development of overt hyperglycaemia[5]. T1DM is an immunologically mediated disease. Prevention and treatment of T1DM are hampered by the fact that the key immunological mechanisms of the pathogenesis of the disease are still under debate[6,7]. However, a Th1 immune response is involved in β-cell destruction[8] and the importance of islet autoantibodies has been highlighted[9-11].

Chronic hepatitis C (CHC) infection has a global prevalence of 2%-3%. Approximately 170 million people are thought to be currently infected (approximately 3% of the world's population), and an additional 3-4 million are infected each year[12,13]. HCV is the main reason for liver transplantation in the developed world and the main cause of liver-related morbidity and mortality in a number of countries, including Italy. This virus is not only a frequent cause of chronic liver diseases, including hepatitis, cirrhosis, and hepatocellular carcinoma (HCC), but it is also involved in the pathogenesis of various autoimmune and rheumatic disorders (*e.g.*, arthritis, vasculitis, sicca syndrome, porphyria cutanea tarda, lichen planus, nephropathies, and lung fibrosis) and in the development of B-cell lymphoproliferative diseases[14,15].

CHC is a multifaceted disorder that is associated with extrahepatic manifestations, including endocrinological disorders, thyroid disorders and diabetes[16,17].

In this paper, we review the increasing evidence linking HCV infection and DM in multiple fields (epidemiology, pathogenesis, clinical aspects, prevention, and treatment).

RELATIONSHIP BETWEEN CHC AND THE DEVELOPMENT OF T2DM

Origins of the hypothesis and epidemiological data in the general population

The liver plays an important role in carbohydrate metabolism, and liver diseases such as chronic hepatitis and cirrhosis are associated with a higher prevalence of disturbed glucose homeostasis, impaired glucose tolerance, and insulin resistance (IR)[18,19], which can eventually lead to DM[20-23]. Asymptomatic, moderate serum aminotransferase elevation has frequently been found in patients with DM, particularly in those with T2DM[24,25]. This phenomenon has often been related to fatty infiltration of the liver without further investigation[26,27]. In particular, steatosis has been related to IR and T2DM, beyond intracellular fat accumulation[28].

Liver fibrosis progression has also long been considered to be responsible for the development of IR and T2DM in patients with chronic liver diseases[29]. However, diabetes often occurs in the early stages of liver disease[30].

The aetiological factors that underlie the development of glucose homeostasis alterations were initially thought to be exclusively related to general long-term hepatocyte damage. However, later studies showed that patients with hepatitis B virus infection have a lower prevalence of T2DM compared with HCV-infected patients[31,32]. Thus, the question is as follows: "Does HCV infection itself have diabetogenic action?"

Since the discovery of HCV in 1989, attention has been paid to the association of CHC with the development of DM. Additionally from 1994[33] until now, several epidemiological studies on the seroprevalence of HCV have shown higher prevalences in diabetic patients than in controls (Figure 1). Moreover, analyses have shown a higher prevalence of DM in patients who are seropositive for HCV than in controls without HCV infection.

To analyse the epidemiological data, we searched for published studies in the PubMed database, covering the period from 1994 to December 2012. The literature search was performed using combinations of the terms "diabetes", "diabetes mellitus", "type 2 diabetes mellitus", "T2DM", "type 2 DM", "non-insulin dependent diabetes", or "NIDDM"; "hepatitis", "hepatitis C", "hepatitis C virus", "HCV", "HVC", or "chronic hepatitis"; and "risk", "risk factor", "case-control", "cohort", "clinical trial", "cross sectional", "epidemiology", "observational", "meta-analysis", "systematic review", or "review". For epidemiological studies, we only searched human studies and publications in English and Italian, the languages understood by the authors.

The data represent a very heterogeneous population regarding gender, age, and ethnic group. Globally, approximately seventy studies are in agreement with an association[18,26,30-96], although not all of them have shown significant data. However, some of the non-significant data may be attributed to small sample sizes and other methodological factors (Figure 1).

Certain negative data that are not in agreement with an association between HCV infection and T2DM have also been reported[97-104]. However, the number of published epidemiological studies that are in agreement with the association between HCV infection and T2DM is higher than the number of studies in disagreement with this hypothesis.

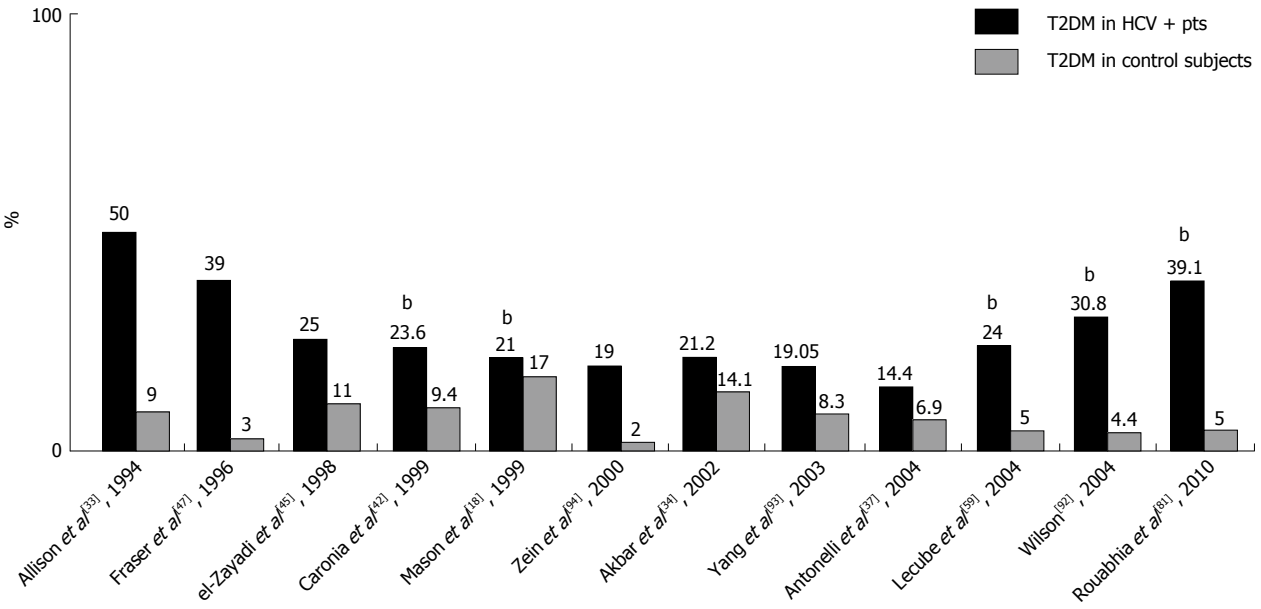

Figure 1 Patients seropositive for hepatitis C virus show a higher prevalence of diabetes mellitus than healthy controls. Twelve representative epidemiological studies demonstrated a relationship between HCV infection and the development of type 2 diabetes mellitus (T2DM). Analyses have shown a higher prevalence of diabetes mellitus in patients who are seropositive for HCV than in controls. $^{b}P < 0.001$, T2DM in HCV+ pts *vs* T2DM in control subjects. HCV+: Hepatitis C virus-infected; pts: Patients.

HCV INFECTION AND T2DM ASSOCIATION: PATHOGENESIS

Direct effects of HCV and IR

HCV is hepatotropic and noncytopathic; nevertheless, its genome has been identified in a number of tissues beyond the liver, including pancreatic acinar cells and epithelial cells of the pancreatic duct[105,106]. Although post-mortem studies have revealed that HCV replicates in the pancreas[107] and animal models have suggested a direct effect of HCV infection on IR in the liver[108], the evidence is scanty.

Of interest are the roles of structural and non-structural HCV proteins. HCV has an RNA genome of 9.6 kb that encodes approximately 3010 amino acids and is translated into structural (core, E1, and E2) and non-structural (NS3-NS5B) proteins. These proteins play a role in the development of IR and oxidative stress *via* reactive oxygen species at the cellular level[109-113]. The HCV core protein, alone or in combination with other viral proteins, increases phosphorylation of insulin receptor substrate-1 (IRS-1), which is the basis of IR[114-116]. Phosphorylated IRS-1 activates phosphatidylinositol 3-kinase (PI3K)[117,118], and the activation of PI3K and one of its downstream targets, Akt, is essential for most of the metabolic effects of insulin[119-126]. Therefore, defects at the level of the association of PI3K with IRS-1 and a lack of PI3K activation may contribute to IR and the increased prevalence of diabetes in HCV-infected patients. Indeed, this mechanism ultimately promotes glucose transporter-4 translocation to the plasma membrane to enhance glucose uptake[127,128]. Within the IR mechanism impairment of the activation of Akt/PKB is the key step that can inhibit glucose uptake[30,129,130].

The detailed molecular events leading to IR in HCV-infected patients are, however, unclear. Recent evidence supports the existence of a significant extrahepatic component of HCV-induced IR. Thus, the molecular pathogenesis of the glucose metabolism disturbances observed in hepatitis C is much more complex than expected[131].

Recently, Eslam *et al*[132] showed that polymorphisms in the IFNL3 (IL28B) region are associated with spontaneous and treatment-induced recovery from HCV infection. Furthermore, circumstantial evidence suggests a link between single-nucleotide polymorphisms in IFNL3 and lipid metabolism, steatosis, and IR in CHC. The emerging picture suggests that the responder genotypes of IFNL3 polymorphisms are associated with higher serum lipid levels and less frequent steatosis and IR[132].

HCV-induced immune responses; cytokines, chemokines-mediated effects

Viral innate immune evasion strategies and human genetic determinants underlie the transition of acute HCV infection into viral persistence and chronic infection. Host genetic factors can influence both the outcome of the infection and the response to antiviral therapy. Recent insights into how HCV regulates immune signalling within the liver reveal a complex interaction of the patient's genetic background with viral and host factors related to the innate immune triggering and control that dictate the outcome of HCV infection and immunity[133].

Beyond the direct effects of HCV on IRS-1/PI3K, the HCV core protein may induce IR indirectly *via* stimulation of the secretion of proinflammatory cytokines[115]. In patients with CHC, most likely due to HCV-induced inflammation, there is hypersecretion of insulin-resistant proinflammatory cytokines such as interleukin (IL)-6 and

tumour necrosis factor (TNF)-α[134-138]. Proinflammatory cytokines also upregulate suppressors of cytokine signalling proteins as part of a negative feedback loop to attenuate cytokine signalling[139,140]. This phenomenon may contribute to increased gluconeogenesis due to a lack of Akt-mediated inhibition of phosphoenolpyruvate carboxykinase gene expression. In this context, it is interesting to note that leptin can modulate the action of insulin in liver cells by antagonising insulin-stimulated IRS-1 tyrosine phosphorylation, increasing phosphoenolpyruvate carboxykinase gene expression, and decreasing glucokinase expression, which results in increased gluconeogenesis[141]. Together with the increase in gluconeogenesis, the enhanced production and accumulation of lipids mediated by inhibition of the AMP-activated protein kinase occur after HCV infection[142]. Additionally TNF-α plays a role in lipid metabolism. Indeed, the lipolysis-stimulating effect of TNF-α leads to increased serum levels of free fatty acids, which reduces insulin sensitivity[143,144].

Cytokines are intercellular mediators involved in viral control and in the liver damage induced by infection with HCV. The complex cytokine network that operates during the initial infection allows the coordinated, effective development of both the innate and the adaptive immune responses. However, HCV interferes with cytokines at various levels and escapes the immune response by inducing a Th2/T cytotoxic 2 cytokine profile. The inability to control infection leads to the recruitment of inflammatory infiltrates into the liver parenchyma by interferon (IFN)-γ-inducible CXC chemokine ligand (CXCL)9, CXCL10, and CXCL11, which result in sustained liver damage and eventually liver cirrhosis. The most important systemic HCV-related extrahepatic diseases (mixed cryoglobulinemia, lymphoproliferative disorders, thyroid autoimmune disorders, and T2DM) are associated with complex dysregulation of the cytokine/chemokine network, involving proinflammatory and Th1 chemokines[145,146].

HCV-INFECTED PATIENTS WITH T1DM

Few data on this association have been reported, and published studies have shown only small proportions of CHC patients positive for one or more markers of pancreatic autoimmunity[18,147-150].

Even rarer are reports on the potential association between autoimmune diabetes and acute HCV infection. Only two cases have been described in the literature[151,152]. Several mechanisms have been postulated to initiate the process. Even if HCV can infect extrahepatic tissue in patients with hepatitis C[16,107,153], no direct involvement of HCV in the onset of T1DM has been clarified yet. Nevertheless, the direct destruction of β-cells by viral infection could be a good explanation. Beyond the undemonstrated direct mechanisms, HCV infection surely initiates an immune reaction against β-cells or causes an acceleration of diabetes onset when an immune reaction against β-cells is already present. Some authors have also suggested the involvement of a process of molecular mimicry as a trigger of HCV-related autoimmunity[154,155]. Indeed, glutamic acid decarboxylase (GAD) 65 shares amino acid sequence similarities with antigenic regions of the HCV polyprotein[156]. Of interest, HCV/self-homologous autoantigenic regions are also mimicked by other microbial agents. Such mimics may give rise to β-cell autoimmunity through a multiple-hit mechanism of molecular mimicry[154,155,157]. Cross-reactive immunity does not exclude the possible involvement of additional factors, such as proinflammatory cytokines, which may act in concert, leading to the development and/or maintenance of pancreatic autoimmunity during acute HCV infection[156]. Another possibility is the induction of antibody reactivity against GAD and the development of full-blown diabetes, mediated by IL-18 and other proinflammatory cytokines. In particular, IL-18 is presumed to play a pathogenetic role in T1DM, specifically because this cytokine appears to be involved in acceleration of the development of overt disease[152,158-160]. IL-18 can induce both Th1 and Th2 responses, depending on the surrounding cytokines[161], and this cytokine plays a pathogenic role in several diseases[161], including acute hepatic injury[162]. Other proinflammatory cytokines, such as TNF-α and IL-1β, which are elevated in patients with acute hepatitis[163], can also induce autoimmune diabetes[164-167].

OTHER IMMUNE ASPECTS OF HCV ASSOCIATED WITH T1DM OR T2DM

Immune aspects have been reported in both T1DM and T2DM, and based on the immunology, it is clear that the lines separating T1DM from latent autoimmune diabetes in adults (LADA) and T2DM are not well delineated[10,11,16,37,145,168-170].

The type of diabetes manifested by patients with CHC is not classical T2DM, and the labelling of HCV patients as having T2DM is purely conventional and possibly inaccurate. The lines separating T1DM from LADA and T2DM are fading away as new pathogenetic information is obtained[170].

Three studies have reported[37,38,171] that HCV patients with T2DM are leaner than T2DM controls and show significantly lower low-density lipoprotein-cholesterol levels and systolic and diastolic blood pressures. Furthermore, patients with HCV-associated mixed cryoglobulinaemia (MC + HCV) and T2DM had non-organ-specific autoantibodies more frequently (34% *vs* 18%, respectively) than did non-diabetic MC + HCV patients[37]. An immune-mediated mechanism for MC + HCV-associated diabetes has been postulated[37], and a similar pathogenesis might be involved in diabetes in HCV patients. This hypothesis is strengthened by the finding that autoimmune phenomena are more common in T2DM patients than previously thought[10]. However, as the prevalence of classic β-cell autoimmune markers is not increased in HCV patients[70], other immune phenomena might be involved[168]. Chemokines could be important in this context. In fact, in children with newly

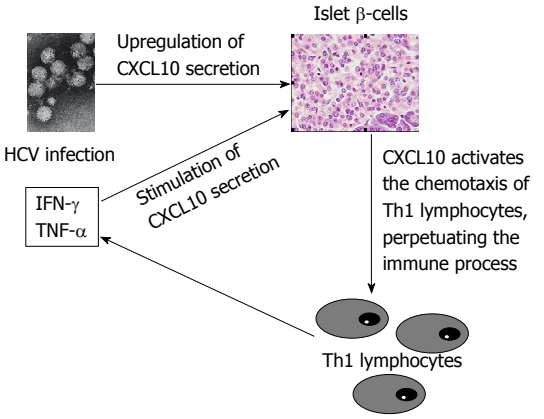

Figure 2 Potential regulation of the endocrine manifestations of hepatitis C virus infection in islet β-cells. Hepatitis C virus (HCV) infection may act by upregulating CXC chemokine ligand (CXCL) 10 gene expression and the subsequent secretion of this chemokine by islet β-cells. These events lead to the recruitment of Th1 lymphocytes that secrete interferon (IFN)-γ and tumour necrosis factor (TNF)-α, which induce chemokine secretion by islet β-cells, thus perpetuating the immune cascade. This cascade may lead to the appearance of autoimmune thyroid disorders in genetically predisposed subjects.

diagnosed T1DM, raised serum CXCL10 and normal chemokine (C-C motif) ligand 2 concentrations signal a predominantly Th1-driven autoimmune process, which shifts toward Th2 immunity 2 years after diagnosis[172].

Based on the abovementioned concepts, HCV infection of β-cells[106] may act by upregulating *CXCL10* gene expression and secretion (as previously shown in human hepatocytes[173]) and recruiting Th1 lymphocytes that secrete IFN-γ and TNF-α, which induce CXCL10 secretion by β-cells and thus perpetuate the immune cascade. This cascade may lead to the appearance of β-cell dysfunction in genetically predisposed subjects (Figure 2). Recently, certain studies have confirmed this hypothesis, demonstrating higher serum levels of CXCL10 in HCV patients with T2DM than in those without[16,169].

T1DM AND T2DM IN HCV-INFECTED PATIENTS TREATED WITH IFN-α

An important research area concerns the relationship between diabetes and IFN-α therapy in HCV-infected patients. In particular, studies have shown a high prevalence of markers of pancreatic autoimmunity in HCV-positive patients after or during IFN-α therapy, most likely due to the immunostimulatory effects of this cytokine. Indeed, IFN-α has antiviral, antiproliferative, and immunomodulatory activities[174]. Thus, in predisposed individuals, IFN-α can either induce a diabetogenic process or accelerate a diabetogenic process that is already underway[18,175,176]. For this reason, islet cell autoantibodies and GADAb should be investigated before and during IFN treatment to identify subjects who are at high risk of developing T1DM[177-180]. A small number of patients can develop *de novo* pancreatic autoimmunity and fall into a group of patients at risk of developing DM. In general, patients who are initially positive for organ-specific autoantibodies (in particular, thyroid- and pancreas-specific autoantibodies) and those who seroconvert seem to be at high risk of developing clinical autoimmune disease after treatment with IFN-α[181]. Timely suspension of IFN-α therapy is rarely accompanied by regression of clinical DM. No correlation has been documented between the response to antiviral therapy and the development of DM.

IFN-α increases HLA class I antigen expression and natural killer cell and T cell activities, and this cytokine may be an important cofactor in the development of a Th1 immune reaction. This reaction can contribute to the development of autoimmune disease by the activation of CD4+ lymphocytes that secrete IL-2, IFN-γ and TNF-β. These cytokines help in the generation of CD8+ cytotoxic T cells[182]. In addition to its immunomodulatory properties, IFN-α can also increase IR and induce hyperglycaemia[183-188]. Fabris *et al*[189] documented the first case of T1DM development during IFN-α therapy. Other studies suggest that IFN-α therapy can stimulate pancreatic autoimmunity and, in certain cases, lead to the development of T1DM[150,175,177,180,181,190-223].

The relationship with T1DM does not account for all of the effects of IFN-α therapy on diabetes. Indeed, from a completely different perspective, antiviral therapy with IFN should also be considered in HCV-positive patients because of its potential role in limiting the progression of this metabolic disturbance (see later discussion).

OUTCOME IN DIABETIC HCV-POSITIVE PATIENTS

CHC is an insidiously progressive form of liver disease that leads to cirrhosis[224-226] and HCC[227-231]. Diabetic HCV-positive patients have increased risk compared with non-diabetic subjects, and DM itself seems to have a selective impact on HCC development[232-251].

The main characteristic of diabetic patients is IR, which plays a crucial role in fibrosis progression and has a negative impact on treatment responses to antiviral therapy in patients with CHC[52,252,253]. Reduced insulin sensitivity is at the basis of compensatory hyperinsulinemia and elevated levels of insulin-like growth factor 1 (IGF-1), which stimulates cell proliferation and inhibits apoptosis. Additionally, this phenomenon has strong mitogenic effects on a wide variety of cancer cell lines[254-256]. At the same time, insulin activates the IGF-1 receptor, which has a growth-promoting effect that includes modulating cell cycle progression. Excess insulin may also indirectly affect the development of cancer by downregulating the level of IGF-binding protein 1, which increases the level and bioavailability of total circulating IGF-1. Additional factors, such as obesity and physical inactivity, also cause hyperinsulinemia and are thus also ultimately associated with accelerated cancer progression[255-258].

Genotype differences in terms of liver disturbance progression have been described as well. Genotype 3a is more strongly correlated with steatosis than other

genotypes[259,260], and the HCV genotype 3 may have a cytopathic effect[261]. Steatosis in genotype 1 infection is instead thought to be an expression of metabolic syndrome caused by the activation of proinflammatory mechanisms as well as underlying obesity and IR[262]. The degree of steatosis in this genotype is independent of the HCV viral load, and antiviral therapy does not improve steatosis in these patients. Similar data have been obtained for genotype 4 infection, whereas few data are available for genotype 2[263].

The presence of HCV infection in patients with DM may also increase the proportion of DM-related chronic nephrologic complications[86,264].

PREVENTION AND TREATMENT

CHC is a complex disease with systemic effects that require a multidisciplinary treatment approach[265].

The potential relationship between HCV infection and the development of DM increases the need for the implementation of prevention measures. Prevention must be directed toward lifestyle changes that can reduce the risk of HCV infection and/or diabetes development[266]; regular diabetes screening for anti-HCV-positive people; and the analysis of other risk factors that can accelerate the progression of both CHC and DM, such as obesity, dyslipidaemia, and alcohol consumption. In these high-risk patients, comprehensive treatment, including lifestyle modifications, must be recommended. Animal models also provide clues regarding the prevention and clinical management of diabetes in the setting of HCV infection[108]. Indeed, identifying patients who are at risk of developing diabetes, and have CHC, reduces liver disturbance progression[267,268], the incidence of HCC and transplant-related morbidity and mortality. Additionally, this identification improves the response to antiviral therapy[269-271], even reducing the side effects of the treatment[270] by encouraging the pretreatment of IR and DM[265].

Moreover, clinical trials on HCV-positive patients have reported improvement in glucose metabolism after antiviral treatment[187]. As discussed earlier, many factors may surely affect the antiviral response that modulates the IFN signalling pathway. Among these factors, the HCV genotype, genetic host factors, and comorbidities have been taken into account. In particular, recent studies have reported obesity[272] and hypercholesterolaemia[273] as potential factors that interfere with a sustained viral response. These observations suggest additional therapeutic options for HCV infection, including dietary changes, anti-diabetic drugs, and statins. Concerning anti-diabetic drugs, it is not currently clear whether the best approach is to use a peroxisome proliferator-activated receptor agonist or a biguanide, such as metformin[274-276]. Concerning statins, these drugs are capable of inhibiting HCV replication *in vitro*[277-279] but not *in vivo*[280].

Further studies are needed to improve prevention policies and to foster adequate and cost-effective programmes for the surveillance and treatment of diabetic CHC patients. The final goal must be to cure two diseases, diabetes and CHC, with one multifaceted treatment.

CONCLUSION

Many epidemiological studies have shown an association between T2DM and CHC. The processes through which HCV is associated with DM seem to involve direct viral effects, IR, proinflammatory cytokines, chemokines, suppressors of cytokine signalling, and other immune-mediated mechanisms. Other factors, such as metabolic syndrome and a family history of diabetes, also seem to be important risk factors for the development of diabetes. Few data on the association of CHC and T1DM have been reported, and reports on the potential association between T1DM and acute HCV infection are even rarer. A small number of studies have indicated that IFN-α therapy can stimulate pancreatic autoimmunity and, in certain cases, lead to the development of T1DM. Diabetes and CHC have important interactions. Diabetic CHC patients have an increased risk of developing cirrhosis and HCC compared with non-diabetic CHC subjects. Additionally, clinical trials on HCV-positive patients have reported improvement in glucose metabolism after antiviral treatment. Further studies are needed to improve prevention policies and to foster adequate and cost-effective programmes for the surveillance and treatment of diabetic CHC patients.

REFERENCES

1 **Lauer GM**, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52 [PMID: 11439948]
2 **Zimmet P**, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; **414**: 782-787 [PMID: 11742409 DOI: 10.1038/414782a]
3 **Alberti KG**, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553 [PMID: 9686693]
4 Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997; **20**: 1183-1197 [PMID: 9203460]
5 **Ferrannini E**. Physiology of glucose homeostasis and insulin therapy in type 1 and type 2 diabetes. *Endocrinol Metab Clin North Am* 2012; **41**: 25-39 [PMID: 22575405 DOI: 10.1016/j.ecl.2012.01.003]
6 **Barbeau WE**. What is the key environmental trigger in type 1 diabetes--is it viruses, or wheat gluten, or both? *Autoimmun Rev* 2012; **12**: 295-299 [PMID: 22633932 DOI: 10.1016/j.autrev.2012.05.003]
7 **Askenasy EM**, Askenasy N. Is autoimmune diabetes caused by aberrant immune activity or defective suppression of physiological self-reactivity? *Autoimmun Rev* 2013; **12**: 633-637 [PMID: 23277162 DOI: 10.1016/j.autrev.2012.12.004]
8 **Lo J**, Clare-Salzler MJ. Dendritic cell subsets and type I diabetes: focus upon DC-based therapy. *Autoimmun Rev* 2006; **5**: 419-423 [PMID: 16890897 DOI: 10.1016/j.autrev.2005.12.001]
9 **Lernmark A**, Larsson HE. Immune therapy in type 1 diabetes mellitus. *Nat Rev Endocrinol* 2013; **9**: 92-103 [PMID: 23296174 DOI: 10.1038/nrendo.2012.237]
10 **Antonelli A**, Tuomi T, Nannipieri M, Fallahi P, Nesti C,

Okamoto H, Groop L, Ferrannini E. Autoimmunity to CD38 and GAD in Type I and Type II diabetes: CD38 and HLA genotypes and clinical phenotypes. *Diabetologia* 2002; **45**: 1298-1306 [PMID: 12242463]
11 **Antonelli A**, Baj G, Marchetti P, Fallahi P, Surico N, Pupilli C, Malavasi F, Ferrannini E. Human anti-CD38 autoantibodies raise intracellular calcium and stimulate insulin release in human pancreatic islets. *Diabetes* 2001; **50**: 985-991 [PMID: 11334442 DOI: 10.2337/diabetes.50.5.985]
12 **Alter MJ**. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; **13**: 2436-2441 [PMID: 17552026]
13 Hepatitis C--global prevalence (update). *Wkly Epidemiol Rec* 1999; **74**: 425-427 [PMID: 10645164]
14 **Antonelli A**, Ferri C, Galeazzi M, Giannitti C, Manno D, Mieli-Vergani G, Menegatti E, Olivieri I, Puoti M, Palazzi C, Roccatello D, Vergani D, Sarzi-Puttini P, Atzeni F. HCV infection: pathogenesis, clinical manifestations and therapy. *Clin Exp Rheumatol* 2008; **26**: S39-S47 [PMID: 18570753]
15 **Ferri C**, Antonelli A, Mascia MT, Sebastiani M, Fallahi P, Ferrari D, Pileri SA, Zignego AL. HCV-related autoimmune and neoplastic disorders: the HCV syndrome. *Dig Liver Dis* 2007; **39** Suppl 1: S13-S21 [PMID: 17936215 DOI: 10.1016/S1590-8658(07)80005-3]
16 **Antonelli A**, Ferri C, Ferrari SM, Colaci M, Fallahi P. Immunopathogenesis of HCV-related endocrine manifestations in chronic hepatitis and mixed cryoglobulinemia. *Autoimmun Rev* 2008; **8**: 18-23 [PMID: 18708169 DOI: 10.1016/j.autrev.2008.07.017]
17 **Ferri C**, Antonelli A, Mascia MT, Sebastiani M, Fallahi P, Ferrari D, Giunti M, Pileri SA, Zignego AL. B-cells and mixed cryoglobulinemia. *Autoimmun Rev* 2007; **7**: 114-120 [PMID: 18035320 DOI: 10.1016/j.autrev.2007.02.019]
18 **Mason AL**, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, Guo L, Jacob S, Regenstein FG, Zimmerman R, Everhart JE, Wasserfall C, Maclaren NK, Perrillo RP. Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; **29**: 328-333 [PMID: 9918906 DOI: 10.1002/hep.510290235]
19 **Weinman SA**, Belalcazar LM. Hepatitis C: a metabolic liver disease. *Gastroenterology* 2004; **126**: 917-919 [PMID: 14988846 DOI: 10.1053/j.gastro.2003.01.001]
20 **Bugianesi E**, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology* 2005; **42**: 987-1000 [PMID: 16250043 DOI: 10.1002/hep.20920]
21 **Cruezfeldt W**, Frerichs H, Sickinger K. Liver diseases and diabetes mellitus. In: Popper H Schaffner F. Progress in Liver Disease. New York: Grune and Stratton, 1970: 371-407
22 **Felig P**, Sherin R. Carbohydrate homeostasis, liver and diabetes. In: Popper H, Schaffner F. Progress in Liver Disease. New York: Grune and Stratton, 1976: 149-171
23 **Kruszynska YT**, McIntyre N. Carbohydrate metabolism. In: McIntyre N, Benhamou J-P, Bircher J, Rizzetto M, Rodes J. Offord Textbook of Clinical Hepatology. Oxford: Oxford University Press, 1991: 129-143
24 **Nagore N**, Scheuer PJ. The pathology of diabetic hepatitis. *J Pathol* 1988; **156**: 155-160 [PMID: 3199264 DOI: 10.1002/path.1711560210]
25 **Morgan C**, Hyland C, Young IF. Hepatitis C antibody and transaminase activities in blood donors. *Lancet* 1990; **335**: 921 [PMID: 1970011 DOI: 10.1016/0140-6736(90)90523-8]
26 **Gray H**, Wreghitt T, Stratton IM, Alexander GJ, Turner RC, O'Rahilly S. High prevalence of hepatitis C infection in Afro-Caribbean patients with type 2 diabetes and abnormal liver function tests. *Diabet Med* 1995; **12**: 244-249 [PMID: 7538925 DOI: 10.1111/j.1464-5491.1995.tb00466.x]
27 **Salmela PI**, Sotaniemi EA, Niemi M, Mäentausta O. Liver function tests in diabetic patients. *Diabetes Care* 1984; **7**: 248-254 [PMID: 6734394 DOI: 10.2337/diacare.7.3.248]
28 **Vozarova B**, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C, Tataranni PA. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 2002; **51**: 1889-1895 [PMID: 12031978 DOI: 10.2337/diabetes.51.6.1889]
29 **Romero-Gómez M**. Insulin resistance and hepatitis C. *World J Gastroenterol* 2006; **12**: 7075-7080 [PMID: 17131467 DOI: 10.1111/j.1478-3231.2010.02365.x]
30 **Petit JM**, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, Brun JM, Hillon P. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. *J Hepatol* 2001; **35**: 279-283 [PMID: 11580152 DOI: 10.1016/S0168-8278(01)00143-X]
31 **Mehta SH**, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, Szklo M, Thomas DL. Hepatitis C virus infection and incident type 2 diabetes. *Hepatology* 2003; **38**: 50-56 [PMID: 12829986 DOI: 10.1053/jhep.2003.50291]
32 **Huang JF**, Dai CY, Hwang SJ, Ho CK, Hsiao PJ, Hsieh MY, Lee LP, Lin ZY, Chen SC, Hsieh MY, Wang LY, Shin SJ, Chang WY, Chuang WL, Yu ML. Hepatitis C viremia increases the association with type 2 diabetes mellitus in a hepatitis B and C endemic area: an epidemiological link with virological implication. *Am J Gastroenterol* 2007; **102**: 1237-1243 [PMID: 17531012 DOI: 10.1111/j.1572-0241.2007.01181.x]
33 **Allison ME**, Wreghitt T, Palmer CR, Alexander GJ. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. *J Hepatol* 1994; **21**: 1135-1139 [PMID: 7699240 DOI: 10.1016/S0168-8278(05)80631-2]
34 **Akbar DH**, Siddique AM, Ahmed MM. Prevalence of Type-2 diabetes in patients with hepatitis C and B virus infection in Jeddah, Saudi Arabia. *Med Princ Pract* 2002; **11**: 82-85 [PMID: 12123108 DOI: 10.1159/000058012]
35 **Alexander GJ**. An association between hepatitis C virus infection and type 2 diabetes mellitus: what is the connection? *Ann Intern Med* 2000; **133**: 650-652 [PMID: 11033595]
36 **AlDosary AA**, Ramji AS, Elliott TG, Sirrs SM, Thompson DM, Erb SR, Steinbrecher UP, Yoshida EM. Post-liver transplantation diabetes mellitus: an association with hepatitis C. *Liver Transpl* 2002; **8**: 356-361 [PMID: 11965580 DOI: 10.1053/jlts.2002.31745]
37 **Antonelli A**, Ferri C, Fallahi P, Sebastiani M, Nesti C, Barani L, Barale R, Ferrannini E. Type 2 diabetes in hepatitis C-related mixed cryoglobulinaemia patients. *Rheumatology* (Oxford) 2004; **43**: 238-240 [PMID: 13130149 DOI: 10.1093/rheumatology/keh011]
38 **Antonelli A**, Ferri C, Fallahi P, Pampana A, Ferrari SM, Goglia F, Ferrannini E. Hepatitis C virus infection: evidence for an association with type 2 diabetes. *Diabetes Care* 2005; **28**: 2548-2550 [PMID: 16186298 DOI: 10.2337/diacare.28.10.2548]
39 **Arao M**, Murase K, Kusakabe A, Yoshioka K, Fukuzawa Y, Ishikawa T, Tagaya T, Yamanouchi K, Ichimiya H, Sameshima Y, Kakumu S. Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. *J Gastroenterol* 2003; **38**: 355-360 [PMID: 12743775 DOI: 10.1007/s005350300063]
40 **Bernsmeier C**, Heim MH. Insulin resistance in chronic hepatitis C: mechanisms and clinical relevance. *Swiss Med Wkly* 2009; **139**: 678-684 [PMID: 20047129]
41 **Boschi-Pinto C**, Stuver S, Okayama A, Trichopoulos D, Orav EJ, Tsubouchi H, Mueller N. A follow-up study of morbidity and mortality associated with hepatitis C virus infection and its interaction with human T lymphotropic virus type I in Miyazaki, Japan. *J Infect Dis* 2000; **181**: 35-41 [PMID: 10608748 DOI: 10.1086/315177]
42 **Caronia S**, Taylor K, Pagliaro L, Carr C, Palazzo U, Petrik J, O'Rahilly S, Shore S, Tom BD, Alexander GJ. Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; **30**: 1059-1063 [PMID: 10498660 DOI: 10.1002/hep.510300416]

43 **Chehadeh W**, Abdella N, Ben-Nakhi A, Al-Arouj M, Al-Nakib W. Risk factors for the development of diabetes mellitus in chronic hepatitis C virus genotype 4 infection. *J Gastroenterol Hepatol* 2009; **24**: 42-48 [PMID: 18717762 DOI: 10.1111/j.1440-1746.2008.05503.x]

44 **Chen HF**, Li CY, Chen P, See TT, Lee HY. Seroprevalence of hepatitis B and C in type 2 diabetic patients. *J Chin Med Assoc* 2006; **69**: 146-152 [PMID: 16689194 DOI: 10.1016/S1726-4901(09)70195-9]

45 **el-Zayadi AR**, Selim OE, Hamdy H, Dabbous H, Ahdy A, Moniem SA. Association of chronic hepatitis C infection and diabetes mellitus. *Trop Gastroenterol* 1998; **19**: 141-144 [PMID: 10228436]

46 **Everhart J**. A confluence of epidemics: does hepatitis C cause type 2 diabetes? *Hepatology* 2001; **33**: 762-763 [PMID: 11230760 DOI: 10.1002/hep.510330336]

47 **Fraser GM**, Harman I, Meller N, Niv Y, Porath A. Diabetes mellitus is associated with chronic hepatitis C but not chronic hepatitis B infection. *Isr J Med Sci* 1996; **32**: 526-530 [PMID: 8756978]

48 **Fukui M**, Kitagawa Y, Nakamura N, Yoshikawa T. Hepatitis C virus and atherosclerosis in patients with type 2 diabetes. *JAMA* 2003; **289**: 1245-1246 [PMID: 12633185 DOI: 10.1001/jama.289.10.1245-b]

49 **Gulcan A**, Gulcan E, Toker A, Bulut I, Akcan Y. Evaluation of risk factors and seroprevalence of hepatitis B and C in diabetic patients in Kutahya, Turkey. *J Investig Med* 2008; **56**: 858-863 [PMID: 18667903 DOI: 10.231/JIM.0b013e3181788d28]

50 **Grimbert S**, Valensi P, Lévy-Marchal C, Perret G, Richardet JP, Raffoux C, Trinchet JC, Beaugrand M. High prevalence of diabetes mellitus in patients with chronic hepatitis C. A case-control study. *Gastroenterol Clin Biol* 1996; **20**: 544-548 [PMID: 8881566]

51 **Howard AA**, Klein RS, Schoenbaum EE. Association of hepatitis C infection and antiretroviral use with diabetes mellitus in drug users. *Clin Infect Dis* 2003; **36**: 1318-1323 [PMID: 12746779 DOI: 10.1086/374838]

52 **Hui JM**, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. *Gastroenterology* 2003; **125**: 1695-1704 [PMID: 14724822 DOI: 10.1053/j.gastro.2003.08.032]

53 **Imazeki F**, Yokosuka O, Fukai K, Kanda T, Kojima H, Saisho H. Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus-infected and hepatitis C virus-cleared patients. *Liver Int* 2008; **28**: 355-362 [PMID: 18290778 DOI: 10.1111/j.1478-3231.2007.01630.x]

54 **Jadoon NA**, Shahzad MA, Yaqoob R, Hussain M, Ali N. Seroprevalence of hepatitis C in type 2 diabetes: evidence for a positive association. *Virol J* 2010; **7**: 304 [PMID: 21054842 DOI: 10.1186/1743-422X-7-304]

55 **Kaabia N**, Ben Jazia E, Slim I, Fodha I, Hachfi W, Gaha R, Khalifa M, Hadj Kilani A, Trabelsi H, Abdelaziz A, Bahri F, Letaief A. Association of hepatitis C virus infection and diabetes in central Tunisia. *World J Gastroenterol* 2009; **15**: 2778-2781 [PMID: 19522029 DOI: 10.3748/wjg.15.2778]

56 **Knobler H**, Schattner A. Association of hepatitis C and diabetes mellitus. *Ann Intern Med* 2001; **135**: 141; author reply 142 [PMID: 11453715 DOI: 10.7326/0003-4819-135-2-200107170-00016]

57 **Knobler H**, Schihmanter R, Zifroni A, Fenakel G, Schattner A. Increased risk of type 2 diabetes in noncirrhotic patients with chronic hepatitis C virus infection. *Mayo Clin Proc* 2000; **75**: 355-359 [PMID: 10761489 DOI: 10.4065/75.4.355]

58 **Labropoulou-Karatza C**, Goritsas C, Fragopanagou H, Repandi M, Matsouka P, Alexandrides T. High prevalence of diabetes mellitus among adult beta-thalassaemic patients with chronic hepatitis C. *Eur J Gastroenterol Hepatol* 1999; **11**: 1033-1036 [PMID: 10503842 DOI: 10.1097/00042737-199909000-00014]

59 **Lecube A**, Hernández C, Genescà J, Esteban JI, Jardí R, Simó R. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. *Diabetes Care* 2004; **27**: 1171-1175 [PMID: 15111540 DOI: 10.2337/diacare.27.5.1171]

60 **Lonardo A**, Adinolfi LE, Petta S, Craxì A, Loria P. Hepatitis C and diabetes: the inevitable coincidence? *Expert Rev Anti Infect Ther* 2009; **7**: 293-308 [PMID: 19344243 DOI: 10.1586/eri.09.3]

61 **Marzouk D**, Sass J, Bakr I, El Hosseiny M, Abdel-Hamid M, Rekacewicz C, Chaturvedi N, Mohamed MK, Fontanet A. Metabolic and cardiovascular risk profiles and hepatitis C virus infection in rural Egypt. *Gut* 2007; **56**: 1105-1110 [PMID: 16956918 DOI: 10.1136/gut.2006.091983]

62 **Mason A**, Nair S. Is type II diabetes another extrahepatic manifestation of HCV infection? *Am J Gastroenterol* 2003; **98**: 243-246 [PMID: 12591036]

63 **Mayo MJ**. Extrahepatic manifestations of hepatitis C infection. *Am J Med Sci* 2003; **325**: 135-148 [PMID: 12640289 DOI: 10.1097/00000441-200303000-00006]

64 **Mehta SH**, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Ann Intern Med* 2000; **133**: 592-599 [PMID: 11033586]

65 **Mehta SH**, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Hepatology* 2001; **33**: 1554 [PMID: 11391549]

66 **Mehta SH**, Strathdee SA, Thomas DL. Association between hepatitis C virus infection and diabetes mellitus. *Epidemiol Rev* 2001; **23**: 302-312 [PMID: 12192739 DOI: 10.1093/oxfordjournals.epirev.a000808]

67 **Negro F**, Alaei M. Hepatitis C virus and type 2 diabetes. *World J Gastroenterol* 2009; **15**: 1537-1547 [PMID: 19340895 DOI: 10.3748/wjg.15.1537]

68 **Ndako JA**, Echeonwu GO, Shidali NN, Bichi IA, Paul GA, Onovoh E, Okeke LA. Occurrence of hepatitis C virus infection in type 2 diabetic patients attending Plateau state specialist hospital Jos Nigeria. *Virol J* 2009; **6**: 98 [PMID: 19586535 DOI: 10.1186/1743-422X-6-98]

69 **Zein NN**. Hepatitis C and diabetes mellitus: an ongoing controversy. *Am J Gastroenterol* 1998; **93**: 2320-2322 [PMID: 9860386 DOI: 10.1111/j.1572-0241.1998.02320.x]

70 **Noto H**, Raskin P. Hepatitis C infection and diabetes. *J Diabetes Complications* 2006; **20**: 113-120 [PMID: 16504840 DOI: 10.1016/j.jdiacomp.2006.01.001]

71 **Nwokediuko SC**, Oli JM. Hepatitis C virus infection in Nigerians with diabetes mellitus. *Niger J Clin Pract* 2008; **11**: 94-99 [PMID: 18817046]

72 **Olokoba AB**, Badung LH, Abdulrahman MB, Salawu FK, Danburam A, Aderibigbe S, Midala J, Tidi SK. Hepatitis C virus infection in Nigerians with diabetes mellitus. *Am J Sci Ind Res* 2010; **1**: 135-138 [DOI: 10.5251/ajsir.2010.1.2.135.138]

73 **Okan V**, Araz M, Aktaran S, Karsligil T, Meram I, Bayraktaroglu Z, Demirci F. Increased frequency of HCV but not HBV infection in type 2 diabetic patients in Turkey. *Int J Clin Pract* 2002; **56**: 175-177 [PMID: 12018820]

74 **Ozyilkan E**, Erbaş T, Simşek H, Telatar F, Kayhan B, Telatar H. Increased prevalence of hepatitis C virus antibodies in patients with diabetes mellitus. *J Intern Med* 1994; **235**: 283-284 [PMID: 8120528 DOI: 10.1111/j.1365-2796.1994.tb01075.x]

75 **Papatheodoridis GV**, Chrysanthos N, Savvas S, Sevastianos V, Kafiri G, Petraki K, Manesis EK. Diabetes mellitus in chronic hepatitis B and C: prevalence and potential association with the extent of liver fibrosis. *J Viral Hepat* 2006; **13**: 303-310 [PMID: 16637860 DOI: 10.1111/j.1365-2893.2005.00677.x]

76 **Parolin MB**, Réa R, Vargas RM, de Almeida AC, Baldanzi GR, Lopes RW. [Prevalence of hepatitis C infection in pa-

tients with type 2 diabetes mellitus]. *Arq Gastroenterol* 2006; **43**: 77-80 [PMID: 17119658]
77 **Picerno I**, Di Pietro A, Spataro P, Di Benedetto A, Romano G, Scoglio ME. Is diabetes mellitus a risk factor for HCV infection? *Ann Ig* 2002; **14**: 473-477 [PMID: 12638350]
78 **Qureshi H**, Ahsan T, Mujeeb SA, Jawad F, Mehdi I, Ahmed W, Alam SE. Diabetes mellitus is equally frequent in chronic HCV and HBV infection. *J Pak Med Assoc* 2002; **52**: 280-283 [PMID: 12481656]
79 **Roaeid RBM**, Ciasuddin ASM, Shakmak AA. Hepatitis C virus: seropositivity and diabetes in Benghazi, Libya. *Diabetes Int* 2002; **12**: 28-29
80 **Ratziu V**, Heurtier A, Bonyhay L, Poynard T, Giral P. Review article: an unexpected virus-host interaction--the hepatitis C virus-diabetes link. *Aliment Pharmacol Ther* 2005; **22** Suppl 2: 56-60 [PMID: 16225475 DOI: 10.1111/j.1365-2036.2005.02598.x]
81 **Rouabhia S**, Malek R, Bounecer H, Dekaken A, Bendali Amor F, Sadelaoud M, Benouar A. Prevalence of type 2 diabetes in Algerian patients with hepatitis C virus infection. *World J Gastroenterol* 2010; **16**: 3427-3431 [PMID: 20632447 DOI: 10.3748/wjg.v16.i27.3427]
82 **Ryu JK**, Lee SB, Hong SJ, Lee S. Association of chronic hepatitis C virus infection and diabetes mellitus in Korean patients. *Korean J Intern Med* 2001; **16**: 18-23 [PMID: 11417300]
83 **Sangiorgio L**, Attardo T, Gangemi R, Rubino C, Barone M, Lunetta M. Increased frequency of HCV and HBV infection in type 2 diabetic patients. *Diabetes Res Clin Pract* 2000; **48**: 147-151 [PMID: 10802152 DOI: 10.1016/S0168-8227(99)00135-7]
84 **Simó R**, Hernández C, Genescà J, Jardí R, Mesa J. High prevalence of hepatitis C virus infection in diabetic patients. *Diabetes Care* 1996; **19**: 998-1000 [PMID: 8875096 DOI: 10.2337/diacare.19.9.998]
85 **Singal AK**, Ayoola AE. Prevalence and factors affecting occurrence of type 2 diabetes mellitus in Saudi patients with chronic liver disease. *Saudi J Gastroenterol* 2008; **14**: 118-121 [PMID: 19568519 DOI: 10.4103/1319-3767.41729]
86 **Soma J**, Saito T, Taguma Y, Chiba S, Sato H, Sugimura K, Ogawa S, Ito S. High prevalence and adverse effect of hepatitis C virus infection in type II diabetic-related nephropathy. *J Am Soc Nephrol* 2000; **11**: 690-699 [PMID: 10752528]
87 **Thuluvath PJ**, John PR. Association between hepatitis C, diabetes mellitus, and race. a case-control study. *Am J Gastroenterol* 2003; **98**: 438-441 [PMID: 12591065]
88 **Wang CS**, Wang ST, Yao WJ, Chang TT, Chou P. Community-based study of hepatitis C virus infection and type 2 diabetes: an association affected by age and hepatitis severity status. *Am J Epidemiol* 2003; **158**: 1154-1160 [PMID: 14652300 DOI: 10.1093/aje/kwg259]
89 **Wang CS**, Wang ST, Yao WJ, Chang TT, Chou P. Hepatitis C virus infection and the development of type 2 diabetes in a community-based longitudinal study. *Am J Epidemiol* 2007; **166**: 196-203 [PMID: 17496314 DOI: 10.1093/aje/kwm061]
90 **Wang LF**, Wu CH, Shan Y, Fan XH, Huo N, Lu HY, Xu XY. Prevalence of abnormal glycometabolism in patients with chronic hepatitis C and related risk factors in China. *Chin Med J* (Engl) 2011; **124**: 183-188 [PMID: 21362362]
91 **White DL**, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. *J Hepatol* 2008; **49**: 831-844 [PMID: 18814931 DOI: 10.1016/j.jhep.2008.08.006]
92 **Wilson C**. Hepatitis C infection and type 2 diabetes in American-Indian women. *Diabetes Care* 2004; **27**: 2116-2119 [PMID: 15333471 DOI: 10.2337/diacare.27.9.2116]
93 **Yang SQ**, Chen HS, Jiang D, Wei L, Ji LN, Wang Y. [Relationship between chronic hepatitis C and type II diabetes mellitus]. *Zhonghua Shiyan He Linchuang Bingduxue Zazhi* 2003; **17**: 46-49 [PMID: 12870018]
94 **Zein NN**, Abdulkarim AS, Wiesner RH, Egan KS, Persing DH. Prevalence of diabetes mellitus in patients with end-stage liver cirrhosis due to hepatitis C, alcohol, or cholestatic disease. *J Hepatol* 2000; **32**: 209-217 [PMID: 10707860 DOI: 10.1016/S0168-8278(00)80065-3]
95 **Zein CO**, Levy C, Basu A, Zein NN. Chronic hepatitis C and type II diabetes mellitus: a prospective cross-sectional study. *Am J Gastroenterol* 2005; **100**: 48-55 [PMID: 15654780 DOI: 10.1111/j.1572-0241.2005.40429.x]
96 **Zhao P**, Wang JB, Jiao J. [Investigation on the incidence of diabetes in chronic hepatitis C patients and their HCV genotypes]. *Zhonghua Ganzangbing Zazhi* 2006; **14**: 86-88 [PMID: 16494773]
97 **Adegoke OA**, Kolawole BA, Ikem RT, Adediran A, Aboderin AO, Salawu A. Seroprevalence of hepatitis C virus infection in Nigerians with type 2 diabetes mellitus. *Niger J Clin Pract* 2008; **11**: 199-201 [PMID: 19140353]
98 **Balogun WO**, Adeleye JO, Akinlade KS, Kuti M, Otegbayo JA. Low prevalence of hepatitis-C viral seropositivity among patients with type-2 diabetes mellitus in a tertiary hospital. *J Natl Med Assoc* 2006; **98**: 1805-1808 [PMID: 17128691]
99 **Costa LM**, Mussi AD, Brianeze MR, Souto FJ. Hepatitis C as a risk factor for diabetes type 2: lack of evidence in a hospital in central-west Brazil. *Braz J Infect Dis* 2008; **12**: 24-26 [PMID: 18553010 DOI: 10.1590/S1413-86702008000100007]
100 **Mangia A**, Schiavone G, Lezzi G, Marmo R, Bruno F, Villani MR, Cascavilla I, Fantasia L, Andriulli A. HCV and diabetes mellitus: evidence for a negative association. *Am J Gastroenterol* 1998; **93**: 2363-2367 [PMID: 9860393]
101 **Sotiropoulos A**, Peppas TA, Skliros E, Apostolou O, Kotsini V, Pappas SI. Low prevalence of hepatitis C virus infection in Greek diabetic patients. *Diabet Med* 1999; **16**: 250-252 [PMID: 10227572 DOI: 10.1046/j.1464-5491.1999.00009.x]
102 **Perret JL**, Moussavou-Kombila JB, Delaporte E, Pemba LF, Larouze B. [Lack of association between hepatitis C virus infection and diabetes mellitus in Gabon]. *Gastroenterol Clin Biol* 2000; **24**: 135-136 [PMID: 10755864]
103 **Vírseda I**, Jaqueti J, Nicolás MD, Prieto RI, Somoza MA, Navarro F. [Hepatitis C virus and type-2 diabetes mellitus. Is there a connection?]. *Enferm Infecc Microbiol Clin* 2002; **20**: 96-97 [PMID: 11886686]
104 **Wolff C**, Moñoz S, Raddatz V. [Is there is association between hepatitis C virus and diabetes?]. *Medicina* (B Aires) 1999; **59**: 315-316 [PMID: 10451579]
105 **Gowans EJ**, Jones KL, Bharadwaj M, Jackson DC. Prospects for dendritic cell vaccination in persistent infection with hepatitis C virus. *J Clin Virol* 2004; **30**: 283-290 [PMID: 15163415]
106 **Masini M**, Campani D, Boggi U, Menicagli M, Funel N, Pollera M, Lupi R, Del Guerra S, Bugliani M, Torri S, Del Prato S, Mosca F, Filipponi F, Marchetti P. Hepatitis C virus infection and human pancreatic beta-cell dysfunction. *Diabetes Care* 2005; **28**: 940-941 [PMID: 15793203]
107 **Laskus T**, Radkowski M, Wang LF, Vargas H, Rakela J. Search for hepatitis C virus extrahepatic replication sites in patients with acquired immunodeficiency syndrome: specific detection of negative-strand viral RNA in various tissues. *Hepatology* 1998; **28**: 1398-1401 [PMID: 9794927 DOI: 10.1002/hep.510280531]
108 **Shintani Y**, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840-848 [PMID: 14988838 DOI: 10.1053/j.gastro.2003.11.056]
109 **Bureau C**, Bernad J, Chaouche N, Orfila C, Béraud M, Gonindard C, Alric L, Vinel JP, Pipy B. Nonstructural 3 protein of hepatitis C virus triggers an oxidative burst in human monocytes via activation of NADPH oxidase. *J Biol Chem* 2001; **276**: 23077-23083 [PMID: 11304537 DOI: 10.1074/jbc.M100698200]
110 **Gale M**, Blakely CM, Kwieciszewski B, Tan SL, Dossett M, Tang NM, Korth MJ, Polyak SJ, Gretch DR, Katze MG. Con-

trol of PKR protein kinase by hepatitis C virus nonstructural 5A protein: molecular mechanisms of kinase regulation. *Mol Cell Biol* 1998; **18**: 5208-5218 [PMID: 9710605]
111 **Mitsuyoshi H**, Itoh Y, Sumida Y, Minami M, Yasui K, Nakashima T, Okanoue T. Evidence of oxidative stress as a cofactor in the development of insulin resistance in patients with chronic hepatitis C. *Hepatol Res* 2008; **38**: 348-353 [PMID: 18021228 DOI: 10.1111/j.1872-034X.2007.00280.x]
112 **Miyamoto H**, Moriishi K, Moriya K, Murata S, Tanaka K, Suzuki T, Miyamura T, Koike K, Matsuura Y. Involvement of the PA28gamma-dependent pathway in insulin resistance induced by hepatitis C virus core protein. *J Virol* 2007; **81**: 1727-1735 [PMID: 17135326]
113 **Sheikh MY**, Choi J, Qadri I, Friedman JE, Sanyal AJ. Hepatitis C virus infection: molecular pathways to metabolic syndrome. *Hepatology* 2008; **47**: 2127-2133 [PMID: 18446789 DOI: 10.1002/hep.22269]
114 **Banerjee S**, Saito K, Ait-Goughoulte M, Meyer K, Ray RB, Ray R. Hepatitis C virus core protein upregulates serine phosphorylation of insulin receptor substrate-1 and impairs the downstream akt/protein kinase B signaling pathway for insulin resistance. *J Virol* 2008; **82**: 2606-2612 [PMID: 18160431 DOI: 10.1128/JVI.01672-07]
115 **Kawaguchi T**, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Baba S, Koga H, Kumashiro R, Ueno T, Ogata H, Yoshimura A, Sata M. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; **165**: 1499-1508 [PMID: 15509521]
116 **Pazienza V**, Clément S, Pugnale P, Conzelman S, Foti M, Mangia A, Negro F. The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms. *Hepatology* 2007; **45**: 1164-1171 [PMID: 17465001 DOI: 10.1002/hep.21634]
117 **Alessi DR**, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA. Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J* 1996; **15**: 6541-6551 [PMID: 8978681]
118 **Manning BD**, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* 2007; **129**: 1261-1274 [PMID: 17604717 DOI: 10.1016/j.cell.2007.06.009]
119 **Burén J**, Liu HX, Jensen J, Eriksson JW. Dexamethasone impairs insulin signalling and glucose transport by depletion of insulin receptor substrate-1, phosphatidylinositol 3-kinase and protein kinase B in primary cultured rat adipocytes. *Eur J Endocrinol* 2002; **146**: 419-429 [PMID: 11888850 DOI: 10.1530/eje.0.1460419]
120 **Cantley LC**. The phosphoinositide 3-kinase pathway. *Science* 2002; **296**: 1655-1657 [PMID: 12040186 DOI: 10.1126/science.296.5573.1655]
121 **Dominici FP**, Hauck S, Argentino DP, Bartke A, Turyn D. Increased insulin sensitivity and upregulation of insulin receptor, insulin receptor substrate (IRS)-1 and IRS-2 in liver of Ames dwarf mice. *J Endocrinol* 2002; **173**: 81-94 [PMID: 11927387 DOI: 10.1677/joe.0.1730081]
122 **Jiang G**, Zhang BB. Pi 3-kinase and its up- and down-stream modulators as potential targets for the treatment of type II diabetes. *Front Biosci* 2002; **7**: d903-d907 [PMID: 11897556]
123 **Kaburagi Y**, Yamauchi T, Yamamoto-Honda R, Ueki K, Tobe K, Akanuma Y, Yazaki Y, Kadowaki T. The mechanism of insulin-induced signal transduction mediated by the insulin receptor substrate family. *Endocr J* 1999; **46** Suppl: S25-S34 [PMID: 12054114 DOI: 10.1507/endocrj.46.Suppl_S25]
124 **Matsumoto M**, Ogawa W, Teshigawara K, Inoue H, Miyake K, Sakaue H, Kasuga M. Role of the insulin receptor substrate 1 and phosphatidylinositol 3-kinase signaling pathway in insulin-induced expression of sterol regulatory element binding protein 1c and glucokinase genes in rat hepatocytes. *Diabetes* 2002; **51**: 1672-1680 [PMID: 12031952 DOI: 10.2337/diabetes.51.6.1672]
125 **Sun Y**, Liu S, Ferguson S, Wang L, Klepcyk P, Yun JS, Friedman JE. Phosphoenolpyruvate carboxykinase overexpression selectively attenuates insulin signaling and hepatic insulin sensitivity in transgenic mice. *J Biol Chem* 2002; **277**: 23301-23307 [PMID: 11964395 DOI: 10.1074/jbc.M200964200]
126 **Ueki K**, Fruman DA, Brachmann SM, Tseng YH, Cantley LC, Kahn CR. Molecular balance between the regulatory and catalytic subunits of phosphoinositide 3-kinase regulates cell signaling and survival. *Mol Cell Biol* 2002; **22**: 965-977 [PMID: 11784871]
127 **Taniguchi CM**, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 2006; **7**: 85-96 [PMID: 16493415 DOI: 10.1038/nrm1837]
128 **Thirone AC**, Huang C, Klip A. Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. *Trends Endocrinol Metab* 2006; **17**: 72-78 [PMID: 16458527 DOI: 10.1016/j.tem.2006.01.005]
129 **Aytug S**, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. *Hepatology* 2003; **38**: 1384-1392 [PMID: 14647049]
130 **Bernsmeier C**, Duong FH, Christen V, Pugnale P, Negro F, Terracciano L, Heim MH. Virus-induced over-expression of protein phosphatase 2A inhibits insulin signalling in chronic hepatitis C. *J Hepatol* 2008; **49**: 429-440 [PMID: 18486982 DOI: 10.1016/j.jhep.2008.04.007]
131 **Negro F**. Mechanisms of hepatitis C virus-related insulin resistance. *Clin Res Hepatol Gastroenterol* 2011; **35**: 358-363 [PMID: 21354385 DOI: 10.1016/j.clinre.2011.01.011]
132 **Eslam M**, Booth DR, George J, Ahlenstiel G. Interaction of IFNL3 with insulin resistance, steatosis and lipid metabolism in chronic hepatitis C virus infection. *World J Gastroenterol* 2013; **19**: 7055-7061 [PMID: 24222948 DOI: 10.3748/wjg.v19.i41.7055]
133 **Horner SM**, Gale M. Regulation of hepatic innate immunity by hepatitis C virus. *Nat Med* 2013; **19**: 879-888 [PMID: 23836238 DOI: 10.1038/nm.3253]
134 **Bastard JP**, Maachi M, Van Nhieu JT, Jardel C, Bruckert E, Grimaldi A, Robert JJ, Capeau J, Hainque B. Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. *J Clin Endocrinol Metab* 2002; **87**: 2084-2089 [PMID: 11994345 DOI: 10.1210/jc.87.5.2084]
135 **Hotamisligil GS**. The role of TNFalpha and TNF receptors in obesity and insulin resistance. *J Intern Med* 1999; **245**: 621-625 [PMID: 10395191 DOI: 10.1046/j.1365-2796.1999.00490.x]
136 **Kern PA**, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 2001; **280**: E745-E751 [PMID: 11287357]
137 **Malaguarnera M**, Di Fazio I, Romeo MA, Restuccia S, Laurino A, Trovato BA. Elevation of interleukin 6 levels in patients with chronic hepatitis due to hepatitis C virus. *J Gastroenterol* 1997; **32**: 211-215 [PMID: 9085170 DOI: 10.1007/BF02936370]
138 **Nelson DR**, Lim HL, Marousis CG, Fang JW, Davis GL, Shen L, Urdea MS, Kolberg JA, Lau JY. Activation of tumor necrosis factor-alpha system in chronic hepatitis C virus infection. *Dig Dis Sci* 1997; **42**: 2487-2494 [PMID: 9440625]
139 **Krebs DL**, Hilton DJ. SOCS proteins: negative regulators of cytokine signaling. *Stem Cells* 2001; **19**: 378-387 [PMID: 11553846 DOI: 10.1634/stemcells.19-5-378]
140 **Naka T**, Tsutsui H, Fujimoto M, Kawazoe Y, Kohzaki H, Morita Y, Nakagawa R, Narazaki M, Adachi K, Yoshimoto T, Nakanishi K, Kishimoto T. SOCS-1/SSI-1-deficient NKT cells participate in severe hepatitis through dysregulated cross-talk inhibition of IFN-gamma and IL-4 signaling in vivo. *Immunity* 2001; **14**: 535-545 [PMID: 11371356]
141 **Oncül O**, Top C, Cavuplu T. Correlation of serum leptin

levels with insulin sensitivity in patients with chronic hepatitis-C infection. *Diabetes Care* 2002; **25**: 937 [PMID: 11978698 DOI: 10.2337/diacare.25.5.937]

142 **Mankouri J**, Tedbury PR, Gretton S, Hughes ME, Griffin SD, Dallas ML, Green KA, Hardie DG, Peers C, Harris M. Enhanced hepatitis C virus genome replication and lipid accumulation mediated by inhibition of AMP-activated protein kinase. *Proc Natl Acad Sci USA* 2010; **107**: 11549-11554 [PMID: 20534540 DOI: 10.1073/pnas.0912426107]

143 **Cheung AT**, Wang J, Ree D, Kolls JK, Bryer-Ash M. Tumor necrosis factor-alpha induces hepatic insulin resistance in obese Zucker (fa/fa) rats via interaction of leukocyte antigen-related tyrosine phosphatase with focal adhesion kinase. *Diabetes* 2000; **49**: 810-819 [PMID: 10905491 DOI: 10.2337/diabetes.49.5.810]

144 **Ruan H**, Lodish HF. Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-alpha. *Cytokine Growth Factor Rev* 2003; **14**: 447-455 [PMID: 12948526 DOI: 10.1016/S1359-6101(03)00052-2]

145 **Fallahi P**, Ferri C, Ferrari SM, Corrado A, Sansonno D, Antonelli A. Cytokines and HCV-related disorders. *Clin Dev Immunol* 2012; **2012**: 468107 [PMID: 22611419]

146 **Antonelli A**, Ferri C, Fallahi P, Ferrari SM, Sebastiani M, Ferrari D, Giunti M, Frascerra S, Tolari S, Franzoni F, Galetta F, Marchi S, Ferrannini E. High values of CXCL10 serum levels in mixed cryoglobulinemia associated with hepatitis C infection. *Am J Gastroenterol* 2008; **103**: 2488-2494 [PMID: 18775023 DOI: 10.1111/j.1572-0241.2008.02040.x]

147 **Ando H**, Nagai Y, Yokoyama M, Takamura T, Kobayashi K. Antibodies to GAD in diabetic patients with chronic hepatitis C. *Diabet Med* 1998; **15**: 797 [PMID: 9737812]

148 **Betterle C**, Zanette F, Pedini B, Presotto F, Rapp LB, Monciotti CM, Rigon F. Clinical and subclinical organ-specific autoimmune manifestations in type 1 (insulin-dependent) diabetic patients and their first-degree relatives. *Diabetologia* 1984; **26**: 431-436 [PMID: 6381190]

149 **Hiéronimus S**, Fredenrich A, Tran A, Benzaken S, Fénichel P. Antibodies to GAD in chronic hepatitis C patients. *Diabetes Care* 1997; **20**: 1044 [PMID: 9167125]

150 **Piquer S**, Hernández C, Enriquez J, Ross A, Esteban JI, Genescà J, Bonifacio E, Puig-Domingo M, Simó R. Islet cell and thyroid antibody prevalence in patients with hepatitis C virus infection: effect of treatment with interferon. *J Lab Clin Med* 2001; **137**: 38-42 [PMID: 11150022 DOI: 10.1067/mlc.2001.111515]

151 **Chen LK**, Chou YC, Tsai ST, Hwang SJ, Lee SD. Hepatitis C virus infection-related Type 1 diabetes mellitus. *Diabet Med* 2005; **22**: 340-343 [PMID: 15717885 DOI: 10.1111/j.1464-5491.2005.01412.x]

152 **Masuda H**, Atsumi T, Fujisaku A, Shimizu C, Yoshioka N, Koike T. Acute onset of type 1 diabetes accompanied by acute hepatitis C: the potential role of proinflammatory cytokine in the pathogenesis of autoimmune diabetes. *Diabetes Res Clin Pract* 2007; **75**: 357-361 [PMID: 16968656 DOI: 10.1016/j.diabres.2006.07.016]

153 **Yan FM**, Chen AS, Hao F, Zhao XP, Gu CH, Zhao LB, Yang DL, Hao LJ. Hepatitis C virus may infect extrahepatic tissues in patients with hepatitis C. *World J Gastroenterol* 2000; **6**: 805-811 [PMID: 11819700]

154 **Bogdanos DP**, Mieli-Vergani G, Vergani D. Virus, liver and autoimmunity. *Dig Liver Dis* 2002; **32**: 440-446 [PMID: 11030191 DOI: 10.1016/S1590-8658(00)80266-2]

155 **Bogdanos DP**, Choudhuri K, Vergani D. Molecular mimicry and autoimmune liver disease: virtuous intentions, malign consequences. *Liver* 2001; **21**: 225-232 [PMID: 11454184 DOI: 10.1034/j.1600-0676.2001.021004225.x]

156 **Bogdanos DP**, Rigopoulou EI. Viral/self-mimicry and immunological cross-reactivity as a trigger of hepatic C virus associated autoimmune diabetes. *Diabetes Res Clin Pract* 2007; **77**: 155-156 [PMID: 17118481 DOI: 10.1016/j.diabres.2006.10.012]

157 **Bogdanos DP**, Lenzi M, Okamoto M, Rigopoulou EI, Muratori P, Ma Y, Muratori L, Tsantoulas D, Mieli- Vergani G, Bianchi FB, Vergani D. Multiple viral/self immunological cross-reactivity in liver kidney microsomal antibody positive hepatitis C virus infected patients is associated with the possession of HLA B51. *Int J Immunopathol Pharmacol* 2004; **17**: 83-92 [PMID: 15000871]

158 **Hanifi-Moghaddam P**, Schloot NC, Kappler S, Seissler J, Kolb H. An association of autoantibody status and serum cytokine levels in type 1 diabetes. *Diabetes* 2003; **52**: 1137-1142 [PMID: 12716743 DOI: 10.2337/diabetes.52.5.1137]

159 **Nicoletti F**, Conget I, Di Marco R, Speciale AM, Morìnigo R, Bendtzen K, Gomis R. Serum levels of the interferon-gamma-inducing cytokine interleukin-18 are increased in individuals at high risk of developing type I diabetes. *Diabetologia* 2001; **44**: 309-311 [PMID: 11317661]

160 **Oikawa Y**, Shimada A, Kasuga A, Morimoto J, Osaki T, Tahara H, Miyazaki T, Tashiro F, Yamato E, Miyazaki J, Saruta T. Systemic administration of IL-18 promotes diabetes development in young nonobese diabetic mice. *J Immunol* 2003; **171**: 5865-5875 [PMID: 14634096]

161 **Nakanishi K**, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine Growth Factor Rev* 2001; **12**: 53-72 [PMID: 11312119]

162 **Yumoto E**, Higashi T, Nouso K, Nakatsukasa H, Fujiwara K, Hanafusa T, Yumoto Y, Tanimoto T, Kurimoto M, Tanaka N, Tsuji T. Serum gamma-interferon-inducing factor (IL-18) and IL-10 levels in patients with acute hepatitis and fulminant hepatic failure. *J Gastroenterol Hepatol* 2002; **17**: 285-294 [PMID: 11982699 DOI: 10.1046/j.1440-1746.2002.02690.x]

163 **Torre D**, Zeroli C, Giola M, Ferrario G, Fiori GP, Bonetta G, Tambini R. Serum levels of interleukin-1 alpha, interleukin-1 beta, interleukin-6, and tumor necrosis factor in patients with acute viral hepatitis. *Clin Infect Dis* 1994; **18**: 194-198 [PMID: 8161626]

164 **Yang XD**, Tisch R, Singer SM, Cao ZA, Liblau RS, Schreiber RD, McDevitt HO. Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J Exp Med* 1994; **180**: 995-1004 [PMID: 8064245 DOI: 10.1084/jem.180.3.995]

165 **Eizirik DL**, Mandrup-Poulsen T. A choice of death--the signal-transduction of immune-mediated beta-cell apoptosis. *Diabetologia* 2001; **44**: 2115-2133 [PMID: 11793013 DOI: 10.1007/s001250100021]

166 **Thomas HE**, Irawaty W, Darwiche R, Brodnicki TC, Santamaria P, Allison J, Kay TW. IL-1 receptor deficiency slows progression to diabetes in the NOD mouse. *Diabetes* 2004; **53**: 113-121 [PMID: 14693705 DOI: 10.2337/diabetes.53.1.113]

167 **Lee LF**, Xu B, Michie SA, Beilhack GF, Warganich T, Turley S, McDevitt HO. The role of TNF-alpha in the pathogenesis of type 1 diabetes in the nonobese diabetic mouse: analysis of dendritic cell maturation. *Proc Natl Acad Sci USA* 2005; **102**: 15995-16000 [PMID: 16247001]

168 **Antonelli A**, Ferri C, Fallahi P, Ferrari SM, Goglia F, Ferrannini E. Hepatitis C virus infection: evidence for an association with type 2 diabetes (Response to Skowronski et al). *Diabetes Care* 2006; **29**: 751 [DOI: 10.2337/diacare.29.03.06.dc05-2420]

169 **Antonelli A**, Ferri C, Ferrari SM, Colaci M, Sansonno D, Fallahi P. Endocrine manifestations of hepatitis C virus infection. *Nat Clin Pract Endocrinol Metab* 2009; **5**: 26-34 [PMID: 19079271 DOI: 10.1038/ncpendmet1027]

170 **Gale EA**. Latent autoimmune diabetes in adults: a guide for the perplexed. *Diabetologia* 2005; **48**: 2195-2199 [PMID: 16193287 DOI: 10.1007/s00125-005-1954-5]

171 **Skowroński M**, Zozulińska D, Juszczyk J, Wierusz-Wysocka B. Hepatitis C virus infection: evidence for an association with type 2 diabetes. *Diabetes Care* 2006; **29**: 750; author

reply 751 [PMID: 16526125 DOI: 10.2337/diacare.29.03.06.dc05-2263]
172 **Antonelli A**, Fallahi P, Ferrari SM, Pupilli C, d'Annunzio G, Lorini R, Vanelli M, Ferrannini E. Serum Th1 (CXCL10) and Th2 (CCL2) chemokine levels in children with newly diagnosed Type 1 diabetes: a longitudinal study. *Diabet Med* 2008; **25**: 1349-1353 [PMID: 19046227 DOI: 10.1111/j.1464-5491.2008.02577.x]
173 **Apolinario A**, Majano PL, Lorente R, Núñez O, Clemente G, García-Monzón C. Gene expression profile of T-cell-specific chemokines in human hepatocyte-derived cells: evidence for a synergistic inducer effect of cytokines and hepatitis C virus proteins. *J Viral Hepat* 2005; **12**: 27-37 [PMID: 15655045 DOI: 10.1111/j.1365-2893.2005.00540.x]
174 **Vial T**, Descotes J. Clinical toxicity of the interferons. *Drug Saf* 1994; **10**: 115-150 [PMID: 7516663 DOI: 10.2165/00002018-199410020-00003]
175 **Fabris P**, Betterle C, Greggio NA, Zanchetta R, Bosi E, Biasin MR, de Lalla F. Insulin-dependent diabetes mellitus during alpha-interferon therapy for chronic viral hepatitis. *J Hepatol* 1998; **28**: 514-517 [PMID: 9551692]
176 **Hadziyannis SJ**. The spectrum of extrahepatic manifestations in hepatitis C virus infection. *J Viral Hepat* 1997; **4**: 9-28 [PMID: 9031061 DOI: 10.1046/j.1365-2893.1997.00120.x]
177 **di Cesare E**, Previti M, Russo F, Brancatelli S, Ingemi MC, Scoglio R, Mazzù N, Cucinotta D, Raimondo G. Interferon-alpha therapy may induce insulin autoantibody development in patients with chronic viral hepatitis. *Dig Dis Sci* 1996; **41**: 1672-1677 [PMID: 8769300 DOI: 10.1007/BF02087923]
178 **Jasonek J**, Kacprzak-Bergman I, Zaleska I. [The treatment of HCV infection with interferon alpha and ribavirin in a child with diabetes I type]. *Przegl Epidemiol* 2006; **60**: 259-263 [PMID: 16964677]
179 **Schories M**, Peters T, Rasenack J, Reincke M. [Autoantibodies against islet cell antigens and type 1 diabetes after treatment with interferon-alpha]. *Dtsch Med Wochenschr* 2004; **129**: 1120-1124 [PMID: 15143453]
180 **Schreuder TC**, Gelderblom HC, Weegink CJ, Hamann D, Reesink HW, Devries JH, Hoekstra JB, Jansen PL. High incidence of type 1 diabetes mellitus during or shortly after treatment with pegylated interferon alpha for chronic hepatitis C virus infection. *Liver Int* 2008; **28**: 39-46 [PMID: 18031478 DOI: 10.1111/j.1478-3231.2007.01610.x]
181 **Betterle C**, Fabris P, Zanchetta R, Pedini B, Tositti G, Bosi E, de Lalla F. Autoimmunity against pancreatic islets and other tissues before and after interferon-alpha therapy in patients with hepatitis C virus chronic infection. *Diabetes Care* 2000; **23**: 1177-1181 [PMID: 10937518 DOI: 10.2337/diacare.23.8.1177]
182 **Chakrabarti D**, Hultgren B, Stewart TA. IFN-alpha induces autoimmune T cells through the induction of intracellular adhesion molecule-1 and B7.2. *J Immunol* 1996; **157**: 522-528 [PMID: 8752897]
183 **Frankart L**, Lejeune D, Donckier J. Diabetes mellitus and interferon therapy. *Diabet Med* 1997; **14**: 405 [PMID: 9171259]
184 **Hayakawa M**, Gando S, Morimoto Y, Kemmotsu O. Development of severe diabetic keto-acidosis with shock after changing interferon-beta into interferon-alpha for chronic hepatitis C. *Intensive Care Med* 2000; **26**: 1008 [PMID: 10990121]
185 **Imano E**, Kanda T, Ishigami Y, Kubota M, Ikeda M, Matsuhisa M, Kawamori R, Yamasaki Y. Interferon induces insulin resistance in patients with chronic active hepatitis C. *J Hepatol* 1998; **28**: 189-193 [PMID: 9514530 DOI: 10.1016/0168-8278(88)80004-7]
186 **Koivisto VA**, Pelkonen R, Cantell K. Effect of interferon on glucose tolerance and insulin sensitivity. *Diabetes* 1989; **38**: 641-647 [PMID: 2653935 DOI: 10.2337/diabetes.38.5.641]
187 **Konrad T**, Zeuzem S, Vicini P, Toffolo G, Briem D, Lormann J, Herrmann G, Berger A, Kusterer K, Teuber G, Cobelli C, Usadel KH. Evaluation of factors controlling glucose tolerance in patients with HCV infection before and after 4 months therapy with interferon-alpha. *Eur J Clin Invest* 2000; **30**: 111-121 [PMID: 10651835 DOI: 10.1046/j.1365-2362.2000.00608.x]
188 **Nemesánszky E**, Pusztay M, Csepregi A. Effects of interferon treatment on the glucose metabolism of patients with chronic hepatitis C. *Eur J Intern Med* 2000; **11**: 151-155 [PMID: 10854821 DOI: 10.1016/S0953-6205(00)00080-7]
189 **Fabris P**, Betterle C, Floreani A, Greggio NA, de Lazzari F, Naccarato R, Chiaramonte M. Development of type 1 diabetes mellitus during interferon alfa therapy for chronic HCV hepatitis. *Lancet* 1992; **340**: 548 [PMID: 1354296 DOI: 10.1016/0140-6736(92)91744-S]
190 **Bhatti A**, McGarrity TJ, Gabbay R. Diabetic ketoacidosis induced by alpha interferon and ribavirin treatment in a patient with hepatitis C. *Am J Gastroenterol* 2001; **96**: 604-605 [PMID: 11232722 DOI: 10.1016/S0002-9270(00)02364-9]
191 **Bosi E**, Minelli R, Bazzigaluppi E, Salvi M. Fulminant autoimmune Type 1 diabetes during interferon-alpha therapy: a case of Th1-mediated disease? *Diabet Med* 2001; **18**: 329-332 [PMID: 11437866 DOI: 10.1046/j.1464-5491.2001.00492.x]
192 **Cozzolongo R**, Betterle C, Fabris P, Paola Albergoni M, Lanzilotta E, Manghisi OG. Onset of type 1 diabetes mellitus during peginterferon alpha-2b plus ribavirin treatment for chronic hepatitis C. *Eur J Gastroenterol Hepatol* 2006; **18**: 689-692 [PMID: 16702861]
193 **Eibl N**, Gschwantler M, Ferenci P, Eibl MM, Weiss W, Schernthaner G. Development of insulin-dependent diabetes mellitus in a patient with chronic hepatitis C during therapy with interferon-alpha. *Eur J Gastroenterol Hepatol* 2001; **13**: 295-298 [PMID: 11293453]
194 **Fattovich G**, Giustina G, Favarato S, Ruol A. A survey of adverse events in 11,241 patients with chronic viral hepatitis treated with alfa interferon. *J Hepatol* 1996; **24**: 38-47 [PMID: 8834023]
195 **Figge E**, Reiser M, Schmiegel W, Nauck MA. Manifestation eines Typ-1-Diabetes bei einem patienten mit hepatitis C wahrend einer therapie mit interferon-a and ribavirin. *Diabetes Stoffwechsel* 2001; **10**: 133-138
196 **Floreani A**, Chiaramonte M, Greggio NA, Fabris P, De Lazzari F, Naccarato R, Betterle C. Organ-specific autoimmunity and genetic predisposition in interferon-treated HCV-related chronic hepatitis patients. *Ital J Gastroenterol Hepatol* 1998; **30**: 71-76 [PMID: 9615270]
197 **Fujioka T**, Honda M, Yoshizaki T, Ogawa M, Matsuno H, Shimokawa K, Koyama K. A case of type 1 diabetes onset and recurrence of Graves' disease during pegylated interferon-α plus ribavirin treatment for chronic hepatitis C. *Intern Med* 2010; **49**: 1987-1990 [PMID: 20847504 DOI: 10.2169/internalmedicine.49.3831]
198 **Giuntoli P**, Mariani S, Avoli D, Giammarco V. Diabete mellito insulino-dipendente indotto da interferon-alfa. *Minerva Endocrinol* 1995; **20**: 243-245
199 **Hayashi M**, Kataoka Y, Tachikawa K, Koguchi H, Tanaka H. Dual onset of type 1 diabetes mellitus and Graves' disease during treatment with pegylated interferon alpha-2b and ribavirin for chronic hepatitis C. *Diabetes Res Clin Pract* 2009; **86**: e19-e21 [PMID: 19744739 DOI: 10.1016/j.diabres.2009.08.009]
200 **Huang X**, Yuang J, Goddard A, Foulis A, James RF, Lernmark A, Pujol-Borrell R, Rabinovitch A, Somoza N, Stewart TA. Interferon expression in the pancreases of patients with type I diabetes. *Diabetes* 1995; **44**: 658-664 [PMID: 7540571 DOI: 10.2337/diabetes.44.6.658]
201 **Imagawa A**, Itoh N, Hanafusa T, Oda Y, Waguri M, Miyagawa J, Kono N, Kuwajima M, Matsuzawa Y. Autoimmune endocrine disease induced by recombinant interferon-alpha therapy for chronic active type C hepatitis. *J Clin Endocrinol Metab* 1995; **80**: 922-926 [PMID: 7883851 DOI: 10.1210/jc.80.3.922]
202 **Kado S**, Miyamoto J, Komatsu N, Iwaki Y, Ozaki H, Taguchi

H, Kure M, Sarashina G, Watanabe T, Katsura Y, Nemoto Y, Noritake M, Matsuoka T. Type 1 diabetes mellitus caused by treatment with interferon-beta. *Intern Med* 2000; **39**: 146-149 [PMID: 10732833 DOI: 10.2169/internalmedicine.39.146]

203 **Mathieu E**, Fain O, Sitbon M, Thomas M. [Autoimmune diabetes after treatment with interferon-alpha]. *Presse Med* 1995; **24**: 238 [PMID: 7899374]

204 **Mofredj A**, Howaizi M, Grasset D, Licht H, Loison S, Devergie B, Demontis R, Cadranel JF. Diabetes mellitus during interferon therapy for chronic viral hepatitis. *Dig Dis Sci* 2002; **47**: 1649-1654 [PMID: 12141831]

205 **Nakamura K**, Kawasaki E, Abiru N, Jo O, Fukushima K, Satoh T, Kuriya G, Kobayashi M, Kuwahara H, Yamasaki H, Ide T, Eguchi K. Trajectories of anti-islet autoantibodies before development of type 1 diabetes in interferon-treated hepatitis C patients. Case reports and a literature review. *Endocr J* 2010; **57**: 947-951 [PMID: 20805634 DOI: 10.1507/endocrj.K10E-207]

206 **Ogihara T**, Katagiri H, Yamada T, Kudo H, Imai J, Ishigaki Y, Hinokio Y, Yamagiwa Y, Ueno Y, Shimosegawa T, Oka Y. Peginterferon (PEG-IFN) plus ribavirin combination therapy, but neither interferon nor PGE-IFN alone, induced type 1 diabetes in a patient with chronic hepatitis C. *Intern Med* 2009; **48**: 1387-1390 [PMID: 19687584 DOI: 10.2169/internalmedicine.48.2220]

207 **Oka R**, Hiroi N, Shigemitsu R, Sue M, Oshima Y, Yoshida-Hiroi M. Type 1 Diabetes Mellitus Associated with Pegylated Interferon-α Plus Ribavirin Treatment for Chronic Hepatitis C: Case Report and Literature Review. *Clin Med Insights Endocrinol Diabetes* 2011; **4**: 39-45 [PMID: 22879793 DOI: 10.4137/CMED.S7815]

208 **Okada K**, Kikuoka H, Kokawa M, Ihozaki M, Takeuchi K, Okamura T, Nakao T, Hoso T, Kondo M. A case of insulin-dependent diabetes mellitus (IDDM) following interferon alpha therapy for type C chronic hepatitis. *J Japan Diab Soc* 1995; **38**: 625-630

209 **Okanoue T**, Sakamoto S, Itoh Y, Minami M, Yasui K, Sakamoto M, Nishioji K, Katagishi T, Nakagawa Y, Tada H, Sawa Y, Mizuno M, Kagawa K, Kashima K. Side effects of high-dose interferon therapy for chronic hepatitis C. *J Hepatol* 1996; **25**: 283-291 [PMID: 8895006 DOI: 10.1016/S0168-8278(96)80113-9]

210 **Recasens M**, Aguilera E, Ampurdanés S, Sánchez Tapias JM, Simó O, Casamitjana R, Conget I. Abrupt onset of diabetes during interferon-alpha therapy in patients with chronic hepatitis C. *Diabet Med* 2001; **18**: 764-767 [PMID: 11606177 DOI: 10.1046/j.1464-5491.2001.00562.x]

211 **Rostaing L**, Oksman F, Izopet J, Baron E, Cisterne JM, Hoff M, Abbal M, Durand D. Serological markers of autoimmunity in renal transplant patients before and after alpha-interferon therapy for chronic hepatitis C. *Am J Nephrol* 1996; **16**: 478-483 [PMID: 8955758 DOI: 10.1159/000169047]

212 **Seifarth C**, Benninger J, Böhm BO, Wiest-Ladenburger U, Hahn EG, Hensen J. [Augmentation of the immune response to islet cell antigens with development of diabetes mellitus caused by interferon-alpha therapy in chronic hepatitis C]. *Z Gastroenterol* 1999; **37**: 235-239 [PMID: 10234796]

213 **Shiba T**, Morino Y, Tagawa K, Fujino H, Unuma T. Onset of diabetes with high titer anti-GAD antibody after IFN therapy for chronic hepatitis. *Diabetes Res Clin Pract* 1995; **30**: 237-241 [PMID: 8861464 DOI: 10.1016/0168-8227(95)01188-9]

214 **Hiltunen Y**, Ala-Korpela M, Jokisaari J, Eskelinen S, Kiviniitty K, Savolainen M, Kesäniemi YA. A lineshape fitting model for 1H NMR spectra of human blood plasma. *Magn Reson Med* 1991; **21**: 222-232 [PMID: 1745121]

215 **Tanaka J**, Sugimoto K, Shiraki K, Beppu T, Yoneda K, Fuke H, Yamamoto N, Ito K, Takei Y. Type 1 diabetes mellitus provoked by peginterferon alpha-2b plus ribavirin treatment for chronic hepatitis C. *Intern Med* 2008; **47**: 747-749 [PMID: 18421192 DOI: 10.2169/internalmedicine.47.0653]

216 **Tohda G**, Oida K, Higashi S, Hayashi T, Miyamori I. Interferon-alpha and development of type 1 diabetes: a case without insulin resistance. *Diabetes Care* 1998; **21**: 1774 [PMID: 9773746 DOI: 10.2337/diacare.21.10.1774b]

217 **Tosone G**, Borgia G, Gentile I, Cerini R, Conte MC, Orlando R, Piazza M. A case of pegylated interferon alpha-related diabetic ketoacidosis: can this complication be avoided? *Acta Diabetol* 2007; **44**: 167-169 [PMID: 17721757 DOI: 10.1007/s00592-007-0259-1]

218 **Uto H**, Matsuoka H, Murata M, Okamoto T, Miyata Y, Hori T, Ido A, Hirono S, Hayashi K, Tsubouchi H. A case of chronic hepatitis C developing insulin-dependent diabetes mellitus associated with various autoantibodies during interferon therapy. *Diabetes Res Clin Pract* 2000; **49**: 101-106 [PMID: 10963820 DOI: 10.1016/S0168-8227(00)00143-1]

219 **Wasmuth HE**, Stolte C, Geier A, Gartung C, Matern S. Induction of multiple autoantibodies to islet cell antigens during treatment with interferon alpha for chronic hepatitis C. *Gut* 2001; **49**: 596-597 [PMID: 11589192 DOI: 10.1136/gut.49.4.596a]

220 **Waguri M**, Hanafusa T, Itoh N, Imagawa A, Miyagawa J, Kawata S, Kono N, Kuwajima M, Matsuzawa Y. Occurrence of IDDM during interferon therapy for chronic viral hepatitis. *Diabetes Res Clin Pract* 1994; **23**: 33-36 [PMID: 8013261 DOI: 10.1016/0168-8227(94)90124-4]

221 **Wesche B**, Jaeckel E, Trautwein C, Wedemeyer H, Falorni A, Frank H, von zur Mühlen A, Manns MP, Brabant G. Induction of autoantibodies to the adrenal cortex and pancreatic islet cells by interferon alpha therapy for chronic hepatitis C. *Gut* 2001; **48**: 378-383 [PMID: 11171829 DOI: 10.1136/gut.48.3.378]

222 **Yamazaki M**, Sato A, Takeda T, Komatsu M. Distinct clinical courses in type 1 diabetes mellitus induced by peg-interferon-alpha treatment for chronic hepatitis C. *Intern Med* 2010; **49**: 403-407 [PMID: 20190473 DOI: 10.2169/internalmedicine.49.2656]

223 **Yanagisawa K**, Amemiya T, Morita Y, Kuroki H, Sanaka M, Uchigata Y, Omori Y, Hashimoto E, Hayashi N. A case of insulindependent diabetes mellitus developing during interferon therapy for chronic hepatitis type C. *J Japan Diab Soc* 1995; **38**: 283-288

224 **Alter MJ**, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE. The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. *N Engl J Med* 1992; **327**: 1899-1905 [PMID: 1280771 DOI: 10.1056/NEJM199212313272702]

225 **Kiyosawa K**, Furuta S. Review of hepatitis C in Japan. *J Gastroenterol Hepatol* 1991; **6**: 383-391 [PMID: 1717034 DOI: 10.1111/j.1440-1746.1991.tb00876.x]

226 **van Rossum TG**, Vulto AG, de Man RA, Brouwer JT, Schalm SW. Review article: glycyrrhizin as a potential treatment for chronic hepatitis C. *Aliment Pharmacol Ther* 1998; **12**: 199-205 [PMID: 9570253]

227 **Colombo M**, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, Dioguardi N, Houghton M. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989; **2**: 1006-1008 [PMID: 2572740 DOI: 10.1016/S0140-6736(89)91016-7]

228 **Hasan F**, Jeffers LJ, De Medina M, Reddy KR, Parker T, Schiff ER, Houghton M, Choo QL, Kuo G. Hepatitis C-associated hepatocellular carcinoma. *Hepatology* 1990; **12**: 589-591 [PMID: 2169456 DOI: 10.1002/hep.1840120323]

229 **Ikeda K**, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H, Kawanishi M. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; **18**: 47-53 [PMID: 7686879 DOI: 10.1016/0270-9139(93)90505-H]

230 **Kew MC**, Houghton M, Choo QL, Kuo G. Hepatitis C virus antibodies in southern African blacks with hepatocellular

carcinoma. *Lancet* 1990; **335**: 873-874 [PMID: 1691422 DOI: 10.1016/0140-6736(90)90474-J]
231 **Tsukuma H**, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797-1801 [PMID: 7684822 DOI: 10.1056/NEJM199306243282501]
232 **Adami HO**, Chow WH, Nyrén O, Berne C, Linet MS, Ekbom A, Wolk A, McLaughlin JK, Fraumeni JF. Excess risk of primary liver cancer in patients with diabetes mellitus. *J Natl Cancer Inst* 1996; **88**: 1472-1477 [PMID: 8841022 DOI: 10.1093/jnci/88.20.1472]
233 **Chen CL**, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, Wang LY, Sun CA, Lu SN, Chen DS, Chen CJ. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. *Gastroenterology* 2008; **135**: 111-121 [PMID: 18505690 DOI: 10.1053/j.gastro.2008.03.073]
234 **Davila JA**, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut* 2005; **54**: 533-539 [PMID: 15753540 DOI: 10.1136/gut.2004.052167]
235 **El-Serag HB**, Richardson PA, Everhart JE. The role of diabetes in hepatocellular carcinoma: a case-control study among United States Veterans. *Am J Gastroenterol* 2001; **96**: 2462-2467 [PMID: 11513191 DOI: 10.1016/S0002-9270(01)02617-X]
236 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576 [PMID: 17570226 DOI: 10.1053/j.gastro.2007.04.061]
237 **Gao C**, Yao SK. Diabetes mellitus: a "true" independent risk factor for hepatocellular carcinoma? *Hepatobiliary Pancreat Dis Int* 2009; **8**: 465-473 [PMID: 19822488]
238 **Hung CH**, Lee CM, Wang JH, Hu TH, Chen CH, Lin CY, Lu SN. Impact of diabetes mellitus on incidence of hepatocellular carcinoma in chronic hepatitis C patients treated with interferon-based antiviral therapy. *Int J Cancer* 2011; **128**: 2344-2352 [PMID: 20669224 DOI: 10.1002/ijc.25585]
239 **Inoue M**, Iwasaki M, Otani T, Sasazuki S, Noda M, Tsugane S. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch Intern Med* 2006; **166**: 1871-1877 [PMID: 17000944 DOI: 10.1001/archinte.166.17.1871]
240 **Jee SH**, Ohrr H, Sull JW, Yun JE, Ji M, Samet JM. Fasting serum glucose level and cancer risk in Korean men and women. *JAMA* 2005; **293**: 194-202 [PMID: 15644546 DOI: 10.1001/jama.293.2.194]
241 **Lagiou P**, Kuper H, Stuver SO, Tzonou A, Trichopoulos D, Adami HO. Role of diabetes mellitus in the etiology of hepatocellular carcinoma. *J Natl Cancer Inst* 2000; **92**: 1096-1099 [PMID: 10880555 DOI: 10.1093/jnci/92.13.1096]
242 **Lai MS**, Hsieh MS, Chiu YH, Chen TH. Type 2 diabetes and hepatocellular carcinoma: A cohort study in high prevalence area of hepatitis virus infection. *Hepatology* 2006; **43**: 1295-1302 [PMID: 16729295 DOI: 10.1002/hep.21208]
243 **La Vecchia C**, Negri E, Franceschi S, D'Avanzo B, Boyle P. A case-control study of diabetes mellitus and cancer risk. *Br J Cancer* 1994; **70**: 950-953 [PMID: 7947103 DOI: 10.1038/bjc.1994.427]
244 **Lawson DH**, Gray JM, McKillop C, Clarke J, Lee FD, Patrick RS. Diabetes mellitus and primary hepatocellular carcinoma. *Q J Med* 1986; **61**: 945-955 [PMID: 2819932]
245 **N'Kontchou G**, Paries J, Htar MT, Ganne-Carrie N, Costentin L, Grando-Lemaire V, Trinchet JC, Beaugrand M. Risk factors for hepatocellular carcinoma in patients with alcoholic or viral C cirrhosis. *Clin Gastroenterol Hepatol* 2006; **4**: 1062-1068 [PMID: 16844421 DOI: 10.1016/j.cgh.2006.05.013]
246 **Taura N**, Ichikawa T, Miyaaki H, Yatsuhashi H, Ishibashi H, Nakao K. Prevalence of type 2 diabetes mellitus in Japanese patients with hepatocellular carcinoma. *Exp Ther Med* 2011; **2**: 81-84 [PMID: 22977473 DOI: 10.3892/etm.2010.167]
247 **Tazawa J**, Maeda M, Nakagawa M, Ohbayashi H, Kusano F, Yamane M, Sakai Y, Suzuki K. Diabetes mellitus may be associated with hepatocarcinogenesis in patients with chronic hepatitis C. *Dig Dis Sci* 2002; **47**: 710-715 [PMID: 11991597]
248 **Veldt BJ**, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, de Knegt RJ, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology* 2008; **47**: 1856-1862 [PMID: 18506898 DOI: 10.1002/hep.22251]
249 **Yu MC**, Tong MJ, Govindarajan S, Henderson BE. Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California. *J Natl Cancer Inst* 1991; **83**: 1820-1826 [PMID: 1660542 DOI: 10.1093/jnci/83.24.1820]
250 **Yuan JM**, Govindarajan S, Arakawa K, Yu MC. Synergism of alcohol, diabetes, and viral hepatitis on the risk of hepatocellular carcinoma in blacks and whites in the U.S. *Cancer* 2004; **101**: 1009-1017 [PMID: 15329910 DOI: 10.1002/cncr.20427]
251 **Wideroff L**, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S, Borch-Johnsen K, Olsen JH. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. *J Natl Cancer Inst* 1997; **89**: 1360-1365 [PMID: 9308706 DOI: 10.1093/jnci/89.18.1360]
252 **D'Souza R**, Sabin CA, Foster GR. Insulin resistance plays a significant role in liver fibrosis in chronic hepatitis C and in the response to antiviral therapy. *Am J Gastroenterol* 2005; **100**: 1509-1515 [PMID: 15984973 DOI: 10.1111/j.1572-0241.2005.41403.x]
253 **Romero-Gómez M**, Del Mar Viloria M, Andrade RJ, Salmerón J, Diago M, Fernández-Rodríguez CM, Corpas R, Cruz M, Grande L, Vázquez L, Muñoz-De-Rueda P, López-Serrano P, Gila A, Gutiérrez ML, Pérez C, Ruiz-Extremera A, Suárez E, Castillo J. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128**: 636-641 [PMID: 15765399 DOI: 10.1053/j.gastro.2004.12.049]
254 **Alexia C**, Fallot G, Lasfer M, Schweizer-Groyer G, Groyer A. An evaluation of the role of insulin-like growth factors (IGF) and of type-I IGF receptor signalling in hepatocarcinogenesis and in the resistance of hepatocarcinoma cells against drug-induced apoptosis. *Biochem Pharmacol* 2004; **68**: 1003-1015 [PMID: 15313394]
255 **Stuver SO**, Kuper H, Tzonou A, Lagiou P, Spanos E, Hsieh CC, Mantzoros C, Trichopoulos D. Insulin-like growth factor 1 in hepatocellular carcinoma and metastatic liver cancer in men. *Int J Cancer* 2000; **87**: 118-121 [PMID: 10861461]
256 **Su TS**, Liu WY, Han SH, Jansen M, Yang-Fen TL, P'eng FK, Chou CK. Transcripts of the insulin-like growth factors I and II in human hepatoma. *Cancer Res* 1989; **49**: 1773-1777 [PMID: 2466561]
257 **Le Roith D**. Seminars in medicine of the Beth Israel Deaconess Medical Center. Insulin-like growth factors. *N Engl J Med* 1997; **336**: 633-640 [PMID: 9032050]
258 **Macaulay VM**. Insulin-like growth factors and cancer. *Br J Cancer* 1992; **65**: 311-320 [PMID: 1313689 DOI: 10.1038/bjc.1992.65]
259 **Ellidokuz E**, Cömlekçi A, Ellidokuz H, Akpinar H, Gökçe C, Tankurt E, Sagol O, Simsek I, Gönen O. The role of serum leptin levels in chronic hepatitis C with steatosis. *Hepatogastroenterology* 2003; **50** Suppl 2: cclxix-cclxxii [PMID: 15244198]
260 **Rubbia-Brandt L**, Quadri R, Abid K, Giostra E, Malé PJ, Mentha G, Spahr L, Zarski JP, Borisch B, Hadengue A, Negro F. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. *J Hepatol* 2000; **33**: 106-115 [PMID: 10905593 DOI: 10.1016/S0168-8278(00)80166-X]
261 **Adinolfi LE**, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; **33**: 1358-1364 [PMID: 11391523 DOI: 10.1053/jhep.2001.24432]

262 **Ratziu V**, Trabut JB, Poynard T. Fat, diabetes, and liver injury in chronic hepatitis C. *Curr Gastroenterol Rep* 2004; **6**: 22-29 [PMID: 14720450 DOI: 10.1007/s11894-004-0022-5]

263 **Tsochatzis E**, Papatheodoridis GV, Manesis EK, Chrysanthos N, Kafiri G, Petraki K, Hadziyannis E, Pandelidaki H, Zafiropoulou R, Savvas S, Koskinas J, Archimandritis AJ. Hepatic steatosis in genotype 4 chronic hepatitis C is mainly because of metabolic factors. *Am J Gastroenterol* 2007; **102**: 634-641 [PMID: 17222326 DOI: 10.1111/j.1572-0241.2006.01025.x]

264 **Crook ED**, Penumalee S, Gavini B, Filippova K. Hepatitis C is a predictor of poorer renal survival in diabetic patients. *Diabetes Care* 2005; **28**: 2187-2191 [PMID: 16123488 DOI: 10.2337/diacare.28.9.2187]

265 **Pattullo V**, Heathcote J. Hepatitis C and diabetes: one treatment for two diseases? *Liver Int* 2010; **30**: 356-364 [PMID: 20040049 DOI: 10.1111/j.1478-3231.2009.02185.x]

266 **Wang CS**, Yao WJ, Chang TT, Wang ST, Chou P. The impact of type 2 diabetes on the development of hepatocellular carcinoma in different viral hepatitis statuses. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2054-2060 [PMID: 19549812 DOI: 10.1158/1055-9965.EPI-08-1131]

267 **Kita Y**, Mizukoshi E, Takamura T, Sakurai M, Takata Y, Arai K, Yamashita T, Nakamoto Y, Kaneko S. Impact of diabetes mellitus on prognosis of patients infected with hepatitis C virus. *Metabolism* 2007; **56**: 1682-1688 [PMID: 17998021 DOI: 10.1016/j.metabol.2007.07.011]

268 **Petta S**, Cammà C, Di Marco V, Alessi N, Cabibi D, Caldarella R, Licata A, Massenti F, Tarantino G, Marchesini G, Craxì A. Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. *Am J Gastroenterol* 2008; **103**: 1136-1144 [PMID: 18477344 DOI: 10.1111/j.1572-0241.2008.01813.x]

269 **Bressler BL**, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; **38**: 639-644 [PMID: 12939590 DOI: 10.1053/jhep.2003.50350]

270 **Elgouhari HM**, Zein CO, Hanouneh I, Feldstein AE, Zein NN. Diabetes mellitus is associated with impaired response to antiviral therapy in chronic hepatitis C infection. *Dig Dis Sci* 2009; **54**: 2699-2705 [PMID: 19148751 DOI: 10.1007/s10620-008-0683-2]

271 **Konishi I**, Horiike N, Hiasa Y, Tokumoto Y, Mashiba T, Michitaka K, Miyake Y, Nonaka S, Joukou K, Matsuura B, Onji M. Diabetes mellitus reduces the therapeutic effectiveness of interferon-alpha2b plus ribavirin therapy in patients with chronic hepatitis C. *Hepatol Res* 2007; **37**: 331-336 [PMID: 17441805]

272 **Walsh MJ**, Jonsson JR, Richardson MM, Lipka GM, Purdie DM, Clouston AD, Powell EE. Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signalling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1. *Gut* 2006; **55**: 529-535 [PMID: 16299039 DOI: 10.1136/gut.2005.069674]

273 **Sanyal AJ**. Role of insulin resistance and hepatic steatosis in the progression of fibrosis and response to treatment in hepatitis C. *Liver Int* 2011; **31** Suppl 1: 23-28 [PMID: 21205134 DOI: 10.1111/j.1478-3231.2010.02397.x]

274 **Gunton JE**, Delhanty PJ, Takahashi S, Baxter RC. Metformin rapidly increases insulin receptor activation in human liver and signals preferentially through insulin-receptor substrate-2. *J Clin Endocrinol Metab* 2003; **88**: 1323-1332 [PMID: 12629126 DOI: 10.1210/jc.2002-021394]

275 **Knowler WC**, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403 [PMID: 11832527]

276 **Shaw RJ**, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, Montminy M, Cantley LC. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* 2005; **310**: 1642-1646 [PMID: 16308421 DOI: 10.1126/science.1120781]

277 **Ikeda M**, Abe K, Yamada M, Dansako H, Naka K, Kato N. Different anti-HCV profiles of statins and their potential for combination therapy with interferon. *Hepatology* 2006; **44**: 117-125 [PMID: 16799963 DOI: 10.1002/hep.21232]

278 **Kapadia SB**, Chisari FV. Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. *Proc Natl Acad Sci USA* 2005; **102**: 2561-2566 [PMID: 15699349 DOI: 10.1073/pnas.0409834102]

279 **Ye J**, Wang C, Sumpter R, Brown MS, Goldstein JL, Gale M. Disruption of hepatitis C virus RNA replication through inhibition of host protein geranylgeranylation. *Proc Natl Acad Sci USA* 2003; **100**: 15865-15870 [PMID: 14668447 DOI: 10.1073/pnas.2237238100]

280 **O'Leary JG**, Chan JL, McMahon CM, Chung RT. Atorvastatin does not exhibit antiviral activity against HCV at conventional doses: a pilot clinical trial. *Hepatology* 2007; **45**: 895-898 [PMID: 17393518]

P- Reviewer: Dashora U **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.601

World J Diabetes 2014 October 15; 5(5): 601-605
ISSN 1948-9358 (online)

TOPIC HIGHLIGHT

WJD 5th Anniversary Special Issues (3): Type 1 diabetes

Prognostic value of endothelial dysfunction in type 1 diabetes mellitus

Ana Marice Ladeia, Raphael Ribeiro Sampaio, Maiara Costa Hita, Luis F Adan

Ana Marice Ladeia, Raphael Ribeiro Sampaio, Maiara Costa Hita, Bahiana School of Medicine and Public Health, Bahia Foundation for the Development of Sciences, FBDC, Salvador, Bahia 40.285-001, Brazil
Luis F Adan, Department of Pediatrics, Federal University of Bahia School of Medicine, Salvador, Bahia 40.026-010, Brazil
Author contributions: Ladeia AM conceived the manuscript, acquired and interpreted the data and drafted the article; Sampaio RR and Hita MC participated in the acquisition and interpretation of data; Adan LF made the final critical review of the manuscript.
Correspondence to: Ana Marice Ladeia, MD, PhD, Bahiana School of Medicine and Public Health, Bahia Foundation for the Development of Sciences, FBDC, Avenida D. João VI 275, Salvador, Bahia 40.285-001, Brazil. anamarice@bahiana.edu.br
Telephone: +55-71-32768265
Received: June 3, 2014 Revised: June 30, 2014
Accepted: July 18, 2014
Published online: October 15, 2014

Abstract

Patients with diabetes mellitus are at high risk of developing atherosclerosis, associated with higher rates of micro and macro vascular involvement such as coronary artery disease and renal disease. The role of hyperglycemia to induce synthesis of reactive oxygen species by the oxidation of glucose, leading to an increased production of advanced glycosylation end products, as well as inflammation and oxidative stress has been proposed as a possible mechanism in the pathogenesis of endothelial dysfunction (ED). The interaction between C-peptide - the connecting segment of pro-insulin-and nitric oxide in vasodilation is also discussed. Therefore, endothelial dysfunction has been identified as an early marker of vascular disorder in type 1 and type 2 diabetes mellitus. In some other diseases, ED has been considered an independent predictor of vascular disease, regardless of the method used. Studies have demonstrated the importance of endothelial dysfunction as an useful tool for identifying the risk of vascular complications in patients with type 1 diabetes mellitus, particularly as regards to renal impairment. The aim of this review is to clarify the prognostic value of endothelial dysfunction as a marker of vascular disease in these subjects.

Key words: Endothelial dysfunction; Type1 diabetes; Prognostic; Cardiovascular disease; Pathogenesis

Core tip: This review is divided into two parts: first we discuss aspects related to the pathogenesis of endothelial dysfunction in type 1 diabetes mellitus. In the second, are pointed out and critically discussed the scientific evidence about the important role of endothelial dysfunction, independent of the method used for its diagnosis, as an early marker of cardiovascular and renal complications in this population.

Ladeia AM, Sampaio RR, Hita MC, Adan LF. Prognostic value of endothelial dysfunction in type 1 diabetes mellitus. *World J Diabetes* 2014; 5(5): 601-605 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/601.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.601

INTRODUCTION

Diabetes mellitus patients have a high risk to develop atherosclerotic disease[1]. The macro- and microvascular complications are the main cause of morbidity and mortality, especially in those with more than five years of disease[2-4]. Endothelial dysfunction (ED) has been identified as an early marker of vascular disease in type 1 diabetes mellitus (T1DM)[5]. In some other conditions, ED has been an independent predictor of cardiovascular risk[6].

This review aims to evaluate the endothelial dysfunc-

tion role as a prognostic factor of vascular complication in patients with T1DM.

PATHOGENESIS OF ENDOTHELIAL DYSFUNCTION IN T1DM

The role of vascular endothelium on the pathogenesis of vascular disease has been better known in the last 30 years. Adequate endothelial function depends on the healthy balance between vasoconstrictor and vasodilator substances that interact in the endovascular environment[7,8]. Nitric oxide (NO), identified by Furchgott *et al*[9], is synthetized from L-arginine by nitric oxide synthase (eNOS) in the presence of oxygen, nicotinamide adenine dinucleotide phosphate and BH4 (tetrahydrobiopterin). This substance produced on endothelial cells diffuses itself into smooth muscle cells and platelets, where it stimulates the activity of the soluble guanylate cyclase and hence production of cyclic GMP promoting, in turn, relaxation of the muscle layer of the vessel and reduces platelet aggregation. On the other hand, NO reduction is associated with increased vascular injury, because it enhances platelet aggregation and increases monocyte adhesion to vascular endothelium; as well it stimulates proliferation of smooth muscle cells[10].

In pathologic situations, as diabetes, numerous mechanisms as: (1) decreased synthesis or inactivation of NO; and (2) increased production and release of vasoconstrictor substances have been proposed to explain the ED. In addition, metabolic changes favoring increased production of free radicals as well as advanced glycosylation end products (AGEs) are able to accelerate the nitric oxide inactivation[10].

Considering that the major metabolic disturbance in diabetes is hyperglycemia, it has been suggested that it may induce the synthesis of reactive oxigen species, by the oxidation of glucose[11], leading to an increased production of AGEs[12], among other mechanisms. On the other hand, a recent study demonstrates that hypoglycemia is also associated with ED, oxidative stress and inflammation. Moreover, worsening of endothelial function was greater in those who went from hypoglycemia to hyperglycemia than those recovered to a state of normoglycemia[13].

Other substances involved in the pathogenesis of endothelial dysfunction in T1DM are insulin and C-peptide. Several studies have shown that the vasodilator effects of insulin depends on the synthesis of nitric oxide, since the use of substances that block eNOS, inhibits the increase of blood flow mediated through the action of insulin[14-16]. Moreover, acute administration of C-peptide-a connecting segment of pro-insulin-is able to increase blood flow in subjects with T1DM after exercise or at rest, but not in normal subjects[17,18]. As well, a prolonged infusion of C-peptide in type 1 diabetic subjects improves kidney function[19] by a mechanism that involves the interaction between nitric oxide activity, and Na^+K^+ATPase[20,21]. So, it is important to understand that the pathogenesis of endothelial dysfunction in T1DM is complex and involves metabolic and hormonal changes; in particular, the role of insulin deficiency that leads to a decreased production of nitric oxide, increased oxidative stress in the vascular milieu with consequent decreased in the ability to promote vessels dilation. Furthermore, it is suggested that a better control of metabolic changes by insulin replacement can decrease the aggression of endothelial cells.

Other aspects of the pathogenesis of vascular abnormalities in diabetic subjects deserve attention. T1DM and T2DM are associated with a reduction in the number of endothelial progenitor cells (EPCs)[22-24]. It is interesting to note that this reduction is related to the severity of peripheral vascular disease which reinforces the importance of EPCs as a marker of vasculopathy in diabetic patients[25]. Moreover, potent vasoconstrictor such as angiotensin II and endothelin promote endothelial dysfunction in the metabolically altered environment of diabetes[26]. This knowledge is relevant since it may allow the emergence of new therapeutic perspectives. It is noteworthy that it has already been demonstrated that oral treatment with bosentan, endothelin receptor antagonist, for 4 wk, improves endothelial function in T2DM[27].

PROGNOSTIC VALUE OF ENDOTHELIAL DYSFUNCTION

The literature clearly suggests that metabolic and hormonal disorders present in T1DM injure the endothelial cells favoring endothelial dysfunction and initiation of the atherogenic process. A longitudinal study published recently suggests that flow-mediated vasodilation is an useful tool to stratify T1DM children according to cardiovascular risk, as well as for the long term follow-up[28]. However, the prognostic value of endothelial dysfunction as a marker of vascular complications should be further analyzed.

A 10-year follow-up prospective cohort study involving young T1DM adults with a mean disease duration of 19 years, evaluated the ability of adhesion molecules in predicting coronary artery disease (CAD) defined by angina, confirmed myocardial infarction, stenosis > 50%, ischemic electrocardiogram, or revascularization. With this purpose, a nested case-control study involving 60 patients who developed CAD and 72 patients without the disease was performed. Dosages of vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 and E-selectin were performed from stored samples prior to the cardiovascular event. Although there was a correlation between adhesion molecules and lipid variables, considered an unquestionable cardiovascular risk factor in type 1 diabetes, only E-selectin was an independent predictor of CAD (HR = 1.07, 95%CI: 1.01-1.15, $P < 0.03$)[29].

Another cross-sectional study that included patients with T1DM without cardiovascular disease and a comparison group of healthy subjects, sought to identify association between endothelial dysfunction [flow-mediated vasodilation (FMD)] and subclinical cardiovascular dis-

ease. It was then observed a strong inverse correlation between FMD and systolic dysfunction (r = -0.70, P < 0.0001), diastolic dysfunction (r = -0.77) and duration of T1DM (r = -0.61,) P < 0.0001 for the three variables[30]. The association between ED and other markers of subclinical CAD, as the carotid intima-media thickness (IMT), was evaluated in a study that included T1DM patients and non-diabetic children-without significant differences in weight, age, blood pressure and gender. This study demonstrated that the T1DM group had lower peaks of FMD response and higher IMT when compared to controls (P < 0.001). Moreover, in the multivariate analysis, there was a strong association between increased IMT and decreased FMD in the group of children with diabetes (P < 0.03). However, the data in the literature are still conflicting. A study involving patients with T1DM and healthy subjects showed no difference between the IMT of the two groups, although endothelial function had been worse in T1DM group and correlated with glycemic control[31].

On the other hand, a recent study that evaluated endothelial function, IMT and ventricular function in 30 children and adolescents with T1DM compared with 30 healthy subjects matched by gender, age, and body mass index, found a lower FMD response, increased IMT and impaired diastolic function with lower early peak flow velocity, decreased E/A ratio, increased early filling deceleration time in T1DM patients. Furthermore, these changes were more evident in patients with poor glycemic control[32].

Several studies have shown the importance of endothelial dysfunction as a marker of renal impairment. In 2005, we demonstrated that FMD had an inverse correlation with microalbuminuria (r = -0.50, P = 0.049) in children and adolescents with a short duration diabetes (2.9 + 1.2 years) calling attention to the value of the endothelial dysfunction as a very early marker of vascular complications[4]. This association was also demonstrated in patients with disease duration > 10 years, with the following features: individuals with proteinuria and chronic renal failure (CRF) had FMD 7% and 4% respectively, while those with normal albumin excretion or microalbuminuria showed FMD > 8%, considered the lower limit of normality for flow-mediated vasodilation in adults[33]. In this study, there was a continuous, progressive and significant increase in the levels of endothelin-1 and C-reactive protein in individuals (1) without microalbuminuria; (2) with microalbuminuria; (3) with proteinuria; and (4) CRF. In addition, the sensitivity coefficient to shear stress endothelium was inversely correlated with glomerular filtration rate (GFR) (r = -0.48, P = 0.03). This aspect can be somewhat reinforced by another recent study that demonstrated that pulse pressure was associated with a decline in estimated GFR (r = 0.26, P = 0.003, adjusted), as well as the higher pressure pulse predicted an increased risk to develop end-stage renal disease: adjusted HR of 1.2 (95%CI: 1.1-1.4, P = 0.011)[34]. In addition, a cohort study of 18 T1DM patients followed for 8 years has shown an association between the expansion of the cortical interstitial volume fraction and PA1-activity and VCAM levels[35].

It is worth noting that changes in endothelial function can be identified regardless of the method used. A sustained hyperaemic stimulation induced by the hand skin heating method, as well as FMD vasodilation, were used to evaluate endothelial dysfunction in T1DM patients with and without microangiopathy and also in healthy controls matched for gender, age and body mass index. It was observed that FMD was lower in the diabetes group compared to controls. Furthermore, the presence of clinical complications was significantly associated with lower FMD and creatinine levels were also negatively correlated with the magnitude of FMD. With regard to the hand skin heating method, it was shown that the radial flow shear stress increased vascular diameter in all groups, however, the amplitude of FMD in diabetic patients were significantly lower than in the control group[36]. This dataset demonstrate the importance of endothelial aggression factors as potential markers of vascular injury.

More recently, longitudinal studies have sought to identify markers of endothelial dysfunction as predictors of long-term cardiovascular events. In a prospective study, T1DM patients with persistent normoalbuminuria and nephropathy, without previous cardiovascular events, were followed for a mean period of 12.3 years. The plasma levels of soluble receptor for advanced glycation end products (sRAGE) and other biomarkers were measured at baseline. High levels of sRAGE as a reflection of RAGE expression, was associated with greater incidence of fatal or nonfatal cardiovascular disease, as well as all-cause mortality. Furthermore, there was a significant association between levels of sRAGE and GRF in patients with nephropathy[37]. These authors, in a prospective study with a similar sample, showed that higher plasma levels of the pro-inflammatory cytokine high -mobility group box 1 was an independent predictor of fatal and non-fatal cardiovascular events and also a high-risk marker for all causes of mortality[38].

According to a recently published review, the mechanisms of endothelial dysfunction and ischemic response in diabetes mellitus is complex, involving inflammation, intercellular signaling peptides and proteins, cell angiogenic potential, among others[39]. It is noteworthy that a prospective study demonstrated a decrease of EPCs in children with T1DM, as well as the association between better glycemic control and increased EPCs after an one-year follow-up, suggesting that knowledge of this mechanism may be a way of mediating the high cardiovascular risk in these patients[40]. Therefore, more knowledge on the balance between vascular homeostasis and cardiometabolic risk factors will certainly improve the monitoring of diabetic patients and reduce vascular complications and consequently morbidity.

CONCLUSION

In summary, the pathogenesis of endothelial dysfunction

in T1DM is complex and involves several mechanisms such as inflammation, oxidative stress, interaction between insulin and C peptide, decreased number of endothelial progenitor cells among others. The prognostic value of assessing endothelial function as a marker of cardiovascular morbidity and risk has been demonstrated by cross-sectional and prospective studies with long follow-up, using various methods to identify subclinical atherosclerosis and endothelial dysfunction. The dataset demonstrate that regardless of the method used, impairment of endothelial function is a predictor of risk for cardiovascular disease and nephropathy. This knowledge suggests that new preventive and therapeutic interventions should be recommended early in order to decrease morbidity in this high-risk population.

REFERENCES

1 **Stehouwer CD**, Nauta JJ, Zeldenrust GC, Hackeng WH, Donker AJ, den Ottolander GJ. Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *Lancet* 1992; **340**: 319-323 [PMID: 1353802 DOI: 10.1016/0140-6736(92)91401-S]

2 **Morrish NJ**, Stevens LK, Fuller JH, Keen H, Jarrett RJ. Incidence of macrovascular disease in diabetes mellitus: the London cohort of the WHO Multinational Study of Vascular Disease in Diabetics. *Diabetologia* 1991; **34**: 584-589 [PMID: 1936662 DOI: 10.1007/BF00400278]

3 **Laing SP**, Swerdlow AJ, Slater SD, Botha JL, Burden AC, Waugh NR, Smith AW, Hill RD, Bingley PJ, Patterson CC, Qiao Z, Keen H. The British Diabetic Association Cohort Study, II: cause-specific mortality in patients with insulin-treated diabetes mellitus. *Diabet Med* 1999; **16**: 466-471 [PMID: 10391393 DOI: 10.1046/j.1464-5491.1999.00076.x]

4 **Ladeia AM**, Ladeia-Frota C, Pinho L, Stefanelli E, Adan L. Endothelial dysfunction is correlated with microalbuminuria in children with short-duration type 1 diabetes. *Diabetes Care* 2005; **28**: 2048-2050 [PMID: 16043758 DOI: 10.2337/diacare.28.8.2048]

5 **Järvisalo MJ**, Raitakari M, Toikka JO, Putto-Laurila A, Rontu R, Laine S, Lehtimäki T, Rönnemaa T, Viikari J, Raitakari OT. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. *Circulation* 2004; **109**: 1750-1755 [PMID: 15023875 DOI: 10.1161/01.CIR.0000124725.46165.2C]

6 **Juonala M**, Viikari JS, Laitinen T, Marniemi J, Helenius H, Rönnemaa T, Raitakari OT. Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study. *Circulation* 2004; **110**: 2918-2923 [PMID: 15505080 DOI: 10.1161/01.CIR.0000147540.88559.00]

7 **Kuo L**, Davis MJ, Chilian WM. Myogenic activity in isolated subepicardial and subendocardial coronary arterioles. *Am J Physiol* 1988; **255**: H1558-H1562 [PMID: 2462367]

8 **Furchgott RF**, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. *FASEB J* 1989; **3**: 2007-2018 [PMID: 2545495]

9 **Furchgott RF**, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; **288**: 373-376 [PMID: 6253831 DOI: 10.1038/288373a0]

10 **Johnstone MT**, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993; **88**: 2510-2516 [PMID: 8080489 DOI: 10.1161/01.CIR.88.6.2510]

11 **Wolff SP**, Dean RT. Glucose autoxidation and protein modification. The potential role of 'autoxidative glycosylation' in diabetes. *Biochem J* 1987; **245**: 243-250 [PMID: 3117042]

12 **Yan SD**, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 1994; **269**: 9889-9897 [PMID: 8144582]

13 **Ceriello A**, Novials A, Ortega E, La Sala L, Pujadas G, Testa R, Bonfigli AR, Esposito K, Giugliano D. Evidence that hyperglycemia after recovery from hypoglycemia worsens endothelial function and increases oxidative stress and inflammation in healthy control subjects and subjects with type 1 diabetes. *Diabetes* 2012; **61**: 2993-2997 [PMID: 22891214 DOI: 10.2337/db12-0224]

14 **Baron AD**. Hemodynamic actions of insulin. *Am J Physiol* 1994; **267**: E187-E202 [PMID: 8074198]

15 **Steinberg HO**, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J Clin Invest* 1994; **94**: 1172-1179 [PMID: 8083357]

16 **Scherrer U**, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 1994; **94**: 2511-2515 [PMID: 7989610 DOI: 10.1172/JCI117621]

17 **Johansson BL**, Pernow J. C-peptide potentiates the vasoconstrictor effect of neuropeptide Y in insulin-dependent diabetic patients. *Acta Physiol Scand* 1999; **165**: 39-44 [PMID: 10072095 DOI: 10.1046/j.1365-201x.1999.00475.x]

18 **Johansson BL**, Linde B, Wahren J. Effects of C-peptide on blood flow, capillary diffusion capacity and glucose utilization in the exercising forearm of type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1992; **35**: 1151-1158 [PMID: 1478367 DOI: 10.1007/BF00401369]

19 **Johansson BL**, Borg K, Fernqvist-Forbes E, Kernell A, Odergren T, Wahren J. Beneficial effects of C-peptide on incipient nephropathy and neuropathy in patients with Type 1 diabetes mellitus. *Diabet Med* 2000; **17**: 181-189 [PMID: 10784221 DOI: 10.1046/j.1464-5491.2000.00274.x]

20 **Wahren J**, Ekberg K, Johansson J, Henriksson M, Pramanik A, Johansson BL, Rigler R, Jörnvall H. Role of C-peptide in human physiology. *Am J Physiol Endocrinol Metab* 2000; **278**: E759-E768 [PMID: 10780930]

21 **Forst T**, Kunt T. Effects of C-peptide on microvascular blood flow and blood hemorheology. *Exp Diabesity Res* 2004; **5**: 51-64 [PMID: 15198371 DOI: 10.1080/15438600490424532]

22 **Tepper OM**, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 2002; **106**: 2781-2786 [PMID: 12451003 DOI: 10.1161/01.CIR.0000039526.42991.93]

23 **Loomans CJ**, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes* 2004; **53**: 195-199 [PMID: 14693715 DOI: 10.2337/diabetes.53.1.195]

24 **Rosso A**, Balsamo A, Gambino R, Dentelli P, Falcioni R, Cassader M, Pegoraro L, Pagano G, Brizzi MF. p53 Mediates the accelerated onset of senescence of endothelial progenitor cells in diabetes. *J Biol Chem* 2006; **281**: 4339-4347 [PMID: 16339764]

25 **Roberts AC**, Porter KE. Cellular and molecular mechanisms of endothelial dysfunction in diabetes. *Diab Vasc Dis Res* 2013; **10**: 472-482 [PMID: 24002671 DOI: 10.1177/1479164113500680]

26 **Tousoulis D**, Papageorgiou N, Androulakis E, Siasos G, Latsios G, Tentolouris K, Stefanadis C. Diabetes mellitus-associated vascular impairment: novel circulating biomarkers and therapeutic approaches. *J Am Coll Cardiol* 2013; **62**:

667-676 [PMID: 23948511 DOI: 10.1016/j.jacc.2013.03.089]
27 **Rafnsson A**, Böhm F, Settergren M, Gonon A, Brismar K, Pernow J. The endothelin receptor antagonist bosentan improves peripheral endothelial function in patients with type 2 diabetes mellitus and microalbuminuria: a randomised trial. *Diabetologia* 2012; **55**: 600-607 [PMID: 22200728 DOI: 10.1007/s00125-011-2415-y]
28 **Bruzzi P**, Predieri B, Patianna VD, Salvini A, Rossi R, Modena MG, Iughetti L. Longitudinal evaluation of endothelial function in children and adolescents with type 1 diabetes mellitus: a long-term follow-up study. *Pediatr Int* 2014; **56**: 188-195 [PMID: 24020908 DOI: 10.1111/ped.12220]
29 **Yishak AA**, Costacou T, Virella G, Zgibor J, Fried L, Walsh M, Evans RW, Lopes-Virella M, Kagan VE, Otvos J, Orchard TJ. Novel predictors of overt nephropathy in subjects with type 1 diabetes. A nested case control study from the Pittsburgh Epidemiology of Diabetes Complications cohort. *Nephrol Dial Transplant* 2006; **21**: 93-100 [PMID: 16144851]
30 **Shivalkar B**, Dhondt D, Goovaerts I, Van Gaal L, Bartunek J, Van Crombrugge P, Vrints C. Flow mediated dilatation and cardiac function in type 1 diabetes mellitus. *Am J Cardiol* 2006; **97**: 77-82 [PMID: 16377288]
31 **Hurks R**, Eisinger MJ, Goovaerts I, van Gaal L, Vrints C, Weyler J, Hendriks J, van Schil P, Lauwers P. Early endothelial dysfunction in young type 1 diabetics. *Eur J Vasc Endovasc Surg* 2009; **37**: 611-615 [PMID: 19297215 DOI: 10.1016/j.ejvs.2009.01.015]
32 **Eltayeb AA**, Ahmad FA, Sayed DM, Osama AM. Subclinical vascular endothelial dysfunctions and myocardial changes with type 1 diabetes mellitus in children and adolescents. *Pediatr Cardiol* 2014; **35**: 965-974 [PMID: 24595824]
33 **Shestakova MV**, Jarek-Martynowa IR, Ivanishina NS, Kuharenko SS, Yadrihinskaya MN, Aleksandrov AA, Dedov II. Role of endothelial dysfunction in the development of cardiorenal syndrome in patients with type 1 diabetes mellitus. *Diabetes Res Clin Pract* 2005; **68** Suppl1: S65-S72 [PMID: 15955379]
34 **Theilade S**, Lajer M, Jorsal A, Tarnow L, Parving HH, Rossing P. Arterial stiffness and endothelial dysfunction independently and synergistically predict cardiovascular and renal outcome in patients with type 1 diabetes. *Diabet Med* 2012; **29**: 990-994 [PMID: 22414297 DOI: 10.1111/j.1464-5491.2012.03633.x]
35 **Bangstad HJ**, Seljeflot I, Berg TJ, Hanssen KF. Renal tubulointerstitial expansion is associated with endothelial dysfunction and inflammation in type 1 diabetes. *Scand J Clin Lab Invest* 2009; **69**: 138-144 [PMID: 18846477 DOI: 10.1080/00365510802444080]
36 **Bellien J**, Costentin A, Dutheil-Maillochaud B, Iacob M, Kuhn JM, Thuillez C, Joannides R. Early stage detection of conduit artery endothelial dysfunction in patients with type 1 diabetes. *Diab Vasc Dis Res* 2010; **7**: 158-166 [PMID: 20382780 DOI: 10.1177/1479164109360470]
37 **Nin JW**, Jorsal A, Ferreira I, Schalkwijk CG, Prins MH, Parving HH, Tarnow L, Rossing P, Stehouwer CD. Higher plasma soluble Receptor for Advanced Glycation End Products (sRAGE) levels are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12-year follow-up study. *Diabetes* 2010; **59**: 2027-2032 [PMID: 20522598 DOI: 10.2337/db09-1509]
38 **Nin JW**, Ferreira I, Schalkwijk CG, Jorsal A, Prins MH, Parving HH, Tarnow L, Rossing P, Stehouwer CD. Higher plasma high-mobility group box 1 levels are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12 year follow-up study. *Diabetologia* 2012; **55**: 2489-2493 [PMID: 22752054 DOI: 10.1007/s00125-012-2622-1]
39 **Howangyin KY**, Silvestre JS. Diabetes mellitus and ischemic diseases: molecular mechanisms of vascular repair dysfunction. *Arterioscler Thromb Vasc Biol* 2014; **34**: 1126-1135 [PMID: 24675660 DOI: 10.1161/ATVBAHA.114.303090]
40 **Hörtenhuber T**, Rami-Mehar B, Satler M, Nagl K, Höbaus C, Höllerl F, Koppensteiner R, Schernthaner G, Schober E, Schernthaner GH. Endothelial progenitor cells are related to glycemic control in children with type 1 diabetes over time. *Diabetes Care* 2013; **36**: 1647-1653 [PMID: 23340890 DOI: 10.2337/dc12-1206]

P- Reviewer: Cosmi E, Okumura K **S- Editor**: Song XX
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.606

World J Diabetes 2014 October 15; 5(5): 606-629
ISSN 1948-9358 (online)

REVIEW

Counterregulation of insulin by leptin as key component of autonomic regulation of body weight

Katarina T Borer

Katarina T Borer, School of Kinesiology, The University of Michigan, Ann Arbor, MI 48109, United States
Author contributions: Borer KT solely contributed to this paper.
Correspondence to: Katarina T Borer, PhD, Professor, School of Kinesiology, The University of Michigan, 401 Washtenaw Avenue, Ann Arbor, MI 48109, United States. katarina@umich.edu
Telephone: +1-734-6472703 Fax: +1-734-9361925
Received: December 7, 2013 Revised: May 15, 2014
Accepted: May 31, 2014
Published online: October 15, 2014

Abstract

A re-examination of the mechanism controlling eating, locomotion, and metabolism prompts formulation of a new explanatory model containing five features: a coordinating joint role of the (1) autonomic nervous system (ANS); (2) the suprachiasmatic (SCN) master clock in counterbalancing parasympathetic digestive and absorptive functions and feeding with sympathetic locomotor and thermogenic energy expenditure within a circadian framework; (3) interaction of the ANS/SCN command with brain substrates of reward encompassing dopaminergic projections to ventral striatum and limbic and cortical forebrain. These drive the nonhomeostatic feeding and locomotor motivated behaviors in interaction with circulating ghrelin and lateral hypothalamic neurons signaling through melanin concentrating hormone and orexin-hypocretin peptides; (4) counterregulation of insulin by leptin of both gastric and adipose tissue origin through: potentiation by leptin of cholecystokinin-mediated satiation, inhibition of insulin secretion, suppression of insulin lipogenesis by leptin lipolysis, and modulation of peripheral tissue and brain sensitivity to insulin action. Thus weight-loss induced hypoleptimia raises insulin sensitivity and promotes its parasympathetic anabolic actions while obesity-induced hyperleptinemia supresses insulin lipogenic action; and (5) inhibition by leptin of bone mineral accrual suggesting that leptin may contribute to the maintenance of stability of skeletal, lean-body, as well as adipose tissue masses.

Key words: Insulin; Leptin; Weight regulation; Autonomic; Circadian

Core tip: The novel proposal for the mechanism of body weight regulation deals with all three components of body mass: bone, lean tissue, and fat depots. It attributes the central control of counterbalancing energy expenditure and intake to an autonomic nervous system-circadian clock command center that encompases brain reward substrates, lateral hypothalamic peptidergic circuits and areas of the cortex. The nonhomeostatic character of feeding and locomotion is driven and controlled by the reward circuits and modulated by shifts in insulin sensitivity induced by counterregulation by leptin of insulin as weight deviates between underweight and overweight and alters basal leptin concentrations.

Borer KT. Counterregulation of insulin by leptin as key component of autonomic regulation of body weight. *World J Diabetes* 2014; 5(5): 606-629 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/606.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.606

INTRODUCTION

Finding and ingesting food and drink are intermittent behaviors essential for individual and species survival against continuous energy cost of staying alive. Our complex physiological design insures that opportunities to ingest food are not missed and that drive to seek food increases and compensatory processes are deployed to counteract substantial losses of body mass. That this behavior supports both growth of body mass as well as its

maintenance when statural growth has ceased only adds to its complexity and challenges our ability to understand its mechanism. Therefore, the transformation from a system in which food abundance drives the acquisition of body mass during statural growth to a system where energy intake is matched to each finite adult physique requires an explanation that integrates both phenomena. In addition, feeding behavior is coupled to spontaneous variations in movement and locomotion in ways that are imperfectly understood, and the two behaviors and control of metabolic heat production also contribute to regulation of body mass. A satisfactory model for the regulation of stable adult body mass must integrate central neural, autonomic, and endocrine controls of feeding, locomotion, and metabolic heat production. But it also needs to account for the prospect that some humans[1,2] and animals[3] can deviate from body mass stability and predictably become obese[1,2] under conditions providing abundant foods of high energy density and palatability along with limited opportunities and incentives for physical activity.

The quest for understanding what guides intermittent meal-to-meal eating and body mass maintainance as well as increased hunger and food intake responding to substantial losses of body mass, has a long history but no satisfactory closure or consensus. Because of its complexity, and relevance to professionals in disconnected fields of psychology, nutrition, gastrointestinal physiology, endocrinology, exercise science, neuroscience, and physiology among others, the wealth of information about the neural, autonomic, and hormonal mechanism of feeding, physical activity, and thermogenesis in body mass regulation has not been satisfactorily integrated. A prevailing preference for a unitary deductive model of body mass regulation has placed emphasis on the presumed metering and matching of energy consumed to energy expended and to the energy content of body fat mass under both ad libitum and underweight conditions[4-9]. The core feature of this model is operation of a negative feedback exerted by adipokine leptin (and in some variations of the hypothesis, also by insulin) over feeding behavior and energy expenditure in response to changes in body fat mass. This widely accepted hypothesis is not supported by the empirical data under conditions of intact neuroendocrine system, environmental abundance of food, reduced opportunities for physical exertion, and rising levels of body fat. Obesity coexists with high basal concentrations of leptin and insulin. Further, administration of leptin to obese humans is ineffective in suppressing feeding and reducing the body or fat mass[10]. On the other hand, two robust findings regarding leptin actions on feeding and body energy status need to be reconciled with its inability to reduce body fat mass in a negative feedback fashion in neurologically normal obese individuals under the ad-libitum feeding conditions. The first finding is a consistent proportional relationship between distributed body fat mass and basal leptin (and insulin) concentrations in humans and animals first clearly demonstrated in humans by Considine[11] and postulated to exert sustained inhibition over feeding and facilitation of energy expenditure[4-9]. The second finding is capacity of leptin to inhibit pronounced and consistently high hunger and suppress high fat mass in freely feeding humans and animals that lack leptin signaling capacity. This was first reported in humans by Farooqi[12,13] and in animals by Pelleymounter[14].

A unitary mechanism of weight regulation that can account for eating and weight changes leading to obesity and in non-deprivation as well as weight-loss conditions needs to account for (1) central neural coordination of this process; (2) interactions of this mechanism with the biological clock in structuring ultradian and nycthemeral rhythms of intermittent hunger and feeding; (3) opportunistic as opposed to homeostatic control of food intake and locomotion; (4) counterregulation by leptin of insulin secretion and actions to fluctuations of short-term energy availability and deviations in body fat mass; and (5) inclusion of skeletal and lean body masses along with the fat mass in the energy regulatory process. The proposed mechanism accounts for these processes in a novel way that differs from the currently prevailing view[4-9]. Its main propositions are that : (1) the autonomic brain centers activate hunger drive in; (2) a circadian pattern suppressed by intermittent inhibition from gastrointestinal (GI) filling and food processing that coordinate anabolic and catabolic processes to produce weight stability; (3) meal-to-meal eating and spontaneous physical activity represent non-homeostatic behaviors motivated through activation of a common brain substrates of reward that are connected to, and controlled by, the autonomic centers and circadian clock and responsive to short-term variations in the filling of the GI tract with food and fluctuations in body fat reserves and body mass; (4) autonomic nervous system (ANS) controls counterregulation by leptin of insulin secretion and tissue sensitivity to insulin actions to yoke leptin's thermogenesis and catabolic metabolism to insulin's anabolic actions; gastric leptin participates in GI processing of ingested nutrients and thus contributes to defining meal size through both anabolic digestive and restrictive satiating effects. It does so in conjunction with leptin of adipose tissue origin to regulate peripheral tissue and ANS/circadian command center sensitivity in response to body fat and body mass deviations from the adult setpoint; and (5) brain defends skeletal and lean body masses along with body fat mass against losses demonstrating that these body components should be integrated along with the adipose tissue in the regulation of adult mammalian body weight. The proposed postulates of this novel formulation of weight regulatory mechanism reconcile the conundrum of central and peripheral resistance to actions of insulin and leptin in obesity that is inherent in the homeostatic negative feedback view and the dichotomy of absence-of-protection model of energy regulation in non-deprivation eating with the central-resistance model of homeostatic leptin negative feedback[15].

CENTRAL COORDINATING ROLE OF THE ANS IN THE CONTROL OF FEEDING

Coordination of parasympathetic functions of nutrient intake, digestion, absorption, storage, and behavioral quiescence with sympathetic control of behavioral and metabolic energy expenditure has been recognized for over half a century. In 1947, Adolph[16] reported that body weight in rats stabilizes and is defended at a given plateau at the end of the growth period when mature rats with unrestricted access to food eat daily an amount of standard lab chow sufficient to maintain a stable weight plateau. Application of various methods of localized brain damage[17] and transsections of neural pathways[18,19] has revealed that ventromedial (VMH) and arcuate (ARC) hypothalamic lesions result in transient hyperphagia and hyperinsulinemia, permanent hypoactivity, and defective postprandial and cold-exposure thermogenesis. This has been interpreted by some to reflect an imbalanced parasympathetic overactivation because insulin oversecretion[20], hyperphagia, deficient thermogenesis[21,22], and spontaneous hypoactivity[23] were preventable by subdiaphragmatic vagotomy. In support of this interpretation, electrical stimulation of ventromedial hypothalamus[24-27] or administration of sympathomimetics[28,29] to neurologically intact animals elicited fuel mobilization and energy expenditure. After the weight in lesioned animals stabilizes, the same amount of food per unit weight is consumed as in intact rats, and the new weight plateau is defended against weight loss[30] indicating that regulation of stable weight is a consequence of balance between parasympathetic and sympathetic actions that is only reset by lesions to a new plateau by damage to the sympathetic controls or pathways. Although the relatively crude methods of brain lesions and neural tract transection initially singled out the VMH in the medial basal hypothalamus as the source of sympathetic actions[31], other lines of evidence identified the paraventricular hypothalamic nucleus (PVN) as the control center of sympathetic outflow and, by inference, dorsal motor nucleus of the vagus as the site of parasympathetic control of visceral actions other than cardiac function. Interest in the role of parasympathetic nervous system in the control of feeding has taken a back seat compared to the focus on leptin actions in the ARC and VMH nuclei. Nevertheless, pharmacological and denervation approaches have shown that suppression of sympathetic tone reduces thermogenesis[32] and increases parasympathetic functions of white adipose tissue (WAT) cell proliferation and body fat accumulation[33].

THE CENTRAL CLOCK COORDINATES ANS CONTROL OF FEEDING

One of the missing pieces in our understanding of energy regulation is the causative stimulus of hunger and meal initiation. The proposition that ghrelin is the key initiator of hunger and feeding[34-37] is challenged by normal food intake and weight maintenance in animals with deficient ghrelin signaling[38] and by a correlational and transient changes in ghrelin concentration and hunger sensations in the course of a meal[34,39]. On the other hand, the proposition that an autonomic controller coordinated by the circadian master clock regulates meal taking, locomotion, and thermogenesis is supported by a wealth of both behavioral, lesioning, and anatomical evidence.

Meal eating is intermittent in contrast to continuous behavioral and metabolic energy expenditure. Its ultradian and circadian patterning is a universal feature of mammalian feeding behavior. Rodents take meals at ultradian intervals of 3 to 4 h with a circadian segregation of eating to only the waking portion of the day[40]. Humans also eat during their nycthemeral wakeful period at 3-h intervals if snacks are included and at about 6-h intervals if more substantive main meals are considered. Circadian control of feeding in mammals is supported by extensive neuroanatomical evidence. Suprachiasmatic nucleus (SCN), the master circadian clock, has multiple ANS interconnections with structures that are implicated in weight regulation. Neural pathways through which the photo-entrainable SCN controls behavioral, endocrine, and metabolic rhythms related to energy balance include direct projections to subparaventricular zone (SPZ), an anterior hypothalamic region that receives innervation from both the PVN and SCN and is therefore thought to integrate circadian and metabolic information[41]. Additional areas receiving SCN innervation include medial preoptic area and dorsomedial hypothalamic nucleus (DMN)[42,43]. DMN, which is innervated both by the SCN and the SPZ, also controls circadian pattern of feeding, sleep-wakefulness, and locomotor activity. SCN also influences the circadian control of food intake, locomotion, and metabolic energy expenditure through its fibers projecting to the ARC, the VMH, and the ventral part of the lateral hypothalamus (LH), all areas implicated in the control of feeding and energy regulation. Interneurons from the SCN inhibit the PVN through γ-aminobutyric acid neurotransmission to facilitate parasympathetic functions. Consequently, most viscera receive SCN-dependent circadian time cues *via* their parasympathetic and/or sympathetic innervations that reflect metabolic and digestive events at peripheral sites[43]. Besides the obligatory periodicity of meal eating, nycthemeral patterning of feeding is necessary for the maintenance of stable body and fat masses. When the nocturnal part of the circadian sleep-wake cycle in humans is truncated, inappropriate overeating during extended wakeful periods ensues contributing to obesity and associated health risk factors[44,45]. Similarly, a seasonal change in the length of circadian exposure to light produces changes in feeding and body fat accumulation in some mammals[46].

Additional evidence for a functional interaction between the circadian clock and the ANS energy regulatory circuits involves loss of feeding, locomotor, and thermogenic periodicities when either the ANS or SCN circuits are disrupted. Destruction of SCN results in the loss of

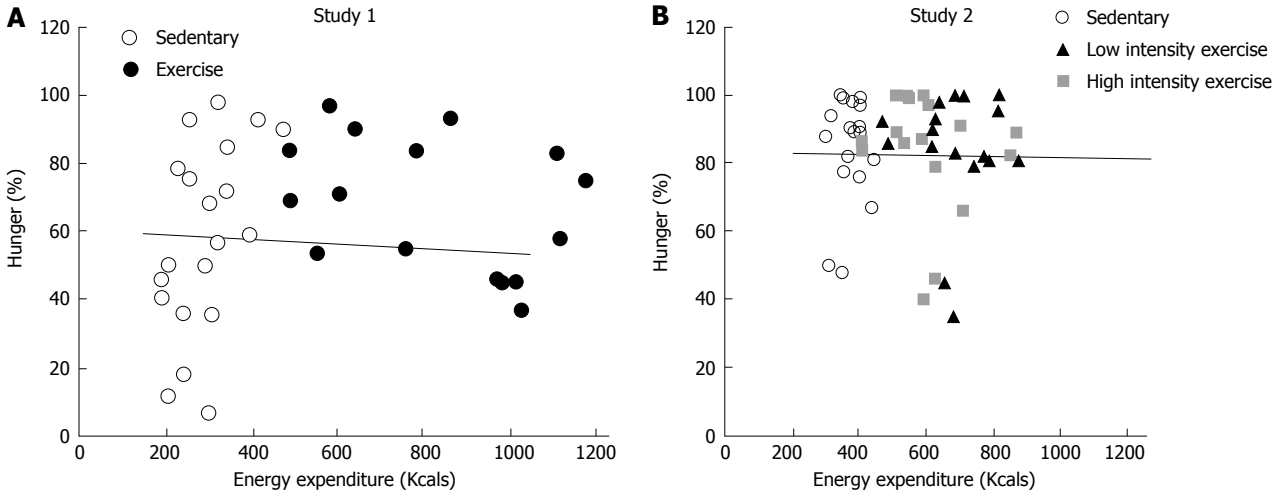

Figure 1 Correlation between energy expenditure and peak hunger in two studies in which exercise energy expenditure of between 2300 and 2500 KJ was inserted between the morning and midday meal (A) or between both morning and midday, and midday and afternoon, meals (B). Correlation coefficient between energy expenditure during overnight fast before the morning meal, and during morning intermeal interval that included exercise on one hand and peak hunger at the next meal was 0.06 in A. In B, the correlation coefficient between intermeal intervals that included exercise and peak hunger at the subsequent meal was 0.0002. Data from Ref. [69].

all bioenergetic circadian responses including circadian pattern of drinking and locomotor activity[47,48]. Destruction of VMH and ARC nuclei within the medial basal hypothalamus disrupts circadian alternation between active and inactive periods of food seeking and eating and results in protracted 24-h extension of meal taking and obesity[49,50]. Postprandial[51] and general thermogenesis also display circadian[52,53] and ultradian[54] rhythms of activation that have an acrophase during the active portion of the day and a nadir during the inactive phase. Metabolic and thermogenic gene expression in brown adipose tissue (BAT) and WAT also follows circadian periodicity[55]. The activation is attributable to stimulation of BAT by sympathetic nerves that originate in PVN, SCN, and DMN[31]. And thermogenesis can be elicited by electrical stimulation of sympathetic nerves to BAT[56], application of sympathomimetics[28,29,57,58] and activation by leptin of sympathetic nerves to BAT[28,29,31,59] when the hormone is applied to DMN, one of key sites involved in circadian components of energy regulation[59]. Leptin itself exhibits a prominent circadian pattern of secretion in humans with an acrophase around midnight and a nadir during mid-day[60-62]. This diurnal pattern is entrained to meal taking and phase shifts by the same number of hours with temporal displacement of meals[62]. In addition to its circadian pattern, leptin secretion is pulsatile with 32 pulses over 24 h, and a mean pulse duration of 33 min[63]. Circadian control of several aspects of energy regulation is seen in circadian changes in postprandial BAT thermogenesis in response to olfactory and gustatory stimulation by hedonic properties of palatable diets. The importance of stimulation of olfactory and gustatory receptors in eliciting postprandial BAT thermogenesis is demonstrated by diet-induced thermogenesis (DIT) attenuation when oral route of food administration is bypassed by tube feeding[64], or by administration of endocannabinoid blocker rimonabant[65,66]. Similarly, overeating palatable diets elicits a greater DIT than does eating diets of lesser olfactory and gustatory appeal[67]. Olfactory responsiveness[68] and hedonic responses to food and associated increases in DIT[67] show a diurnal rhythm with an acrophase during the active portion of the circadian period. The rhythm and the magnitude of thermogenic response are abolished by SCN lesion, sympathetic denervation of BAT[66], or deletion of β_1 receptors in BAT[60]. Endocannabinoid blockade of DIT thermogenesis is more effective during the active that during the inactive phase of the circadian cycle[66].

Circadian influence in human meal eating is evident by comparing the effect of energy expenditure during long nocturnal inter-meal interval (IMI) on morning hunger[69]. We determined that the nocturnal IMI generated expenditure of between 710 and 750 Kcal in healthy postmenopausal women as compared to 340 to 450 Kcal expended during diurnal sedentary 6-h IMIs. Yet hunger rating at the end of 11 to 12-h long nocturnal IMI was only half as large as the hunger rating recorded at the end of individual diurnal IMIs and approximately as low as the evening hunger rating. Even more remarkably, the quantity of food consumed at the end of two mid-diurnal IMIs bore no relationship to the magnitude of preceding energy expenditure (Figure 1). These data support the operation of a circadian control of hunger with an acrophase at mid-day, a presumed nadir in the middle of sleep period, and transitional effects at dawn and dusk. They also indicate that the quantity of food eaten at a meal bears no homeostatic relationship to preceding energy balance but is influenced by time of day.

The universal circadian and ultradian patterning of mammalian feeding behavior suggests the operation of a central circadian meal- and hunger-timing mechanism where the signals related to meal digestion may be entrained to an ultradian gastric-contraction oscillator. The circadian clock restricts the predisposition to seek

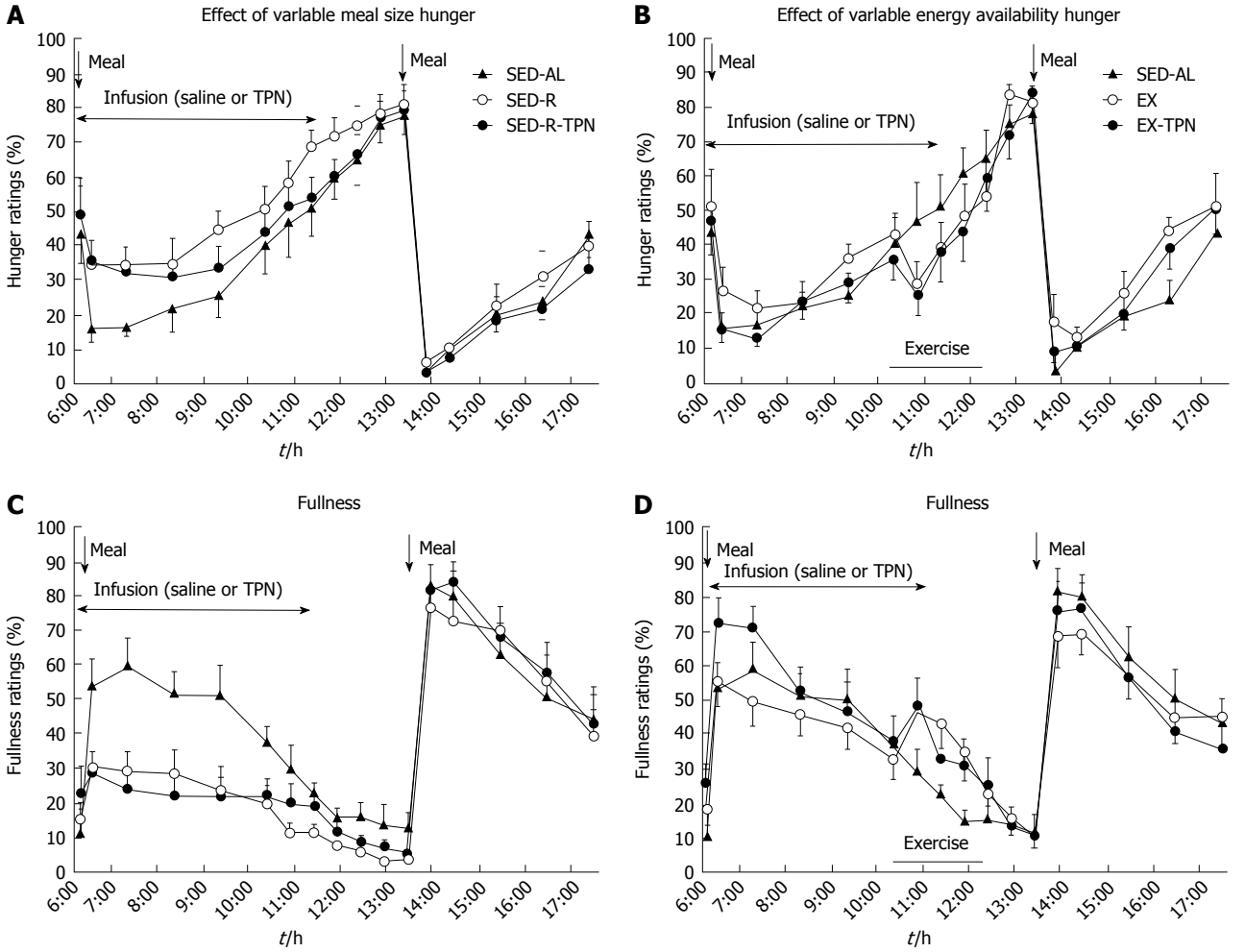

Figure 2 The effects of variable meal size (A) and energy availability (B) on the psychophysical ratings of hunger (A and B) and fullness (C and D) in 10 postmenopausal women subjected to a sedentary trial with a large morning meal (SED-AL), or a small morning meal SED-R, 2 h of moderate intensity exercise after a large morning meal (EX), and *iv* nutrient infusion (TPN) as a replacement of energy withheld from a morning meal (SED-R-TPN) or expended through exercise (EX-TPN). Meal size had a negative effect on hunger ($F_{df4,36}$ = 39.3, P < 0.0001) and a positive effect on fullness ($F_{df4,36}$ = 115.3, P < 0.0001). Exercise energy expenditure had a negative effect on hunger ($F_{df4,36}$ = 25.5, P < 0.0001), and a positive effect on fullness ($F_{df4,36}$ = 42.8; P < 0.0001). TPN had no effect on psychophysical ratings. Data from Ref. [39].

and take food to the active portion of the day when it is interrupted only by the GI signals of fullness and suppresses it during the inactive phase. The uniformity and regularity in the postprandial rise in hunger and attainment of peak hunger regardless of the pre-meal energy balance is consistent with suppression by the GI stimuli of the influence of a central food-seeking command. Energy content of orally taken food appears responsible for partial suppression of hunger when the stomach is incompletely filled (Figure 2A and B). Here, GI nutrient sensing and the rate of stomach emptying according to the energy content of the meal may affect the predisposition for supplementary food intake. Circadian control of hunger and initiation of eating is inferred from low morning and evening hunger and a hunger acrophase between 10 and 19 h[69] that are independent of variations in pre-meal energy availability[39] (Figure 1). An empty stomach and completed GI transit of food generate peak pre-meal hunger during wakeful portion of diurnal cycle (Figure 2A and B) and could do so through removal of gastrointestinal inhibition over the central circadian command guiding the predisposition to eat.

OPPORTUNISTIC AND HEDONISTIC CONTROL OF MEAL-TO-MEAL FEEDING: THE ROLES OF TASTE, OLFACTION, GI NUTRIENT SENSING, AND SOCIAL FACILITATION

In contrast to much of our physiology that operates automatically, we have an innate capacity to consciously detect and prefer foods with sweet and savory taste[70] that leads to predisposition for acceptance and intake of palatable food. Sweet and savory nutrients elicit swallowing even at a fetal stage of development[71], positive facial expressions and sucking in newborn infants[72], and acceptance of palatable foods by children[73]. Sampled nutrients bind to five different populations of taste receptors in the mouth.

Their gustatory properties are signaled in the afferents of facial (VIIth), glossopharyngeal (IXth), and vagus (X th) nerves and are relayed to the rostral two thirds of the nucleus of the solitary tract (NTS) in medulla oblongata[74]. Gustatory information also reaches parabrachial nucleus in the pons[75], ventral tegmental area[76], and several regions of the cortex to elicit hedonic appreciation of the properties of the food. The amygdala and medial and mid-anterior edge of orbitofrontal cortex, and anterior cingulate and insular cortex contribute the emotional component of hedonic responses. The nucleus accumbens (NA) in ventral pallidum contributes to hedonic reinforcement of intake of palatable food through the release of endocannabinoids[76-78]. These innate properties justify the hypothesis that non-homeostatic olfactory and gustatory stimuli provide incentives for non-homeostatic intake of food.

Olfactory and gustatory stimuli complement sensing by the GI tract of food properties and eliciting digestive and absorptive endocrine reflexes[79]. Chemosensory receptors for sugars, amino acids, and fatty acids are located in the neuroendocrine epithelium of the stomach, duodenum, and small intestine. By sensing ingested nutrients, chemosensory neuroendocrine cells in the stomach secrete gastrin from G cells. In the intestine, ghrelin is released from P or X/A cells, somatostatin from D cells, cholecystokinin (CCK) from I cells, serotonin from enterochromaffin cells, glucose-dependent insulinotropic peptide (GIP) from K cells in the proximal small intestine, while glucagon-like peptides (GLPs) and peptide tyrosine tyrosine (PYY) are released from L cells in the distal small intestine. These GI hormones bind to receptors on the afferent vagal fibers that are located in the lamina propria[80]. Stoichiometric GI endocrine responses to energy content of ingested nutrients affect the rate and duration of nutrient digestion and absorption. Some digestive hormones also elicit conscious sensation. Ghrelin increases olfactory salience of food stimuli, decreases olfactory detection threshold, and elicits sniffing[81] as its secretion rises in parallel with pre-meal appetite and declines with meal completion. This action is its most probable contribution to facilitation of the pre-meal appetite[34,35]. Besides their digestive roles in promoting enzyme release and slowing the rate of stomach emptying, CCK[82-85], GLP-1[86-88], and PYY[89], also contribute to the conscious detection of stomach fullness and therefore participate in short-term meal-associated control of post-meal satiation.

Opportunistic characteristic of feeding also is revealed in its responsiveness to the abundance of food and communal food setting. More fluid is consumed if presented in tall, rather than short, glasses[90]. Savory food is consumed in greater amounts from larger platters than from small ones[91]. More food is eaten in company of others[92-96], a social facilitation phenomenon widely shared by mammals[97-99] and even birds[100]. Further, increasing the number of palatable food choices in all-you-can-eat settings leads to overeating in animals[3] and humans[101-103]. In effect, that represents the basis for producing experimental obesity by providing animals fat-enriched, in addition to standard laboratory, diet[3].

A direct test of the homeostatic metering of energy during feeding requires either changing the caloric density of food or the magnitude of pre-meal energy expenditure (EE). Studies manipulating the energy content of food and the meal size indicate that sensations of fullness after the meal and the amount eaten in the subsequent meal are guided by the volume of food eaten rather than its energy content[104-106]. That such non-homeostatic eating bears no direct relationship to the energy content of ingested food also during a longer time frame was suggested by an 11-wk study in which 13 females were provided with either low-fat (20%-25% of energy as fat), or a higher fat, diet (35%-40% fat)[107]. The volume or weight of food eaten daily was comparable on the two diets resulting in a daily energy intake error of 1.22 KJ. Only 35% of this caloric error on a low-fat diet was compensated by the end of 11 wk resulting in a weight loss of 2.5 kg, twice the amount of weight lost on a higher-fat diet.

A more rigorous test of human ability to homeostatically sense energy availability in non-deprived state requires that hunger and food consumption show evidence of caloric compensation when oral, olfactory, and GI sensing is bypassed. Three circumstances that meet that criterion include already mentioned prolonged nocturnal period without food, exercise energy expenditure (EEE), and intravenous supplementation of withheld or expended calories in the form of total parenteral nutrition (TPN). Examination of the effects of between 2300 to 2500 KJ of EEE inserted during morning and afternoon IMIs reveals that this increase in energy expenditure does not influence peak hunger ratings at the onset of the next meal[69] (Figure 1). A similar lack of a relationship between pre-meal energy expenditure and the size of spontaneous meal was previously described in rats[40]. In another study, the search for compensatory changes in food intake was extended to manipulations of EEE, intravenous TPN supplementation for energy withheld in a small meal or for EEE, and the size of meals taken by oral and intragastric route. In this crossover study[39], ten overweight postmenopausal women were provided with a large breakfast containing 2100 KJ in three trials and a small one containing 420 KJ in two trials. The energy supply in the large breakfast in one trial was cancelled by 2270 KJ EEE in another, and EEE was largely replaced by intravenous infusion of 1530 KJ of TPN in the third trial. The low energy content in the small 420 KJ breakfast in the fourth trial, was supplemented with the intravenous infusion of 1530 KJ of TPN in the fifth. The results showed unequivocally that changes in the sensations of hunger (Figure 2A and B) and fullness (Figure 2C and D) were only elicited by the size of the meals taken by oral, and processed by GI, route but not by energy lost exercising or supplemented intravenously. Moreover, the quantity of food eaten, and peak hunger rating at the onset of the next ad-libitum meal is indistinguishable

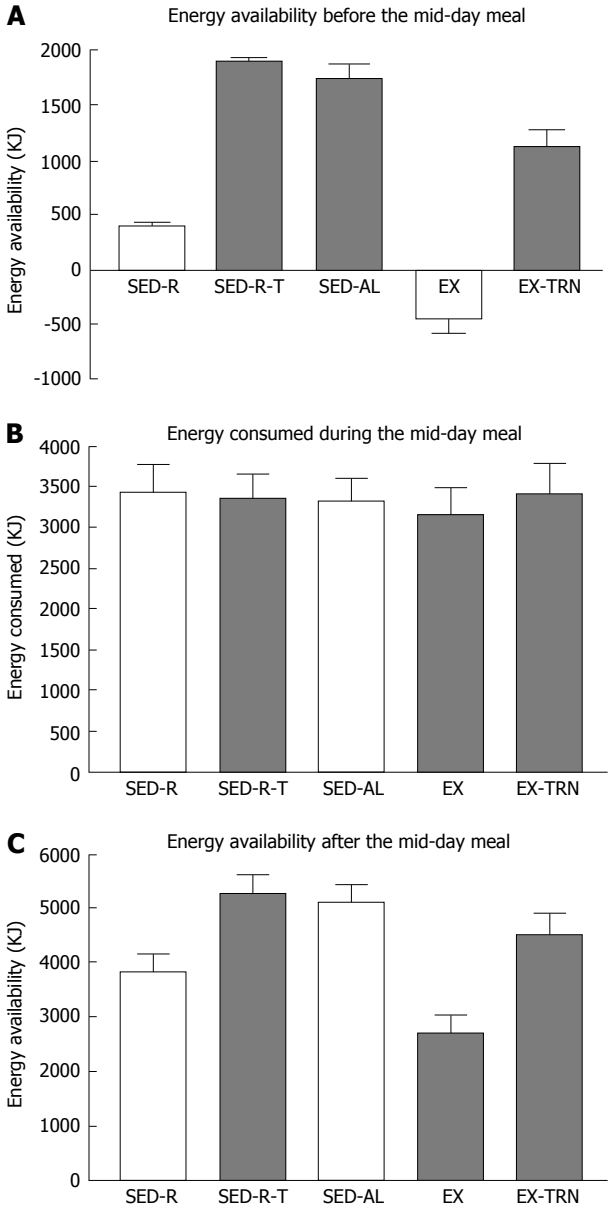

Figure 3 Effect of the morning energy availability (A) on the energy consumed during the midday meal (B) and the residual postmeal energy balance (C) in 10 women subjected to small (SED-R) or large (SED) morning meals, exercise (EX), and TPN (SED-R-T, EX-TPN). Midday meal did not compensate for the significantly lower energy balance in SED-R and EX trials ($F_{df4,45}$ = 77.2; P < 0.0001), which remained uncorrected after the meal ($F_{df4,45}$ = 10.2; P < 0.0001). Data from Ref. [39].

among the five conditions, two of which generated substantial negative energy balance (Figure 3). Furthermore, hormones insulin and leptin tracked accurately changes in energy balance that resulted from unequal meal size, energy lost exercising, and energy supplemented intravenously (Figure 4), but the changes in their plasma concentrations bore no apparent relationship to conscious sensations of hunger and fullness (Figure 2).

Collectively, the above studies support the hypothesis that intermittent meal-to-meal eating under unrestricted access to food is guided by cues provided by oral and GI processing of food. Hunger and fullness ratings, the conscious guides for food intake and meal termination, are affected by the size of the orally ingested nutrients (Figure 2) but not by fluctuation in short-term energy availability caused by intravenous nutrient infusion or by EEE, or by changes in the plasma concentrations of insulin and leptin[39]. Moreover, the peak hunger rating at the onset of the next meal, and the amount eaten during that meal are not responsive to preceding energy imbalance[39,69,104-107]. Stomach filling as a guide to meal size held true in the 11-wk study in which the subjects were largely unresponsive to the energy content of the food[107].

Additional supportive evidence for the role of GI signaling rather than for homeostatic metering of premeal caloric deficit in the control of ad-libitum meal-to-meal eating is available in the singular success of various forms of bariatric surgery in curbing hunger and reducing food intake. A common feature of several variants of bariatric surgery is reduction in stomach capacity to hold food and associated suppression of appetite and hunger[108] or increased nausea and vomiting[109]. The efficacy of stomach fullness as a deterrent for hunger and food intake is also evident in successful application of inflatable balloons to induce weight loss[110,111]. A century ago, Cannon and Washburn[112] demonstrated a striking concordance between episodic bursts of gastric contractions and intermittent sensations of hunger using intragastric balloons as pressure gauges. In addition to Cannon's classic demonstration of the correlation between the ultradian periodicity of empty stomach contractions and hunger, connections of mechanosensitive elements in the smooth muscle of the stomach with the afferent vagus also have been documented more recently[113-115]. Further, these GI smooth muscle mechanoreceptors inhibit eating in response to volume of food introduced into the stomach without regard to its nutritional properties[116,117]. On the other hand, nutrient quality and energy content are sensed by vagal receptors in the intestine and lead to secretion of digestive hormones such as CCK/gastric leptin, GLP-1 and PYY[117]. More recently, pooled data from 8 studies on 67 healthy humans confirmed Cannon and Washburn observation by identifying pyloric pressure waves and peak CCK concentrations as predictors of food intake while finding intravenous nutrient infusions ineffective[118].

In its basic outline, the blueprint of human non-deprivation meal-to-meal eating bears a striking resemblance to the feeding mechanism of a blowfly[119,120]. The insect whose adult body mass is confined within a rigid exoskeleton, accepts sapid solutions whenever its crop is empty. Similar to ad-libitum feeding humans in whom termination of growth imposes a finite body mass, blowfly's food acceptance operates on an opportunistic and hedonic principle, and feeding termination on a GI negative feedback. The fly will ingest to capacity higher concentrations of sweet solutions rather than larger quantities of more dilute solutions. It stops feeding when its full crop inhibits a brain mechanism responsible for predisposition to seek and ingest nutrients whenever the crop is empty. If the recurrent nerve that provides the negative feedback from the crop to the brain is severed, the animal overeats,

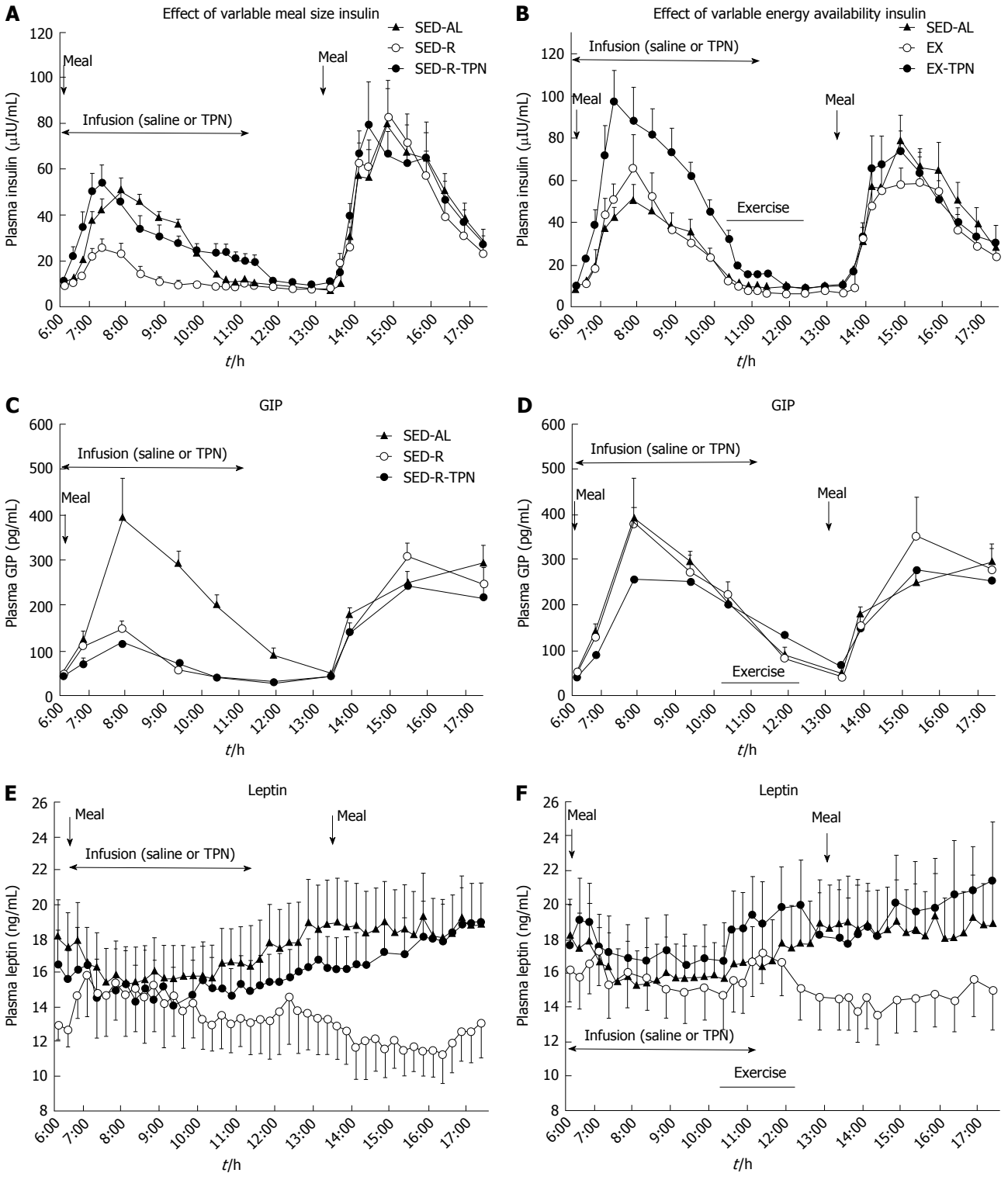

Figure 4 The effects of variable meal size (A) and energy variability (B) on the plasma concentrations of insulin (A and B), GIP (C and D) and leptin (E and F) under the conditions described in Figure 2. Insulin showed significant postprandial increases to meal size (A) and TPN (B) ($F_{df4,45}$ = 25.7; P < 0.0001), whereas GIP responded only to meal size ($F_{df4,45}$ = 42.3; P < 0.0001). Neither insulin nor GIP responded to exercise energy expenditure. Plasma leptin concentration slowly and progressively decreased in response to reduced energy availability caused by small meal ($F_{df4,36}$ = 48.1; P < 0.0001) and exercise ($F_{df4,36}$ = 39.1; P < 0.0001), and this response was abolished by large meal and TPN. Data from Ref. [39].

and with sufficiently high sugar concentrations, will rupture its crop. Presented evidence supports the conclusion that a similar system of nonhomeostatic meal-to-meal eating operates in humans. However, these considerations still leave unanswered the question regarding the signal initiating hunger and food intake. The present reinterpretation of energy regulation proposes that a central ANS command mechanism, given temporal structure by the SCN master circadian clock is responsible for sustained food seeking and meal intake interrupted intermittently

by the inhibition from the signaling of gastric distension as sensations of satiation and fullness associated with GI processing of food. This proposition is consistent with close anatomical connections between SCN and the ANS energy regulatory circuits, circadian and ultradian pattern of meal eating and sympathetic activation of BAT thermogenesis, and disruption of both feeding pattern and thermogenesis and DIT in particular with lesions of either the master clock or the ANS energy regulatory substrates.

COUNTERREGULATION BY LEPTIN OF INSULIN SECRETION, ACTION, AND SENSITIVITY

The key feature of the proposed novel view of body weight regulation is the counterregulation of insulin by leptin under the control of the ANS-circadian command mechanism. Leptin counterregulates insulin in four ways, by (1) acting as a gut peptide signaling satiating fullness and contributing to termination of meals; (2) suppressing insulin secretion; (3) counteracting insulin anabolic actions; and (4) regulating ANS and peripheral tissue sensitivity to insulin in response to downward or upward deflections in the components of body mass. Through these counterregulatory interactions with insulin, leptin matches its sympathetic energy expending actions to the parasympathetic energy conserving actions of insulin.

The sustained stoichiometric relationship between the body fat mass and basal leptin secretion[11] has strongly influenced formulation of a homeostatic lipostatic hypothesis of body fat regulation featuring leptin negative-feedback from WAT to the brain. Integration of short-term secretory responsiveness of leptin to fasting[121-124], meal intake[123,125,126], glucose[127-129], pyruvate[128], insulin secretion[121,130], and insulin-stimulated carbohydrate metabolism[39,127,131-133] with the long-term parallel shifts in plasma leptin concentration and body fat mass has largely escaped scrutiny. To update the understanding about leptin physiology, it should be pointed out that, besides the WAT[134], the hormone also is secreted from the stomach[135-143], placenta[144], and lactating mammary glands[145]. Since leptin of gastric origin is likely to react more rapidly to short-term fluctuations in prandial state than leptin of WAT origin, and both may contribute to short-term changes in circulating leptin concentration, it is useful to briefly review how gastric leptin secretion and appearance in circulation differs from that arising in WAT.

Gastric leptin is rapidly mobilized by cholinergic neurotransmission, nutrient entry into the stomach[139], and release of CCK[135]. Its release is distinctly regulated by these stimuli in contrast to the leptin release from the WAT which is predominantly released in a constitutive fashion[128,134,140,146,147]. Leptin is released into the stomach lumen in exocrine fashion from the chief cells in gastric mucosa. Complexing of gastric leptin with its soluble receptor (LepR) prior to being released from the Golgi apparatus protects it from denaturation by gastric acid[141]. It then is transported to the duodenum where it binds with LepR on the luminal membrane and is transcytozed into the Golgi apparatus of the duodenal enterocyte. There it again binds with LepR and leaves the intestinal mucosa for systemic circulation[139-141].

The first counterregulation of insulin by leptin is clearly of gastric origin and consists of its counteracting the absorptive actions of insulin during a meal. Gastric leptin, mobilized by ingested nutrients and CCK, potentiates the satiating effects of CCK[148,149] and GLP-1[150,151], actions that the two hormones exert in part by slowing the rate of gastric emptying[82-85,88,151], trigerring a sensation of fullness and thus contributing to the termination of a meal. The potentiation by leptin of satiating properties of CCK is mediated by vagal primary afferent neurons selectively responsive to both hormones and to gastric distension and transmitting gastric stretch information to NTS[152] *via* vagal sensory nodose ganglion[153]. Activation of gastric smooth muscle mechanoreceptors is sensitive only to volume of food introduced into the stomach without regard to its nutritional properties[116] while vagal intestinal receptors sense directly the nutrient quality and energy content of ingested food[117]. The potentiation by leptin of CCK satiating effect is activated by nutrient intake while fasting and obesity attenuate vagal afferent stretch signaling[154]. Repeated gastric overstretching, common in overeating and some eating disorders, delays onset of feeding, suppresses leptin concentration and reduces neuropeptide Y levels in ARC and NTS after meal intake as compared to no stomach overstretching[155]. The indirect involvement of leptin in the control of postprandial insulin response and the meal size explains the lack of a relationship between its postprandial concentration (Figure 4C and D) and sensation of fullness (Figure 2C and D). The role of gastric leptin in curtailing postprandial insulin actions may contribute to increased food consumption in free feeding individuals[12,13] and animals[14] who have an inability to produce leptin or leptin receptors. In line with the parasympathetic source of gastric leptin elicitation, the sympathetic actions of leptin suppress cardiac rate by acting on the rostral ventrolateral medullary heart pacer[156,157].

Since leptin of both gastric and WAT origin reaches systemic circulation, it is difficult to distinguish their relative role in the remaining three counterregulations of insulin by leptin. Similar to the responsiveness of gastric leptin to meal ingestion, secretion of leptin from WAT adipocytes also is responsive to short-term fluctuations in prandial state and a number of hormones[160,161]. Feeding increases leptin secretion from WAT cells[134,140,147] and fasting decreases both[147]. Endocrine secretagogues are insulin[128,158,159] and cortisol[158-160], and inhibitors are β-adrenergic stimulation, adrenocorticotropic hormone (ACTH), alpha melanocyte stimulating hormone (αMSH)[161] and testosterone[161,162]. Furthermore, carbohydrate metabolism has to be present for insulin to

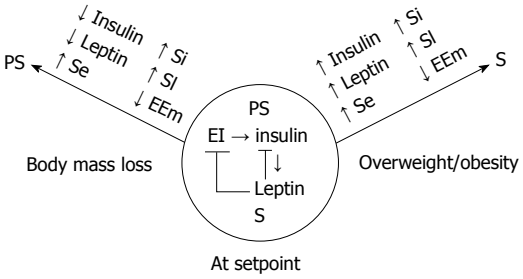

Figure 5 The conceptual model of the autonomic regulation of body weight. Autonomic nervous system regulates the energy flux through the energy conserving actions of insulin that are counterbalanced by the energy expending actions of leptin to match energy intake (EI) and expenditure (EE) and maintain stable body weight (center circle). The counterbalancing is achieved by the upregulation of leptin by the glycolytic energy flux in the WAT stimulated by insulin. Leptin, in turn, inhibits insulin secretion and actions in several organs. If dieting or food scarcity cause weight loss (left arrow), energy conservation is achieved, in part, by reduced Symp activity and EE (EEm). Predominance of Parasymp actions are manifested in reduced fasting insulin and leptin concentrations, increased tissue sensitivities to insulin (Si) and leptin (Sl) along with increased sensitivity in enzymatic nutrient sensing (Se) of energy depletion. In addition, energy is conserved through reduced S activation of metabolism. When overeating and reduced physical activity result in obesity (right arrow), there is a reverse change in fasting insulin and leptin concentrations, tissues become resistant to both hormones as well as to S elicitation of metabolic EE. Due to insulin and leptin resistance, the ineffective compensatory increase in S activity to counteract further body fat, lean body mass, and bone accretion, mainly causes vasoconstriction and hypertension.

increase leptin secretion[129,133], linking the WAT cell responses to short-term metabolic changes. The uncertainty as to the origin of circulating leptin particularly arises when the hormone is being stimulated by systemic administration of insulin in hyperinsulinemic euglycemia. This stimulus applied for longer than 3 to 4 h increases leptin concentration in the plasma[130,163,164] but not if the duration of the clamp[165-170] or of the postprandial period[170,171] is shorter or if hyperinsulinemia is accompanied by hypoglycemia[124].

The second way that leptin counterregulates insulin is by suppressing its secretion in pancreatic β cells[172-176] as shown by insulin oversecretion after deletion of leptin receptors in these cells[176] (Figure 5, circle). Thus, after leptin gene deletion or pharmacological antagonism of leptin action, insulin secretion is supranormal, and leptin administration in *ob/ob* mice that are unable to produce leptin suppresses it[172-177]. Insulin oversecretion results from leptin counterregulation of insulin secretion and not from obesity because it occurs before any significant tissue fat accumulation takes place[176].

The third way that leptin counterregulates insulin is by suppressing its lipogenic and other anabolic actions. While the catecholamines and growth hormone facilitate lipolysis and lipid utilization to systemic signals of energy deficit[178,179] and actually decrease leptin gene expression in WAT[180-182] and its circulating concentration[183-187], leptin binds to adipocytes to selectively counteract insulin-stimulated lipogenesis and activate lipolysis and lipid utilization in WAT[188], especially in its visceral conpartment[189]. It similarly counterregulates insulin lipogenesis in other tissues and thus reduces triglyceride (TG) content in pancreas[190], liver[189-193], and the muscle[190,194-198]. In the liver[192,199], the skeletal muscle[197,200], the BAT[201] and WAT[202], leptin shifts the metabolism from insulin-mediated carbohydrate utilization and TG synthesis toward free fatty acid (FFA) uptake and increased lipid utilization. In the skeletal muscle, leptin activates the enzyme 5'-AMP-activated protein kinase (AMPK) that is capable of sensing metabolic energy depletion[190,194,195]. AMPK in turn inhibits fat synthesis and facilitates FFA entry into the mitochondria for fat oxidation[195-198,203,204]. While some of these metabolic leptin actions result from the hormone binding directly to its receptors in peripheral target organs such as the pancreas[190] the WAT[188,189], the liver[205,206], and the muscle[198,207], the same actions also can be achieved by leptin binding to its receptors in the brain. Suppression by leptin of lipogenic actions of insulin in the WAT[205,208-211] and liver[205,206] is controlled both by the brain, particularly the VMH[208-211] and also is effected at the tissue level[205], particularly in the liver[206].

Leptin counteracts insulin's postprandial anabolic effects by stimulating DIT. It does so by upregulating the thermogenic uncoupling protein UCP1 in BAT by increasing sympathetic nerve activity[123,124,212,213] and norepinephrine turnover in BAT[214]. It also upregulates UCP2 in WAT[215,216], and UCP3 in skeletal muscle[217]. Leptin increases muscle thermogenesis by stimulating substrate cycling[218,219], both lipid and carbohydrate oxidation[200], and expression of genes for anaerobic glycolysis, a metabolic pathway that is bioenergetically less efficient than lipid oxidation[203,204]. While insulin increases postprandial metabolism and thermogenesis through its stimulation of carbohydrate oxidation and sympathetic activation of fat oxidation in BAT[220-222], thermogenic actions of leptin are yoked to postprandial insulin release.

The fourth way that leptin counterregulates insulin action is by controlling the sensitivity of peripheral tissues and the brain to insulin actions as body fat and body masses deviate from the adult plateau. Considering first the peripheral tissues, it is well established that insulin sensitivity increases with body fat and body mass losses, and insulin resistance increases with body fat and body mass gains. Tissues such as the liver, muscle and the WAT display direct autoregulatory increases in numbers of spare receptors, hormone-receptor binding[223], and enzyme sensitivity to nutrients as they are depleted of storage molecules and structural proteins. After glycogen-depleting exercise, activity of glycogen synthase increases in proportion to the magnitude of glycogen depletion which leads to a faster rate of glycogen resynthesis during recovery from exercise[224,225]. As they are depleted of storage nutrients, liver, muscle, and WAT develop direct and autoregulatory increases in sensitivities to the anabolic actions of insulin[191,223,225-227] and catabolic actions of catecholamines[228] some of which are induced by counterregulatory actions of leptin[190-193]. Changes in hormone sensitivities and responses are greater to more rapid rather than to gradual or prolonged reductions in

energy availability. Insulin sensitivity (IS) increases more during the initial weight loss than during maintenance of reduced body weight[229]. Declines in leptin concentration are greater during faster weight loss over a two-day food restriction[230] than to a slower but cumulatively larger energy deficit extended over a 4-[231] or 7-d period[122]. During weight loss, sympathetic activation of metabolic EE is suppressed and only the release of adrenal epinephrine[232] regulates the metabolic shift to predominant lipid utilization[222].

The insulin sensitizing effect of leptin in peripheral tissues becomes manifest as body mass index (BMI) declines below 25 kg/m^2 and fasting plasma leptin concentration drops below 15 ng/dL[233,234]. At its low plasma concentrations, leptin contributes to insulin's parasympathetic actions by increasing muscle glucose uptake[201,235,236] achieved in part by inhibiting the expression of negative regulators of glucose transporter type 4 (GLUT4) translocation to the membrane[237]. By restraining visceral fat accumulation and insulin oversecretion[191-226], leptin preserves insulin sensitivity in the liver[191,226,238] implicating hyperinsulinemia in resistance to insulin action. When the visceral fat is surgically removed[226], reduced glycogenolysis and hepatic glucose production, increased glucose uptake, and reduced insulin requirements to maintain euglycemia are all markers of increased IS. In addition, metabolic gene expression in favor of reduced WAT fat synthesis also results from visceral fat removal[226]. In the oxidative skeletal muscle, leptin counteracts insulin facilitation of intramyocellular triglyceride synthesis and storage by activating AMPK[200]. Through this action, leptin preserves the sensitivity of muscle to insulin leading to increased glucose uptake and glycogen synthesis[175,225]. In addition to being able to exert some of these actions directly in respective tissues studied *in vitro*[175,209], most of leptin actions are contingent on its systemic counteraction of insulin secretion and actions.

The physiological significance of insulin sensitizing actions of low leptin concentrations in weight-reduced state is that it contributes to increased metabolic efficiency that facilitates weight regain and a shift in the ANS balance in favor of the parasympathetic activation[239] (Figure 5, left arrow). A rebound increase in carbohydrate utilization and insulin oversecretion in insulin-sensitive state during post-deprivation overeating in the rats[240,241] is comparable to the postlesion insulin oversecretion after VMH-ARC damage that is prevented by subdiaphragmatic vagotomy[20].

With weight gain at body mass indices above 25 to 27 kg/m^2[233,234] caused by the oportunistic and hedonic design of human meal-to-meal eating where energy intake and expenditure are loosely coupled[242-245], rising basal plasma concentrations of insulin and leptin lead to peripheral tissue resistance to the two hormones[233,234,246,247]. Although adult human adipose tissue retains some capacity to expand both through hyperplasia and hypertrophy[248-250] and is refractory to reductions in adipocyte numbers[251], the parallel rises in obesity and tissue resistance to high plasma leptin and insulin concentrations limit additional body fat and mass accumulation. Resistance to both hormones[246,252] has several causes. An enzymatic resistance to anabolic actions of insulin and counterregulatory actions of leptin[198,207,253] develops in part due to downregulation of respective receptors exposed to prolonged high insulin[173] and leptin[179,181] concentrations. Insulin resistance (IR) also develops due to impaired hormone signaling that results from the action of intermediates of fat biosynthesis driven by high circulating lipid concentrations[253] and accumulation of TG in peripheral organs[200,209,252,253]. Although IR and leptin resistance (LR) increase in parallel with the rise in adiposity, they differ in the timing of their development and their relationship to WAT mass[163,254,255]. Hyperinsulinemia causes hyperleptinemia[163] and both lead to IR and LR. A decline in insulin signaling and IS is a consequence of hyperinsulinemia rather than of IR, since its correction with insulin-lowering diazoxide restores IS and prevents development of obesity while treatment of IR with metformin does not[173]. IR has received a lot of medical attention as a gateway to type 2 diabetes. However, development of IR and LR can also be viewed as an important compensatory processes in autonomic regulation of energy flux in the form of both enzymatic[15,256,257] and sympathetic resistance against additional accretion of body fat. The autonomic resistance to accretion of additional energy storage involves an increase in sympathetic activation of thermogenesis[258] (Figure 5, right arrow), the action of which is rendered ineffective by resistance of enlarged adipocytes to actions of catecholamines[193,228]. The deleterious health consequence of sympathetic overactivation and tissue resistance to hormones in obesity are increased vasoconstriction and hypertension[259-262]. Finally, peripheral LR is possibly dissociable from the resistance of the brain and ANS to leptin actions because of its origin from two different sources, stomach and the WAT, and different routes of accessing the brain, vagal transmission of gastric leptin signals to the NTS, and endocrine signaling of both gastric and WAT leptin to the hypothalamus. This dissociation is suggested by continued effectiveness of leptin when administered intracerebroventricularly at the time dietary obesity has rendered leptin applied intraperitoneally ineffective[263].

Remarkably and importantly leptin controls insulin sensitivity of the ANS energy regulatory command center as body fat and body masses deviate from the norm. The brain substrate that is responsive to changes in body fat and body mass is midbrain ventral tegmental (VTA) dopaminergic and opioidergic projection to the NA in the ventral striatum[76,77] that has rich interconnections with hypothalamic and cortical circuits responsible for activation and inhibition of feeding, voluntary activity, and thermogenesis. The key neurotransmitter mediating behavioral reinforcements is dopamine (DA)[264,265], originating in medial VTA and projecting to ventromedial striatum including medial olfactory tubercle and medial shell of the NA[265]. Activation of these midbrain

DA neural projections to ventral striatum supports nonhomeostatic motivating, rewarding, and incentive properties of food and drives locomotor and eating behaviors[76,77,266-268]. Functional connections between the hypothalamus and this motivational circuitry is illustrated by the LH being the key effective site for behavioral self stimulation with mild electric current[269,270]. LH area also is responsible for arousal and incentive activation of locomotion probably linked to search for food through its component ghrelin[271], melanin concentrating hormone (MCH), and orexin/hypocretin[272-274] neural circuits. LH ghrelin is involved in anticipatory meal-associated increase in locomotion[271] and increases in olfactory stimulus salience during intermeal intervals[81]. MCH neurons regulate olfactory locomotor food-seeking behaviors[272]. In addition to motivating feeding[273], MCH neurons affect energy metabolism[274-277], and their secretion is regulated by gut peptide GLP-1[276], leptin[277], and β_3 adrenergic stimulation[278]. Distinct presympathetic-premotor neurons in LH express both orexin and MCH[279]. Orexin-hypocretin neurotransmission elicits circadian periodicity of locomotion[280], locomotor food seeking, and sequencing of postprandial behavioral satiety and grooming[281,282]. Activation of LH orexin-hypocretin neurons is functionally connected to DA reward circuit[282]. Further, the hyperactivity in anorexia nervosa is hypothesized to be driven in part by increased ghrelin signaling to DA neurons in ventral tegmental area during underweight and hypoleptinemia[283].

At this point, the attention should be brought to the fact that spontaneous locomotion and physical activity levels are, like meal-to-meal eating, under nonhomeostatic control although their interaction brings about the stability of adult body weight[284]. Cross-sectional human data show that total non-basal energy expenditure normalized for body mass is inversely related to body fat[285,286], and that morbidly obese individuals are almost completely inactive[287]. On the other extreme, underweight subjects with anorexia nervosa are known for compulsive running, "drive for activity", and "restlessness"[288,289]. This paradox where overweight and obese subjects reduce locomotor energy expenditure while the underweight ones are hyperactive, defies the homeostatic expectations. Several lines of experimental animal research confirm the inverse relationship between spontaneous physical activity and body fatness. Obesity induced by either VMH lesions in rats[23], rostromedial septal lesions[290] and hippocampal[291] or septo-hypothalamic transections[292] in hamsters, or cafeteria and high-fat diets in neurologically intact animals[3,293,294] reduce spontaneous running activity. On the other hand, severe dietary restriction consisting of only 2-h access to food, leads to weight loss in rats and up to 300% to 500% increase in spontaneous running activity to the point of emaciation[295]. Spontaneous running by rodents in wheels is a motivated behavior amplified by the device challenges[296] and mediated in part by μ opioids[297]. The inverse relationship between body fat and activity levels is associated with neurochemical changes in brain areas where damage produces obesity and hypoactivity[293]. Obesity-inducing brain lesions in hamsters abolish the inverse relationship to body fat[292]. This then indicates a neurochemical link between the nonhomeostatic physical activity and body fat and body mass.

The motivational basis of spontaneous activity can be demonstrated by placing obese and hypoactive lesioned animals on a motor-driven treadmill. Mild electrical shock at the base of treadmill provides external motivation for animals to keep running on the moving track. Compared to neurologically intact animals, obese hypoactive animals can be compelled by such external negative incentive to run on a treadmill as long and as fast as the intact controls[298]. In a similar vein, rats displaying hyperphagia during ad libitum access to food following a weight loss, display reduced willingness to run and need external incentives to increase their activity[241].

So how then do non-homeostatic feeding and non-homeostatic spontaneous physical activity add up to maintenance of stable adult body mass and composition? The evidence presented so far permits a conclusion that the intermittent feeding and locomotor and other physical activities are loosely coupled with continuous body energy drain[244-246,248]. The way they result in stable body mass setpoint is by sharing the same neural substrate which provides variable reward for these behaviors based on the changes in the brain substrate's sensitivity to circulating concentrations of insulin and leptin. The brain substrate that supports motivations to locomote and search and ingest food is richly populated by insulin[299] and leptin[300] receptors and consists of dopaminergic projections from VTA to NA in the ventral striatum, to limbic forebrain structures and to orbitofrontal cortex[265-267]. Endogenous opiates and cannabinoids[301,302] also are components of this DA reward circuitry, with most of NA, but also some of its parts in particular, showing increased hedonic responding to sweets after stimulation of μ opioid receptors[303]. Mu opioids are also implicated in the motivation for spontaneous running[297]. LH circuits responsive to circulating ghrelin and signaling through MCH and orexin-hypocretin neurons[304] also are associated with DA reward system[273,282,283] in supporting behavioral activation and search for food. The basis of changes in incentive value for locomotor search and ingestion of food[76,77,264-268,302] is vested in changes in the brain substrate's sensitivity to changes in the concentrations of the two hormones as body mass undergoes deviations from the adult norm. Withdrawal of leptin during weight loss reduces its counterregulation of insulin actions, increases the sensitivity of the brain reward substrates to locomotor, olfactory, and gustatory rewards and increases the efficiency of insulin actions leading to lipogenesis and recovery of depleted body energy reservoirs. Leptin administration to underweight humans and animals suppresses the motivation to eat[305], insulin metabolic efficiency[203,204,305], and motivation for spontaneous locomotion[306-309]. With body mass loss and declines in leptin and insulin concentration, increased parasympathetic activation and sensitivities of tissues to insulin and leptin action

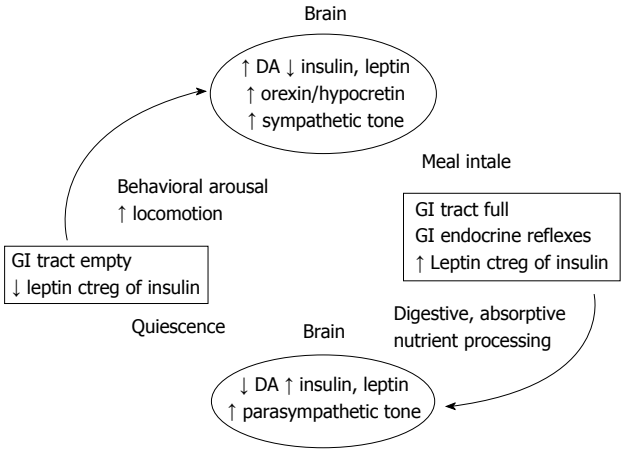

Figure 6 The conceptual model of the linkage between nonhomeostatic meal eating and nonhomeostatic facilitation of physical activity. Completion of gastrointestinal (GI) transit of food removes the inhibitory influence of volumetric and nutritional afferent information mediated by the vagus nerve from reaching nucleus tractus solitaries and DA and opioidergic brain centers of reward.This allows activation of sympathetic actions over fuel mobilization, full operation of behavioral arousal, nonhomeostatic increase in locomotion in quest of food associated with activation of orexin/hypocretin and ghrelin. Completion of nonhomeostatically controled meal results in filling of the stomach, activation of GI nutrient sensing, and increases in postprandial plasma concentrations of insulin and leptin. Vagal projections of this information to the brain reinstate the inhibition over autonomic sympathetic actions and activate parasympathetic control of food digestion and absorption and behavioral quiescence. It is probable that weight loss increases postprandial events linked by the left arrow, and that obesity increases the postprandial events linked by right arrow. Additional consequences of weight loss and weight gain are mediated by changes in tissue sensitivities to leptin and insulin actions altering the prevailing sympathovagal balance and illustrated in Figure 5. DA: Dopaminergic.

facilitate efficient energy storage. Insulin actions are enhanced by reduced leptin counterregulation of its secretion and actions (Figure 5). The parasympathetic dominance in underweight state is reflected in hyperphagia, insulin oversecretion to food intake, and increased efficiency of energy storage that prevail as long as peripheral and central insulin and leptin concentrations remain low and tissue and ANS sensitivity to their actions high.

As increased hunger and metabolic efficiency drive restoration of body fat and body stores to pre-deprivation plateau, the sensitivity of the brain reward circuit declines. The transport of both hormones into the brain also declines[310,311], a process that most likely signals that predeprivation body weight setpoint has been attained. Accrual of excess body fat and body mass along with increases in basal insulin and leptin concentrations leads to reduced motivation to locomote, while feeding is supported in part by palatability of food rather than responsiveness to hunger[76,77]. When excess fat is gained, increased basal concentrations of both insulin and leptin lead to reduced peripheral tissue sensitivity to their actions, and increased activation of sympathetic tone develops as a countermeasure against further body fat and body mass accretion (Figure 5). Thus the brain reward circuit is a component of the autonomic-circadian command center responsible for balancing of sympathetic and parasympathetic processes in part by controlling the secretion of insulin and leptin.

Alternating cycles of famine and feast very likely produced the evolutionary pressure toward coupling of nonhomeostatic search for food opportunities with variable incentive rewards associated with these behaviors[312]. Meal taking and meal processing represent shorter cycles of intermittent refueling of the body that expends energy continuously (Figure 6). Pre-meal behavioral arousal and increased nonhomeostatic locomotion may reflect the activation by the central ANS/circadian command center of lateral hypothalamic neurons responsive to ghrelin, and signaling through MCH and orexin/hypocretin neurons as well as ultradian contractile activity of the empty stomach. The activation of these processes increases locomotor behavior and responsiveness to olfactory gustatory and other signals of food availability. Meal eating inhibits the ANS/circadian command center by GI signals of fullness and satiation. Post-meal grooming in animals and somnolence is induced in part by postprandial insulin secretion[313] and activation of orexin-hypocretin circuits in the LH[280]. The inhibition of the ANS/circadian command center by volumetric and hormonal signals of GI repletion declines progressively as the GI processing of food is completed allowing the sensation of hunger to progressively rise (Figure 2).

REGULATION OF SKELETAL, LEAN AND FAT BODY MASS

Body weight losses or gains along with accumulation of excess fat by either damage to the sympathetic and circadian components of the ANS or cafeteria or high-fat food are viewed by some as a pathological dysfunction of brain substrates where leptin and insulin fail to exert a negative feedback over feeding due to neural inflammation[9]. What this formulation fails to take into account is that weight regulatory mechanism is in full operation at either starvation or obesity extreme of energy balance. Animals rendered obese by medial basal (or in the case of hamsters, septal) lesions or by cafeteria and fat diets defend their new elevated body weight plateau after it has been attained against downward deflections[3,314,315]. The lesions and hedonic nonhomeostatic overeating therefore only raise the plateau at which WAT mass is defended and do not interfere with the body mass regulatory mechanism per se. The clearest demonstration of the integrity of the body mass regulatory defenses after VMH lesions is absence of hyperphagia and hyperinsulinemia (or even presence of hypophagia) if animals are rendered obese by prolonged insulin injections prior to VMH lesion. The change in their feeding behavior lasts until they attain the usual obese body mass plateau characteristic of lesioned animals and thus demonstrate its regulatory defense[316].

The origin of the signal for body mass recovery can therefore not reside exclusively in the size of WAT or adipocyte fat content but requires consideration of the role of the other two body components, the bone and lean tissues. The bone is the probable source of such sig-

nals as its mass changes in parallel with changes in body fat. With each kilogram of body fat gained or lost, 16.5 g of bone mineral is gained or lost[317], and changes in body fat level are accompanied by changes in lean mass. In the hamster, rostral septal lesions[290] and hippocampal[291] and septo-hypothalamic[292] transections increase obesity but also elicit bone and lean body mass growth and an upward displacement of regulated body mass setpoint. Remarkably, increases in body mass setpoint without obesity also can be triggered by voluntary running in this species[318] proving Gordon Kennedy right about the interconnectedness of voluntary activity, weight regulation, and body growth[30,319-321]. The facility of producing upward displacement of body mass setpoint by voluntary running in neurologically intact mature animals provides an exceptional opportunity to examine the location and nature of neural changes responsible for termination of statural growth and initiation of regulatory defenses of the adult body mass setpoint[322,323]. That this is a maturational event is seen by growth acceleration not being possible during animals' natural early rapid growth[323]. The requirement for growth cessation before the defenses of stable adult weight against downward deflections are initiated is shown by the necessity of pituitary gland presence for acceleration of growth by exercise[324], increased pulsatile oversecretion of growth hormone during that growth[325,326], and increased pulsatile growth hormone secretion after the disruption of the brain substrates involved in the maintenance of weight stability in non-growing animals[290-292]. Finally, the permanence of neural changes involved in the defense of the growth-induced upward displacement of body mass setpoint is seen in the phenomenon of catch-up growth[325]. If the hamsters are not given enough food during exercise, their bones and other body components cannot grow as they do when the food is available in unlimited amounts[327]. When the unlimited food is restored, previously exercised hamsters now execute catch-up growth to the approximate body mass plateau they achieve in the absence of food restriction[325]. These data thus demonstrate that exercise has raised mature hamster weight setpoint, and the catch-up growth represents a compensatory process of attaining it.

The supporting inference that leptin is involved in the regulation of lean tissues of the adult body, in particular the bone, should be credited to Gerard Karsenty. He and his research team demonstrated that leptin and sympathetic nerves regulate bone mass in adult mammals by affecting the bioactivity of the bone hormone osteocalcin (OCN)[328]. Although his studies do not include measurements of physical activity, they suggest that signals from the bone osteoblasts influence ANS circuits involved in the regulation of adult body mass. The key finding of Karsenty research was that leptin-induced increase in sympathetic stimulation of the bone suppresses its mineralization and growth. It does so by blocking bioactivity of OCN by activation of an Esp gene in osteoblasts and gamma carboxylation of the hormone. Upregulation of this gene reduces osteoblast numbers and blocks increases in bone mineralization and size. The effect requires β adrenergic receptors on the osteoblasts in the absence of which a high-bone, obese, and hypoactive phenotype is observed similar to that of VMH lesioned animals or mice with deficient leptin signaling (*ob/ob* and *db/db* mice). These findings help explain why with each kg of body fat lost, 16.5 g of bone mineral is lost, and then gained back with body fat regain[317]. Acknowledgment that all three compartments of body mass are regulated extends our understanding of the scope of the roles of leptin and ANS both in short-term nonhomeostatic behaviors and in maintenance of adult weight stability.

The proposed re-interpretation of body weight regulation presents it as a counterpoint between the sympathetic and parasympathetic actions of the ANS/circadian command center in which counterregulation by leptin of insulin secretion and actions and change in tissue sensitivities to the two hormones influence nonhomeostatic locomotor and ingestive behaviors as body fat and body mass are displaced from the stable adult norm. This novel integration offers an opportunity to revise the prevailing homeostatic view of energy regulation and to refocus weight regulation research. The inclusion of body components other than fat stores in body weight regulation expands the scope of study of this mechanism. The proposal that the role of leptin is to counterbalance energy storage associated with insulin secretion as well as help guide lost body mass to pre-deprivation setpoint prompts new hypotheses and research about its possible role in termination of growth and initiation of the maintenance of a stable adult body mass.

REFERENCES

1 **Ogden CL**, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity among adults: United States, 2011-2012. *NCHS Data Brief* 2013; (**131**): 1-8 [PMID: 24152742]

2 **Malik VS**, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. *Nat Rev Endocrinol* 2013; **9**: 13-27 [PMID: 23165161 DOI: 10.1038/nrendo.2012.199]

3 **Sclafani A**, Springer D. Dietary obesity in adult rats: Similarity to hypothalamic and human obesity syndromes. *Physiol Behav* 1976; **17**: 461-471 [DOI: 10.1016/0031-9384(76)90109-8]

4 **Schwartz MW**, Woods SC, Porte D, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; **404**: 661-671 [PMID: 10766253]

5 **Woods SC**, Schwartz MW, Baskin DG, Seeley RJ. Food intake and the regulation of body weight. *Annu Rev Psychol* 2000; **51**: 255-277 [PMID: 10751972 DOI: 10.1146/annurev.psych.51.1.255]

6 **Benoit SC**, Clegg DJ, Seeley RJ, Woods SC. Insulin and leptin as adiposity signals. *Recent Prog Horm Res* 2004; **59**: 267-285 [PMID: 14749506 DOI: 10.1210/rp.59.1.267]

7 **Woods SC**, D'Alessio DA. Central control of body weight and appetite. *J Clin Endocrinol Metab* 2008; **93**: S37-S50 [PMID: 18987269 DOI: 10.1210/jc.2008-1630]

8 **Ryan KK**, Woods SC, Seeley RJ. Central nervous system mechanisms linking the consumption of palatable high-fat diets to the defense of greater adiposity. *Cell Metab* 2012; **15**: 137-149 [PMID: 22244528 DOI: 10.1016/j.cmet.2011.12.013]

9 **Guyenet SJ**, Schwartz MW. Clinical review: Regulation of food intake, energy balance, and body fat mass: implications for the pathogenesis and treatment of obesity. *J Clin Endocrinol Metab* 2012; **97**: 745-755 [PMID: 22238401 DOI: 10.1210/

jc.2011-2525]
10 **Heymsfield SB**, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA* 1999; **282**: 1568-1575 [PMID: 10546697 DOI: 10.1001/jama.282.16.1568]
11 **Considine RV**, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996; **334**: 292-295 [PMID: 8532024 DOI: 10.1056/NEJM199602013340503]
12 **Montague CT**, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 1997; **387**: 903-908 [PMID: 9202122 DOI: 10.1038/43185]
13 **Farooqi IS**, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* 1999; **341**: 879-884 [PMID: 10486419 DOI: 10.1056/NEJM199909163411204]
14 **Pelleymounter MA**, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995; **269**: 540-543 [PMID: 7624776 DOI: 10.1126/science.7624776]
15 **Schwartz MW**, Niswender KD. Adiposity signaling and biological defense against weight gain: absence of protection or central hormone resistance? *J Clin Endocrinol Metab* 2004; **89**: 5889-5897 [PMID: 15579732 DOI: 10.1210/jc.2004-0906]
16 **Adolph EF**. Urges to eat and drink in rats. *Am J Physiol* 1947; **151**: 110-125 [PMID: 20271650]
17 **Bray GA**, York DA. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol Rev* 1979; **59**: 719-809 [PMID: 379887]
18 **Bray GA**, Sclafani A, Novin D. Obesity-inducing hypothalamic knife cuts: effects on lipolysis and blood insulin levels. *Am J Physiol* 1982; **243**: R445-R449 [PMID: 7051866]
19 **Gold RM**. Hypothalamic hyperphagia produced by parasagittal knife cuts. *Physiol Behav* 1970; **5**: 23-25 [PMID: 4950994 DOI: 10.1016/0031-9384(70)90007-7]
20 **Berthoud HR**, Jeanrenaud B. Acute hyperinsulinemia and its reversal by vagotomy after lesions of the ventromedial hypothalamus in anesthetized rats. *Endocrinology* 1979; **105**: 146-151 [PMID: 446404 DOI: 10.1210/endo-105-1-146]
21 **Nishizawa Y**, Bray GA. Ventromedial hypothalamic lesions and the mobilization of fatty acids. *J Clin Invest* 1978; **61**: 714-721 [PMID: 641150 DOI: 10.1172/JCI108984]
22 **Inoue S**, Bray GA. The effects of subdiaphragmatic vagotomy in rats with ventromedial hypothalamic obesity. *Endocrinology* 1977; **100**: 108-114 [PMID: 830533 DOI: 10.1210/endo-100-1-108]
23 **Eng R**, Gold RM, Sawchenko PE. Hypothalamic hypoactivity prevented but not reversed by subdiaphragmatic vagotomy. *Physiol Behav* 1978; **20**: 637-641 [PMID: 684098 DOI: 10.1016/0031-9384(78)90257-3]
24 **Takahashi A**, Shimazu T. Hypothalamic regulation of lipid metabolism in the rat: effect of hypothalamic stimulation on lipolysis. *J Auton Nerv Syst* 1981; **4**: 195-205 [PMID: 7299038 DOI: 10.1016/0165-1838(81)90044-8]
25 **Saito M**, Minokoshi Y, Shimazu T. Ventromedial hypothalamic stimulation accelerates norepinephrine turnover in brown adipose tissue of rats. *Life Sci* 1987; **41**: 193-197 [PMID: 2885704 DOI: 10.1016/0024-3205(87)90493-0]
26 **Iwai M**, Hell NS, Shimazu T. Effect of ventromedial hypothalamic stimulation on blood flow of brown adipose tissue in rats. *Pflugers Arch* 1987; **410**: 44-47 [PMID: 3684509 DOI: 10.1007/BF00581894]
27 **Sudo M**, Minokoshi Y, Shimazu T. Ventromedial hypothalamic stimulation enhances peripheral glucose uptake in anesthetized rats. *Am J Physiol* 1991; **261**: E298-E303 [PMID: 1887876]
28 **Dulloo AG**, Miller DS. The thermogenic properties of ephedrine/methylxanthine mixtures: animal studies. *Am J Clin Nutr* 1986; **43**: 388-394 [PMID: 3953478]
29 **Dulloo AG**, Miller DS. The thermogenic properties of ephedrine/methylxanthine mixtures: human studies. *Int J Obes* 1986; **10**: 467-481 [PMID: 3804564]
30 **Kennedy GC**. The hypothalamic control of food intake in rats. *Proc R Soc Lond B Biol Sci* 1950; **137**: 535-549 [PMID: 14797669 DOI: 10.1098/rspb.1950.0065]
31 **Bamshad M**, Song CK, Bartness TJ. CNS origins of the sympathetic nervous system outflow to brown adipose tissue. *Am J Physiol* 1999; **276**: R1569-R1578 [PMID: 10362733]
32 **Dulloo AG**, Miller DS. The effect of parasympathetic drugs on energy expenditure: relevance to the autonomic hypothesis. *Can J Physiol Pharmacol* 1986; **64**: 586-591 [PMID: 3730944 DOI: 10.1139/y86-097]
33 **Bowers RR**, Festuccia WT, Song CK, Shi H, Migliorini RH, Bartness TJ. Sympathetic innervation of white adipose tissue and its regulation of fat cell number. *Am J Physiol Regul Integr Comp Physiol* 2004; **286**: R1167-R1175 [PMID: 15142857]
34 **Cummings DE**, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714-1719 [PMID: 11473029 DOI: 10.2337/diabetes.50.8.1714]
35 **Wren AM**, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001; **86**: 5992 [PMID: 11739476 DOI: 10.1210/jcem.86.12.8111]
36 **Patterson M**, Bloom SR, Gardiner JV. Ghrelin and appetite control in humans--potential application in the treatment of obesity. *Peptides* 2011; **32**: 2290-2294 [PMID: 21835215 DOI: 10.1016/j.peptides.2011.07.021]
37 **Parker JA**, Bloom SR. Hypothalamic neuropeptides and the regulation of appetite. *Neuropharmacology* 2012; **63**: 18-30 [PMID: 22369786 DOI: 10.1016/j.neuropharm.2012.02.004]
38 **Wortley KE**, Anderson KD, Garcia K, Murray JD, Malinova L, Liu R, Moncrieffe M, Thabet K, Cox HJ, Yancopoulos GD, Wiegand SJ, Sleeman MW. Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. *Proc Natl Acad Sci USA* 2004; **101**: 8227-8232 [PMID: 15148384 DOI: 10.1073/pnas.0402763101]
39 **Borer KT**, Wuorinen E, Ku K, Burant C. Appetite responds to changes in meal content, whereas ghrelin, leptin, and insulin track changes in energy availability. *J Clin Endocrinol Metab* 2009; **94**: 2290-2298 [PMID: 19401375 DOI: 10.1210/jc.2008-2495]
40 **Le Magnen J**. Peripheral and systemic actions of food in the caloric regulation of intake. *Ann N Y Acad Sci* 1969; **157**: 1126-1157 [PMID: 5255624 DOI: 10.1111/j.1749-6632.1969.tb12940.x]
41 **Elmquist JK**, Ahima RS, Elias CF, Flier JS, Saper CB. Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proc Natl Acad Sci USA* 1998; **95**: 741-746 [PMID: 9435263 DOI: 10.1073/pnas.95.2.741]
42 **Maywood ES**, O'Neill JS, Chesham JE, Hastings MH. Minireview: The circadian clockwork of the suprachiasmatic nuclei--analysis of a cellular oscillator that drives endocrine rhythms. *Endocrinology* 2007; **148**: 5624-5634 [PMID: 17901233 DOI: 10.1210/en.2007-0660]
43 **Froy O**. Metabolism and circadian rhythms--implications for obesity. *Endocr Rev* 2010; **31**: 1-24 [PMID: 19854863 DOI: 10.1210/er.2009-0014]
44 **Nedeltcheva AV**, Kilkus JM, Imperial J, Schoeller DA, Penev PD. Insufficient sleep undermines dietary efforts to reduce adiposity. *Ann Intern Med* 2010; **153**: 435-441 [PMID: 20921542 DOI: 10.7326/0003-4819-153-7-201010050-00006]
45 **Van Cauter E**, Spiegel K, Tasali E, Leproult R. Metabolic

consequences of sleep and sleep loss. *Sleep Med* 2008; **9** Suppl 1: S23-S28 [PMID: 18929315]
46 **Dark J**, Zucker I, Wade GN. Photoperiodic regulation of body mass, food intake, and reproduction in meadow voles. *Am J Physiol* 1983; **245**: R334-R338 [PMID: 6351638]
47 **Kriegsfeld LJ**, LeSauter J, Silver R. Targeted microlesions reveal novel organization of the hamster suprachiasmatic nucleus. *J Neurosci* 2004; **24**: 2449-2457 [PMID: 15014120 DOI: 10.1523/JNEUROSCI.5323-03.2004]
48 **Stephan FK**, Zucker I. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc Natl Acad Sci USA* 1972; **69**: 1583-1586 [PMID: 4556464 DOI: 10.1073/pnas.69.6.1583]
49 **Egawa M**, Inoue S, Satoh S, Takamura Y, Nagai K, Nakagawa H. Acute and chronic effects of VMH lesions on circadian rhythms in food intake and metabolites. *Brain Res Bull* 1993; **31**: 293-299 [PMID: 8490728 DOI: 10.1016/0361-9230(93)90220-6]
50 **Kakolewski JW**, Deaux E, Christensen J, Case B. Diurnal patterns in water and food intake and body weight changes in rats with hypothalamic lesions. *Am J Physiol* 1971; **221**: 711-718 [PMID: 5570328]
51 **Sakaguchi T**, Takahashi M, Bray GA. Diurnal changes in sympathetic activity. Relation to food intake and to insulin injected into the ventromedial or suprachiasmatic nucleus. *J Clin Invest* 1988; **82**: 282-286 [PMID: 3292586 DOI: 10.1172/JCI113584]
52 **van der Veen DR**, Shao J, Chapman S, Leevy WM, Duffield GE. A diurnal rhythm in glucose uptake in brown adipose tissue revealed by in vivo PET-FDG imaging. *Obesity* (Silver Spring) 2012; **20**: 1527-1529 [PMID: 22447290 DOI: 10.1038/oby.2012.78]
53 **Redlin U**, Nuesslein B, Schmidt I. Circadian changes of brown adipose tissue thermogenesis in juvenile rats. *Am J Physiol* 1992; **262**: R504-R508 [PMID: 1558221]
54 **Ootsuka Y**, de Menezes RC, Zaretsky DV, Alimoradian A, Hunt J, Stefanidis A, Oldfield BJ, Blessing WW. Brown adipose tissue thermogenesis heats brain and body as part of the brain-coordinated ultradian basic rest-activity cycle. *Neuroscience* 2009; **164**: 849-861 [PMID: 19679172 DOI: 10.1016/j.neuroscience.2009.08.013]
55 **Zvonic S**, Ptitsyn AA, Conrad SA, Scott LK, Floyd ZE, Kilroy G, Wu X, Goh BC, Mynatt RL, Gimble JM. Characterization of peripheral circadian clocks in adipose tissues. *Diabetes* 2006; **55**: 962-970 [PMID: 16567517 DOI: 10.2337/diabetes.55.04.06.db05-0873]
56 **Freeman PH**, Wellman PJ. Brown adipose tissue thermogenesis induced by low level electrical stimulation of hypothalamus in rats. *Brain Res Bull* 1987; **18**: 7-11 [PMID: 3828843 DOI: 10.1016/0361-9230(87)90026-8]
57 **Ueta CB**, Fernandes GW, Capelo LP, Fonseca TL, Maculan FD, Gouveia CH, Brum PC, Christoffolete MA, Aoki MS, Lancellotti CL, Kim B, Bianco AC, Ribeiro MO. β(1) Adrenergic receptor is key to cold- and diet-induced thermogenesis in mice. *J Endocrinol* 2012; **214**: 359-365 [PMID: 22728333]
58 **Haynes WG**, Morgan DA, Walsh SA, Mark AL, Sivitz WI. Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest* 1997; **100**: 270-278 [PMID: 9218503 DOI: 10.1172/JCI119532]
59 **Enriori PJ**, Sinnayah P, Simonds SE, Garcia Rudaz C, Cowley MA. Leptin action in the dorsomedial hypothalamus increases sympathetic tone to brown adipose tissue in spite of systemic leptin resistance. *J Neurosci* 2011; **31**: 12189-12197 [PMID: 21865462 DOI: 10.1523/JNEUROSCI.2336-11.2011]
60 **Sinha MK**, Ohannesian JP, Heiman ML, Kriauciunas A, Stephens TW, Magosin S, Marco C, Caro JF. Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. *J Clin Invest* 1996; **97**: 1344-1347 [PMID: 8636448 DOI: 10.1172/JCI118551]
61 **Saad MF**, Riad-Gabriel MG, Khan A, Sharma A, Michael R, Jinagouda SD, Boyadjian R, Steil GM. Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity. *J Clin Endocrinol Metab* 1998; **83**: 453-459 [PMID: 9467557]
62 **Schoeller DA**, Cella LK, Sinha MK, Caro JF. Entrainment of the diurnal rhythm of plasma leptin to meal timing. *J Clin Invest* 1997; **100**: 1882-1887 [PMID: 9312190 DOI: 10.1172/JCI119717]
63 **Licinio J**, Mantzoros C, Negrão AB, Cizza G, Wong ML, Bongiorno PB, Chrousos GP, Karp B, Allen C, Flier JS, Gold PW. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nat Med* 1997; **3**: 575-579 [PMID: 9142131 DOI: 10.1038/nm0597-575]
64 **Saito M**, Minokoshi Y, Shimazu T. Metabolic and sympathetic nerve activities of brown adipose tissue in tube-fed rats. *Am J Physiol* 1989; **257**: E374-E378 [PMID: 2506762]
65 **Bajzer M**, Olivieri M, Haas MK, Pfluger PT, Magrisso IJ, Foster MT, Tschöp MH, Krawczewski-Carhuatanta KA, Cota D, Obici S. Cannabinoid receptor 1 (CB1) antagonism enhances glucose utilisation and activates brown adipose tissue in diet-induced obese mice. *Diabetologia* 2011; **54**: 3121-3131 [PMID: 21987346 DOI: 10.1007/s00125-011-2302-6]
66 **Verty AN**, Allen AM, Oldfield BJ. The effects of rimonabant on brown adipose tissue in rat: implications for energy expenditure. *Obesity* (Silver Spring) 2009; **17**: 254-261 [PMID: 19057531 DOI: 10.1038/oby.2008.509]
67 **Rothwell NJ**, Stock MJ, Warwick BP, Winter PD. Diurnal variations in circulating hormone levels and brown adipose tissue activity in "cafeteria"-fed rats. *Comp Biochem Physiol A Comp Physiol* 1983; **75**: 461-465 [PMID: 6136383]
68 **Tanida M**, Shen J, Nakamura T, Niijima A, Nagai K. Day-night difference in thermoregulatory responses to olfactory stimulation. *Neurosci Lett* 2008; **439**: 192-197 [PMID: 18514405 DOI: 10.1016/j.neulet.2008.05.021]
69 **Wuorinen EC**, Borer KT. Circadian and ultradian components of hunger in human non-homeostatic meal-to-meal eating. *Physiol Behav* 2013; **122**: 8-16 [PMID: 23973366 DOI: 10.1016/j.physbeh.2013.08.001]
70 **Steiner JE**, Glaser D, Hawilo ME, Berridge KC. Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. *Neurosci Biobehav Rev* 2001; **25**: 53-74 [PMID: 11166078 DOI: 10.1016/S0149-7634(00)00051-8]
71 **DeSnoo K**. Das trinkende Kind im Uterus. *Monats Geburtsh Gynaekol* 1937; **105**: 88-97
72 **Desor JA**, Maller O, Andrews K. Ingestive responses of human newborns to salty, sour, and bitter stimuli. *J Comp Physiol Psychol* 1975; **89**: 966-970 [PMID: 1184802 DOI: 10.1037/h0077171]
73 **Lipchock SV**, Reed DR, Mennella JA. The gustatory and olfactory systems during infancy: implications for development of feeding behaviors in the high-risk neonate. *Clin Perinatol* 2011; **38**: 627-641 [PMID: 22107894 DOI: 10.1016/j.clp.2011.08.008]
74 **Norgren R**. The gustatory system in mammals. *Am J Otolaryngol* 1983; **4**: 234-237 [PMID: 6312827 DOI: 10.1016/S0196-0709(83)80064-7]
75 **Dutta TM**, Josiah AF, Cronin CA, Wittenberg GF, Cole JW. Altered taste and stroke: a case report and literature review. *Top Stroke Rehabil* 2013; **20**: 78-86 [PMID: 23340074 DOI: 10.1310/tsr2001-78.]
76 **Berridge KC**. 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. *Physiol Behav* 2009; **97**: 537-550 [PMID: 19336238 DOI: 10.1016/j.physbeh.2009.02.044]
77 **Berridge KC**. Food reward: brain substrates of wanting and liking. *Neurosci Biobehav Rev* 1996; **20**: 1-25 [PMID: 8622814 DOI: 10.1016/0149-7634(95)00033-B]
78 **Cardinal RN**, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci Biobehav Rev* 2002; **26**: 321-352 [PMID: 12034134 DOI: 10.1016/S0149-7634(02)00007-6]

79 **Janssen S**, Depoortere I. Nutrient sensing in the gut: new roads to therapeutics? *Trends Endocrinol Metab* 2013; **24**: 92-100 [PMID: 23266105 DOI: 10.1016/j.tem.2012.11.006]

80 **Berthoud HR**, Kressel M, Raybould HE, Neuhuber WL. Vagal sensors in the rat duodenal mucosa: distribution and structure as revealed by in vivo DiI-tracing. *Anat Embryol* (Berl) 1995; **191**: 203-212 [PMID: 7771683 DOI: 10.1007/BF00187819]

81 **Tong J**, Mannea E, Aimé P, Pfluger PT, Yi CX, Castaneda TR, Davis HW, Ren X, Pixley S, Benoit S, Julliard K, Woods SC, Horvath TL, Sleeman MM, D'Alessio D, Obici S, Frank R, Tschöp MH. Ghrelin enhances olfactory sensitivity and exploratory sniffing in rodents and humans. *J Neurosci* 2011; **31**: 5841-5846 [PMID: 21490225 DOI: 10.1523/JNEUROSCI.5680-10.2011]

82 **Gibbs J**, Smith GP. Cholecystokinin and satiety in rats and rhesus monkeys. *Am J Clin Nutr* 1977; **30**: 758-761 [PMID: 404865]

83 **Muurahainen N**, Kissileff HR, Derogatis AJ, Pi-Sunyer FX. Effects of cholecystokinin-octapeptide (CCK-8) on food intake and gastric emptying in man. *Physiol Behav* 1988; **44**: 645-649 [PMID: 3237850]

84 **Melton PM**, Kissileff HR, Pi-Sunyer FX. Cholecystokinin (CCK-8) affects gastric pressure and ratings of hunger and fullness in women. *Am J Physiol* 1992; **263**: R452-R456 [PMID: 1510184]

85 **Smith GP**, Gibbs J. Satiating effect of cholecystokinin. *Ann N Y Acad Sci* 1994; **713**: 236-241 [PMID: 8185165 DOI: 10.1111/j.1749-6632.1994.tb44071.x]

86 **van Dijk G**, Thiele TE, Seeley RJ, Woods SC, Bernstein IL. Glucagon-like peptide-1 and satiety. *Nature* 1997; **385**: 214 [PMID: 9000071 DOI: 10.1038/385214a0]

87 **Flint A**, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 1998; **101**: 515-520 [PMID: 9449682 DOI: 10.1172/JCI990]

88 **Näslund E**, Gutniak M, Skogar S, Rössner S, Hellström PM. Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. *Am J Clin Nutr* 1998; **68**: 525-530 [PMID: 9734726]

89 **Batterham RL**, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 2002; **418**: 650-654 [PMID: 12167864 DOI: 10.1038/nature00887]

90 **Walker D**, Smarandescu L, Wansink B. Half Full or Empty: Cues that lead wine drinkers to unintentionally overpour. *Subst Use Misuse* 2013

91 **van Kleef E**, Shimizu M, Wansink B. Serving bowl selection biases the amount of food served. *J Nutr Educ Behav* 2012; **44**: 66-70 [PMID: 21982579 DOI: 10.1016/j.jneb.2011.03.001]

92 **De Castro JM**. Social facilitation of duration and size but not rate of the spontaneous meal intake of humans. *Physiol Behav* 1990; **47**: 1129-1135 [PMID: 2395917 DOI: 10.1016/0031-9384(90)90363-9]

93 **De Castro JM**. Social facilitation of food intake in humans. *Appetite* 1995; **24**: 260 [PMID: 7574575]

94 **De Castro JM**. Socio-cultural determinants of meal size and frequency. *Br J Nutr* 1997; **77** Suppl 1: S39-S54; discussion S54-S55 [PMID: 9155493]

95 **Lumeng JC**, Hillman KH. Eating in larger groups increases food consumption. *Arch Dis Child* 2007; **92**: 384-387 [PMID: 17301111 DOI: 10.1136/adc.2006.103259]

96 **Pliner P**, Bell R, Hirsch ES, Kinchla M. Meal duration mediates the effect of "social facilitation" on eating in humans. *Appetite* 2006; **46**: 189-198 [PMID: 16500000 DOI: 10.1016/j.appet.2005.12.003]

97 **Ferrari PF**, Maiolini C, Addessi E, Fogassi L, Visalberghi E. The observation and hearing of eating actions activates motor programs related to eating in macaque monkeys. *Behav Brain Res* 2005; **161**: 95-101 [PMID: 15904715 DOI: 10.1016/j.bbr.2005.01.009]

98 **James WT**. Social facilitation of eating behavior in puppies after satiation. *J Comp Physiol Psychol* 1953; **46**: 427-428 [PMID: 13109065 DOI: 10.1037/h0056028]

99 **Sweeting MP**, Houpt CE, Houpt KA. Social facilitation of feeding and time budgets in stabled ponies. *J Anim Sci* 1985; **60**: 369-374 [PMID: 3988629]

100 **Strobel MG**, Macdonald GE. Induction of eating in newly hatched chicks. *J Comp Physiol Psychol* 1974; **86**: 493-502 [PMID: 4814765 DOI: 10.1037/h0036170]

101 **Levitsky DA**, Youn T. The more food young adults are served, the more they overeat. *J Nutr* 2004; **134**: 2546-2549 [PMID: 15465745]

102 **Levitsky DA**, Halbmaier CA, Mrdjenovic G. The freshman weight gain: a model for the study of the epidemic of obesity. *Int J Obes Relat Metab Disord* 2004; **28**: 1435-1442 [PMID: 15365585 DOI: 10.1038/sj.ijo.0802776]

103 **Levitsky DA**, Iyer S, Pacanowski CR. Number of foods available at a meal determines the amount consumed. *Eat Behav* 2012; **13**: 183-187 [PMID: 22664394 DOI: 10.1016/j.eatbeh.2012.01.006]

104 **Blatt AD**, Williams RA, Roe LS, Rolls BJ. Effects of energy content and energy density of pre-portioned entrées on energy intake. *Obesity* (Silver Spring) 2012; **20**: 2010-2018 [PMID: 22262161]

105 **Williams RA**, Roe LS, Rolls BJ. Comparison of three methods to reduce energy density. Effects on daily energy intake. *Appetite* 2013; **66**: 75-83 [PMID: 23523752 DOI: 10.1016/j.appet.2013.03.004.]

106 **Williams RA**, Roe LS, Rolls BJ. Assessment of satiety depends on the energy density and portion size of the test meal. *Obesity* (Silver Spring) 2014; **22**: 318-324 [PMID: 23929544]

107 **Kendall A**, Levitsky DA, Strupp BJ, Lissner L. Weight loss on a low-fat diet: consequence of the imprecision of the control of food intake in humans. *Am J Clin Nutr* 1991; **53**: 1124-1129 [PMID: 2021123]

108 **Schindler K**, Prager G, Ballaban T, Kretschmer S, Riener R, Buranyi B, Maier C, Luger A, Ludvik B. Impact of laparoscopic adjustable gastric banding on plasma ghrelin, eating behaviour and body weight. *Eur J Clin Invest* 2004; **34**: 549-554 [PMID: 15305889 DOI: 10.1111/j.1365-2362.2004.01382.x]

109 **Sinha AC**, Singh PM, Williams NW, Ochroch EA, Goudra BG. Aprepitant's prophylactic efficacy in decreasing postoperative nausea and vomiting in morbidly obese patients undergoing bariatric surgery. *Obes Surg* 2014; **24**: 225-231 [PMID: 23990451 DOI: 10.1007/s11695-013-1065-1]

110 **Mathus-Vliegen EM**, Tytgat GN, Veldhuyzen-Offermans EA. Intragastric balloon in the treatment of super-morbid obesity. Double-blind, sham-controlled, crossover evaluation of 500-milliliter balloon. *Gastroenterology* 1990; **99**: 362-369 [PMID: 2194894 DOI: 10.1002/bjs.1800770127]

111 **Mathus-Vliegen EM**, Tytgat GN. Intragastric balloon for treatment-resistant obesity: safety, tolerance, and efficacy of 1-year balloon treatment followed by a 1-year balloon-free follow-up. *Gastrointest Endosc* 2005; **61**: 19-27 [PMID: 15672051 DOI: 10.1016/S0016-5107(04)02406-X]

112 **Cannon WB**, Washburn AL. An explanation of hunger. 1911. *Obes Res* 1993; **1**: 494-500 [PMID: 16350324]

113 **Berthoud HR**, Lynn PA, Blackshaw LA. Vagal and spinal mechanosensors in the rat stomach and colon have multiple receptive fields. *Am J Physiol Regul Integr Comp Physiol* 2001; **280**: R1371-R1381 [PMID: 11294756]

114 **Phillips RJ**, Powley TL. Tension and stretch receptors in gastrointestinal smooth muscle: re-evaluating vagal mechanoreceptor electrophysiology. *Brain Res Brain Res Rev* 2000; **34**: 1-26 [PMID: 11086184 DOI: 10.1016/S0165-0173(00)00036-9]

115 **Powley TL**, Phillips RJ. Vagal intramuscular array afferents form complexes with interstitial cells of Cajal in gastroin-

testinal smooth muscle: analogues of muscle spindle organs? *Neuroscience* 2011; **186**: 188-200 [PMID: 21530617 DOI: 10.1016/j.neuroscience.2011.04.036]
116 **Phillips RJ**, Powley TL. Gastric volume rather than nutrient content inhibits food intake. *Am J Physiol* 1996; **271**: R766-R769 [PMID: 8853402]
117 **Powley TL**, Phillips RJ. Gastric satiation is volumetric, intestinal satiation is nutritive. *Physiol Behav* 2004; **82**: 69-74 [PMID: 15234593 DOI: 10.1016/j.physbeh.2004.04.037]
118 **Seimon RV**, Lange K, Little TJ, Brennan IM, Pilichiewicz AN, Feltrin KL, Smeets AJ, Horowitz M, Feinle-Bisset C. Pooled-data analysis identifies pyloric pressures and plasma cholecystokinin concentrations as major determinants of acute energy intake in healthy, lean men. *Am J Clin Nutr* 2010; **92**: 61-68 [PMID: 20484444 DOI: 10.3945/ajcn.2009.29015]
119 **Dethier VG**, Solomon RL, Turner LH. Sensory input and central excitation and inhibition in the blowfly. *J Comp Physiol Psychol* 1965; **60**: 303-313 [PMID: 5839258 DOI: 10.1037/h0022557]
120 **Dethier VG**, Solomon RL, Turner LH. Central inhibition in the blowfly. *J Comp Physiol Psychol* 1968; **66**: 144-150 [PMID: 5672621 DOI: 10.1037/h0025957]
121 **Boden G**, Chen X, Mozzoli M, Ryan I. Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab* 1996; **81**: 3419-3423 [PMID: 8784108]
122 **Dubuc GR**, Phinney SD, Stern JS, Havel PJ. Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women. *Metabolism* 1998; **47**: 429-434 [PMID: 9550541 DOI: 10.1016/S0026-0495(98)90055-5]
123 **Kolaczynski JW**, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, Myint M, Caro JF. Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. *Diabetes* 1996; **45**: 1511-1515 [PMID: 8866554 DOI: 10.2337/diab.45.11.1511]
124 **Schmitz O**, Fisker S, Orskov L, Hove KY, Nyholm B, Møller N. Effects of hyperinsulinaemia and hypoglycaemia on circulating leptin levels in healthy lean males. *Diabetes Metab* 1997; **23**: 80-83 [PMID: 9059771]
125 **Patel BK**, Koenig JI, Kaplan LM, Hooi SC. Increase in plasma leptin and Lep mRNA concentrations by food intake is dependent on insulin. *Metabolism* 1998; **47**: 603-607 [PMID: 9591754 DOI: 10.1016/S0026-0495(98)90247-5]
126 **Kolaczynski JW**, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. *J Clin Endocrinol Metab* 1996; **81**: 4162-4165 [PMID: 8923877]
127 **Casabiell X**, Piñeiro V, De la Cruz LF, Gualillo O, Folgar L, Diéguez C, Casanueva FF. Dual effect of insulin on in vitro leptin secretion by adipose tissue. *Biochem Biophys Res Commun* 2000; **276**: 477-482 [PMID: 11027500 DOI: 10.1006/bbrc.2000.3506]
128 **Levy JR**, Stevens W. The effects of insulin, glucose, and pyruvate on the kinetics of leptin secretion. *Endocrinology* 2001; **142**: 3558-3562 [PMID: 11459803 DOI: 10.1210/endo.142.8.8313]
129 **Mueller WM**, Gregoire FM, Stanhope KL, Mobbs CV, Mizuno TM, Warden CH, Stern JS, Havel PJ. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. *Endocrinology* 1998; **139**: 551-558 [PMID: 9449624]
130 **Malmström R**, Taskinen MR, Karonen SL, Yki-Järvinen H. Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. *Diabetologia* 1996; **39**: 993-996 [PMID: 8858224 DOI: 10.1007/BF00403921]
131 **Rayner DV**, Trayhurn P. Regulation of leptin production: sympathetic nervous system interactions. *J Mol Med* (Berl) 2001; **79**: 8-20 [PMID: 11327106 DOI: 10.1007/s001090100198]
132 **Tsai M**, Asakawa A, Amitani H, Inui A. Stimulation of leptin secretion by insulin. *Indian J Endocrinol Metab* 2012; **16**: S543-S548 [PMID: 23565488 DOI: 10.4103/2230-8210.105570]
133 **Wellhoener P**, Fruehwald-Schultes B, Kern W, Dantz D, Kerner W, Born J, Fehm HL, Peters A. Glucose metabolism rather than insulin is a main determinant of leptin secretion in humans. *J Clin Endocrinol Metab* 2000; **85**: 1267-1271 [PMID: 10720074 DOI: 10.1210/jcem.85.3.6483]
134 **Cammisotto PG**, Bukowiecki LJ, Deshaies Y, Bendayan M. Leptin biosynthetic pathway in white adipocytes. *Biochem Cell Biol* 2006; **84**: 207-214 [PMID: 16609702 DOI: 10.1139/o06-032]
135 **Bado A**, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, Moizo L, Lehy T, Guerre-Millo M, Le Marchand-Brustel Y, Lewin MJ. The stomach is a source of leptin. *Nature* 1998; **394**: 790-793 [PMID: 9723619 DOI: 10.1038/29547]
136 **Mix H**, Widjaja A, Jandl O, Cornberg M, Kaul A, Göke M, Beil W, Kuske M, Brabant G, Manns MP, Wagner S. Expression of leptin and leptin receptor isoforms in the human stomach. *Gut* 2000; **47**: 481-486 [PMID: 10986207 DOI: 10.1136/gut.47.4.481]
137 **Sobhani I**, Bado A, Vissuzaine C, Buyse M, Kermorgant S, Laigneau JP, Attoub S, Lehy T, Henin D, Mignon M, Lewin MJ. Leptin secretion and leptin receptor in the human stomach. *Gut* 2000; **47**: 178-183 [PMID: 10896907 DOI: 10.1136/gut.47.2.178]
138 **Cammisotto PG**, Renaud C, Gingras D, Delvin E, Levy E, Bendayan M. Endocrine and exocrine secretion of leptin by the gastric mucosa. *J Histochem Cytochem* 2005; **53**: 851-860 [PMID: 15995144 DOI: 10.1369/jhc.5A6620.2005]
139 **Cammisotto PG**, Gingras D, Bendayan M. Transcytosis of gastric leptin through the rat duodenal mucosa. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G773-G779 [PMID: 17673543]
140 **Cammisotto PG**, Bendayan M. Leptin secretion by white adipose tissue and gastric mucosa. *Histol Histopathol* 2007; **22**: 199-210 [PMID: 17149693]
141 **Cammisotto PG**, Levy E, Bukowiecki LJ, Bendayan M. Cross-talk between adipose and gastric leptins for the control of food intake and energy metabolism. *Prog Histochem Cytochem* 2010; **45**: 143-200 [PMID: 20621336 DOI: 10.1016/j.proghi.2010.06.001]
142 **Cammisotto P**, Bendayan M. A review on gastric leptin: the exocrine secretion of a gastric hormone. *Anat Cell Biol* 2012; **45**: 1-16 [PMID: 22536547 DOI: 10.5115/acb.2012.45.1.1]
143 **Yarandi SS**, Hebbar G, Sauer CG, Cole CR, Ziegler TR. Diverse roles of leptin in the gastrointestinal tract: modulation of motility, absorption, growth, and inflammation. *Nutrition* 2011; **27**: 269-275 [PMID: 20947298 DOI: 10.1016/j.nut.2010.07.004]
144 **Hassink SG**, de Lancey E, Sheslow DV, Smith-Kirwin SM, O'Connor DM, Considine RV, Opentanova I, Dostal K, Spear ML, Leef K, Ash M, Spitzer AR, Funanage VL. Placental leptin: an important new growth factor in intrauterine and neonatal development? *Pediatrics* 1997; **100**: E1 [PMID: 9200375 DOI: 10.1542/peds.100.1.e1]
145 **Pico C**, Jilkova ZM, Kus V, Palou A, Kopecky J. Perinatal programming of body weight control by leptin: putative roles of AMP kinase and muscle thermogenesis. *Am J Clin Nutr* 2011; **94**: 1830S-1837S [PMID: 21543529 DOI: 10.3945/ajcn.110.000752]
146 **Zheng D**, Jones JP, Usala SJ, Dohm GL. Differential expression of ob mRNA in rat adipose tissues in response to insulin. *Biochem Biophys Res Commun* 1996; **218**: 434-437 [PMID: 8561773 DOI: 10.1006/bbrc.1996.0077]
147 **Lee MJ**, Fried SK. Multilevel regulation of leptin storage, turnover, and secretion by feeding and insulin in rat adipose tissue. *J Lipid Res* 2006; **47**: 1984-1993 [PMID: 16738357 DOI: 10.1194/jlr.M600065-JLR200]
148 **Matson CA**, Ritter RC. Long-term CCK-leptin synergy suggests a role for CCK in the regulation of body weight. *Am J Physiol* 1999; **276**: R1038-R1045 [PMID: 10198383]
149 **Matson CA**, Reid DF, Cannon TA, Ritter RC. Cholecystokinin and leptin act synergistically to reduce body weight.

Am J Physiol Regul Integr Comp Physiol 2000; **278**: R882-R890 [PMID: 10749775]
150 **Williams DL**, Baskin DG, Schwartz MW. Leptin regulation of the anorexic response to glucagon-like peptide-1 receptor stimulation. *Diabetes* 2006; **55**: 3387-3393 [PMID: 17130484 DOI: 10.2337/db06-0558]
151 **Nowak A**, Bojanowska E. Effects of peripheral or central GLP-1 receptor blockade on leptin-induced suppression of appetite. *J Physiol Pharmacol* 2008; **59**: 501-510 [PMID: 18953093]
152 **Williams DL**, Baskin DG, Schwartz MW. Hindbrain leptin receptor stimulation enhances the anorexic response to cholecystokinin. *Am J Physiol Regul Integr Comp Physiol* 2009; **297**: R1238-R1246 [PMID: 19726710]
153 **Li Y**, Wu X, Zhou S, Owyang C. Low-affinity CCK-A receptors are coexpressed with leptin receptors in rat nodose ganglia: implications for leptin as a regulator of short-term satiety. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G217-G227 [PMID: 21109591 DOI: 10.1152/ajpgi.00356.2010]
154 **Kentish SJ**, O'Donnell TA, Isaacs NJ, Young RL, Li H, Harrington AM, Brierley SM, Wittert GA, Blackshaw LA, Page AJ. Gastric vagal afferent modulation by leptin is influenced by food intake status. *J Physiol* 2013; **591**: 1921-1934 [PMID: 23266933 DOI: 10.1113/jphysiol.2012.247577]
155 **Hargrave SL**, Kinzig KP. Repeated gastric distension alters food intake and neuroendocrine profiles in rats. *Physiol Behav* 2012; **105**: 975-981 [PMID: 22115950 DOI: 10.1016/j.physbeh.2011.11.006]
156 **Sartor DM**, Verberne AJ. Gastric leptin: a novel role in cardiovascular regulation. *Am J Physiol Heart Circ Physiol* 2010; **298**: H406-H414 [PMID: 19940076]
157 **How JM**, Fam BC, Verberne AJ, Sartor DM. High-fat diet is associated with blunted splanchnic sympathoinhibitory responses to gastric leptin and cholecystokinin: implications for circulatory control. *Am J Physiol Heart Circ Physiol* 2011; **300**: H961-H967 [PMID: 21239630]
158 **Russell CD**, Petersen RN, Rao SP, Ricci MR, Prasad A, Zhang Y, Brolin RE, Fried SK. Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone. *Am J Physiol* 1998; **275**: E507-E515 [PMID: 9725819]
159 **Wabitsch M**, Jensen PB, Blum WF, Christoffersen CT, Englaro P, Heinze E, Rascher W, Teller W, Tornqvist H, Hauner H. Insulin and cortisol promote leptin production in cultured human fat cells. *Diabetes* 1996; **45**: 1435-1438 [PMID: 8826983 DOI: 10.2337/diab.45.10.1435]
160 **Bradley RL**, Cheatham B. Regulation of ob gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. *Diabetes* 1999; **48**: 272-278 [PMID: 10334301 DOI: 10.2337/diabetes.48.2.272]
161 **Norman D**, Isidori AM, Frajese V, Caprio M, Chew SL, Grossman AB, Clark AJ, Michael Besser G, Fabbri A. ACTH and alpha-MSH inhibit leptin expression and secretion in 3T3-L1 adipocytes: model for a central-peripheral melanocortin-leptin pathway. *Mol Cell Endocrinol* 2003; **200**: 99-109 [PMID: 12644303 DOI: 10.1016/S0303-7207(02)00410-0]
162 **Machinal F**, Dieudonne MN, Leneveu MC, Pecquery R, Giudicelli Y. In vivo and in vitro ob gene expression and leptin secretion in rat adipocytes: evidence for a regional specific regulation by sex steroid hormones. *Endocrinology* 1999; **140**: 1567-1574 [PMID: 10098489 DOI: 10.1210/endo.140.4.6617]
163 **Boden G**, Chen X, Kolaczynski JW, Polansky M. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. *J Clin Invest* 1997; **100**: 1107-1113 [PMID: 9276727 DOI: 10.1172/JCI119621]
164 **Utriainen T**, Malmström R, Mäkimattila S, Yki-Järvinen H. Supraphysiological hyperinsulinemia increases plasma leptin concentrations after 4 h in normal subjects. *Diabetes* 1996; **45**: 1364-1366 [PMID: 8826972 DOI: 10.2337/diab.45.10.1364]
165 **Pratley RE**, Nicolson M, Bogardus C, Ravussin E. Effects of acute hyperinsulinemia on plasma leptin concentrations in insulin-sensitive and insulin-resistant Pima Indians. *J Clin Endocrinol Metab* 1996; **81**: 4418-4421 [PMID: 8954052]
166 **Muscelli E**, Camastra S, Masoni A, Baldi S, Sironi AM, Natali A, Ferrannini E. Acute insulin administration does not affect plasma leptin levels in lean or obese subjects. *Eur J Clin Invest* 1996; **26**: 940-943 [PMID: 8911870 DOI: 10.1111/j.1365-2362.1996.tb02142.x]
167 **Clapham JC**, Smith SA, Moore GB, Hughes MG, Azam H, Scott A, Jung RT. Plasma leptin concentrations and OB gene expression in subcutaneous adipose tissue are not regulated acutely by physiological hyperinsulinaemia in lean and obese humans. *Int J Obes Relat Metab Disord* 1997; **21**: 179-183 [PMID: 9080255 DOI: 10.1038/sj.ijo.0800384]
168 **Vidal H**, Auboeuf D, De Vos P, Staels B, Riou JP, Auwerx J, Laville M. The expression of ob gene is not acutely regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. *J Clin Invest* 1996; **98**: 251-255 [PMID: 8755631 DOI: 10.1172/JCI118786]
169 **Carlson JJ**, Turpin AA, Wiebke G, Hunt SC, Adams TD. Pre- and post- prandial appetite hormone levels in normal weight and severely obese women. *Nutr Metab* (Lond) 2009; **6**: 32 [PMID: 19671157 DOI: 10.1186/1743-7075-6-32]
170 **Dagogo-Jack S**, Fanelli C, Paramore D, Brothers J, Landt M. Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes* 1996; **45**: 695-698 [PMID: 8621026 DOI: 10.2337/diab.45.5.695]
171 **Karhunen L**, Haffner S, Lappalainen R, Turpeinen A, Miettinen H, Uusitupa M. Serum leptin and short-term regulation of eating in obese women. *Clin Sci* (Lond) 1997; **92**: 573-578 [PMID: 9205417]
172 **Ishida K**, Murakami T, Mizuno A, Iida M, Kuwajima M, Shima K. leptin suppresses basal insulin secretion from rat pancreatic islets. *Regul Pept* 1997; **70**: 179-182 [PMID: 9272631]
173 **Gray SL**, Donald C, Jetha A, Covey SD, Kieffer TJ. Hyperinsulinemia precedes insulin resistance in mice lacking pancreatic beta-cell leptin signaling. *Endocrinology* 2010; **151**: 4178-4186 [PMID: 20631001 DOI: 10.1210/en.2010-0102]
174 **Kieffer TJ**, Heller RS, Leech CA, Holz GG, Habener JF. Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic beta-cells. *Diabetes* 1997; **46**: 1087-1093 [PMID: 9166685 DOI: 10.2337/diab.46.6.1087]
175 **Covey SD**, Wideman RD, McDonald C, Unniappan S, Huynh F, Asadi A, Speck M, Webber T, Chua SC, Kieffer TJ. The pancreatic beta cell is a key site for mediating the effects of leptin on glucose homeostasis. *Cell Metab* 2006; **4**: 291-302 [PMID: 17011502 DOI: 10.1016/j.cmet.2006.09.005]
176 **Levi J**, Gray SL, Speck M, Huynh FK, Babich SL, Gibson WT, Kieffer TJ. Acute disruption of leptin signaling in vivo leads to increased insulin levels and insulin resistance. *Endocrinology* 2011; **152**: 3385-3395 [PMID: 21750049 DOI: 10.1210/en.2011-0185]
177 **Harris RB**, Zhou J, Redmann SM, Smagin GN, Smith SR, Rodgers E, Zachwieja JJ. A leptin dose-response study in obese (ob/ob) and lean (+/?) mice. *Endocrinology* 1998; **139**: 8-19 [PMID: 9421392 DOI: 10.1210/endo.139.1.5675]
178 **Stich V**, Berlan M. Physiological regulation of NEFA availability: lipolysis pathway. *Proc Nutr Soc* 2004; **63**: 369-374 [PMID: 15294057 DOI: 10.1079/PNS2004350]
179 **McMurray RG**, Hackney AC. Interactions of metabolic hormones, adipose tissue and exercise. *Sports Med* 2005; **35**: 393-412 [PMID: 15896089 DOI: 10.2165/00007256-200535050-00003]
180 **Moinat M**, Deng C, Muzzin P, Assimacopoulos-Jeannet F, Seydoux J, Dulloo AG, Giacobino JP. Modulation of obese gene expression in rat brown and white adipose tissues. *FEBS Lett* 1995; **373**: 131-134 [PMID: 7589451 DOI: 10.1016/0014-5793(95)01030-I]
181 **Trayhurn P**, Duncan JS, Rayner DV. Acute cold-induced suppression of ob (obese) gene expression in white adipose tissue of mice: mediation by the sympathetic system. *Biochem*

J 1995; **311** (Pt 3): 729-733 [PMID: 7487925]
182 **Trayhurn P**, Duncan JS, Hoggard N, Rayner DV. Regulation of leptin production: a dominant role for the sympathetic nervous system? *Proc Nutr Soc* 1998; **57**: 413-419 [PMID: 9793999 DOI: 10.1079/PNS19980060]
183 **Zheng D**, Wooter MH, Zhou Q, Dohm GL. The effect of exercise on ob gene expression. *Biochem Biophys Res Commun* 1996; **225**: 747-750 [PMID: 8780684 DOI: 10.1006/bbrc.1996.1245]
184 **Pérusse L**, Collier G, Gagnon J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Nadeau A, Zimmet PZ, Bouchard C. Acute and chronic effects of exercise on leptin levels in humans. *J Appl Physiol* (1985) 1997; **83**: 5-10 [PMID: 9216937]
185 **Pinkney JH**, Coppack SW, Mohamed-Ali V. Effect of isoprenaline on plasma leptin and lipolysis in humans. *Clin Endocrinol* (Oxf) 1998; **48**: 407-411 [PMID: 9640406 DOI: 10.1046/j.1365-2265.1998.00480.x]
186 **Carulli L**, Ferrari S, Bertolini M, Tagliafico E, Del Rio G. Regulation of ob gene expression: evidence for epinephrine-induced suppression in human obesity. *J Clin Endocrinol Metab* 1999; **84**: 3309-3312 [PMID: 10487703 DOI: 10.1210/jcem.84.9.6007]
187 **Donahoo WT**, Jensen DR, Yost TJ, Eckel RH. Isoproterenol and somatostatin decrease plasma leptin in humans: a novel mechanism regulating leptin secretion. *J Clin Endocrinol Metab* 1997; **82**: 4139-4143 [PMID: 9398728]
188 **Walder K**, Filippis A, Clark S, Zimmet P, Collier GR. Leptin inhibits insulin binding in isolated rat adipocytes. *J Endocrinol* 1997; **155**: R5-R7 [PMID: 9488006 DOI: 10.1677/joe.0.155R005]
189 **Barzilai N**, Wang J, Massilon D, Vuguin P, Hawkins M, Rossetti L. Leptin selectively decreases visceral adiposity and enhances insulin action. *J Clin Invest* 1997; **100**: 3105-3110 [PMID: 9399957 DOI: 10.1172/JCI119865]
190 **Shimabukuro M**, Koyama K, Chen G, Wang MY, Trieu F, Lee Y, Newgard CB, Unger RH. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci USA* 1997; **94**: 4637-4641 [PMID: 9114043 DOI: 10.1073/pnas.94.9.4637]
191 **Barzilai N**, She L, Liu L, Wang J, Hu M, Vuguin P, Rossetti L. Decreased visceral adiposity accounts for leptin effect on hepatic but not peripheral insulin action. *Am J Physiol* 1999; **277**: E291-E298 [PMID: 10444425]
192 **Huang W**, Dedousis N, O'Doherty RM. Hepatic steatosis and plasma dyslipidemia induced by a high-sucrose diet are corrected by an acute leptin infusion. *J Appl Physiol* (1985) 2007; **102**: 2260-2265 [PMID: 17363621 DOI: 10.1152/japplphysiol.01449.2006]
193 **Kraegen EW**, Saha AK, Preston E, Wilks D, Hoy AJ, Cooney GJ, Ruderman NB. Increased malonyl-CoA and diacylglycerol content and reduced AMPK activity accompany insulin resistance induced by glucose infusion in muscle and liver of rats. *Am J Physiol Endocrinol Metab* 2006; **290**: E471-E479 [PMID: 16234268]
194 **Ceci R**, Sabatini S, Duranti G, Savini I, Avigliano L, Rossi A. Acute, but not chronic, leptin treatment induces acyl-CoA oxidase in C2C12 myotubes. *Eur J Nutr* 2007; **46**: 364-368 [PMID: 17571209 DOI: 10.1007/s00394-007-0664-9]
195 **Janovská A**, Hatzinikolas G, Staikopoulos V, McInerney J, Mano M, Wittert GA. AMPK and ACC phosphorylation: effect of leptin, muscle fibre type and obesity. *Mol Cell Endocrinol* 2008; **284**: 1-10 [PMID: 18255222 DOI: 10.1016/j.mce.2007.12.013]
196 **Linares C**, Su D. Body mass index and health among Union Army veterans: 1891-1905. *Econ Hum Biol* 2005; **3**: 367-387 [PMID: 16046203 DOI: 10.2337/diabetes.54.8.2365]
197 **Todd MK**, Yaspelkis BB, Turcotte LP. Short-term leptin treatment increases fatty acids uptake and oxidation in muscle of high fat-fed rats. *Metabolism* 2005; **54**: 1218-1224 [PMID: 16125533 DOI: 10.1016/j.metabol.2005.04.007]
198 **Steinberg GR**, Dyck DJ. Development of leptin resistance in rat soleus muscle in response to high-fat diets. *Am J Physiol Endocrinol Metab* 2000; **279**: E1374-E1382 [PMID: 11093926]
199 **Fishman S**, Muzumdar RH, Atzmon G, Ma X, Yang X, Einstein FH, Barzilai N. Resistance to leptin action is the major determinant of hepatic triglyceride accumulation in vivo. *FASEB J* 2007; **21**: 53-60 [PMID: 17099068 DOI: 10.1096/fj.06-6557com]
200 **Ceddia RB**. Direct metabolic regulation in skeletal muscle and fat tissue by leptin: implications for glucose and fatty acids homeostasis. *Int J Obes* (Lond) 2005; **29**: 1175-1183 [PMID: 16030519 DOI: 10.1038/sj.ijo.0803025]
201 **Rouru J**, Cusin I, Zakrzewska KE, Jeanrenaud B, Rohner-Jeanrenaud F. Effects of intravenously infused leptin on insulin sensitivity and on the expression of uncoupling proteins in brown adipose tissue. *Endocrinology* 1999; **140**: 3688-3692 [PMID: 10433228]
202 **Suzuki R**, Tobe K, Aoyama M, Sakamoto K, Ohsugi M, Kamei N, Nemoto S, Inoue A, Ito Y, Uchida S, Hara K, Yamauchi T, Kubota N, Terauchi Y, Kadowaki T. Expression of DGAT2 in white adipose tissue is regulated by central leptin action. *J Biol Chem* 2005; **280**: 3331-3337 [PMID: 15550388 DOI: 10.1074/jbc.M410955200]
203 **Baldwin KM**, Joanisse DR, Haddad F, Goldsmith RL, Gallagher D, Pavlovich KH, Shamoon EL, Leibel RL, Rosenbaum M. Effects of weight loss and leptin on skeletal muscle in human subjects. *Am J Physiol Regul Integr Comp Physiol* 2011; **301**: R1259-R1266 [PMID: 21917907]
204 **Goldsmith R**, Joanisse DR, Gallagher D, Pavlovich K, Shamoon E, Leibel RL, Rosenbaum M. Effects of experimental weight perturbation on skeletal muscle work efficiency, fuel utilization, and biochemistry in human subjects. *Am J Physiol Regul Integr Comp Physiol* 2010; **298**: R79-R88 [PMID: 19889869]
205 **Jiang L**, Wang Q, Yu Y, Zhao F, Huang P, Zeng R, Qi RZ, Li W, Liu Y. Leptin contributes to the adaptive responses of mice to high-fat diet intake through suppressing the lipogenic pathway. *PLoS One* 2009; **4**: e6884 [PMID: 19727392 DOI: 10.1371/journal.pone.0006884]
206 **Bingham NC**, Anderson KK, Reuter AL, Stallings NR, Parker KL. Selective loss of leptin receptors in the ventromedial hypothalamic nucleus results in increased adiposity and a metabolic syndrome. *Endocrinology* 2008; **149**: 2138-2148 [PMID: 18258679 DOI: 10.1210/en.2007-1200]
207 **Steinberg GR**, Parolin ML, Heigenhauser GJ, Dyck DJ. Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. *Am J Physiol Endocrinol Metab* 2002; **283**: E187-E192 [PMID: 12067860]
208 **Buettner C**, Muse ED, Cheng A, Chen L, Scherer T, Pocai A, Su K, Cheng B, Li X, Harvey-White J, Schwartz GJ, Kunos G, Rossetti L, Buettner C. Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms. *Nat Med* 2008; **14**: 667-675 [PMID: 18516053 DOI: 10.1038/nm1775]
209 **Boghossian S**, Dube MG, Torto R, Kalra PS, Kalra SP. Hypothalamic clamp on insulin release by leptin-transgene expression. *Peptides* 2006; **27**: 3245-3254 [PMID: 16962683 DOI: 10.1016/j.peptides.2006.07.022]
210 **Pérez C**, Fernández-Galaz C, Fernández-Agulló T, Arribas C, Andrés A, Ros M, Carrascosa JM. Leptin impairs insulin signaling in rat adipocytes. *Diabetes* 2004; **53**: 347-353 [PMID: 14747284 DOI: 10.2337/diabetes.53.2.347]
211 **Tortoriello DV**, McMinn JE, Chua SC. Increased expression of hypothalamic leptin receptor and adiponectin accompany resistance to dietary-induced obesity and infertility in female C57BL/6J mice. *Int J Obes* (Lond) 2007; **31**: 395-402 [PMID: 16865100 DOI: 10.1038/sj.ijo.0803392]
212 **Scarpace PJ**, Matheny M, Pollock BH, Tümer N. Leptin increases uncoupling protein expression and energy expenditure. *Am J Physiol* 1997; **273**: E226-E230 [PMID: 9252501]
213 **Scarpace PJ**, Matheny M. Leptin induction of UCP1 gene expression is dependent on sympathetic innervation. *Am J Physiol* 1998; **275**: E259-E264 [PMID: 9688627]

214 **Casas AT**, Hubsch AP, Doran JE. Effects of reconstituted high-density lipoprotein in persistent gram-negative bacteremia. *Am Surg* 1996; **62**: 350-355 [PMID: 8615560 DOI: 10.1038/380677a0]

215 **Zhou YT**, Shimabukuro M, Koyama K, Lee Y, Wang MY, Trieu F, Newgard CB, Unger RH. Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. *Proc Natl Acad Sci USA* 1997; **94**: 6386-6390 [PMID: 9177227 DOI: 10.1073/pnas.94.12.6386]

216 **Scarpace PJ**, Nicolson M, Matheny M. UCP2, UCP3 and leptin gene expression: modulation by food restriction and leptin. *J Endocrinol* 1998; **159**: 349-357 [PMID: 9795377 DOI: 10.1677/joe.0.1590349]

217 **Liu Q**, Bai C, Chen F, Wang R, MacDonald T, Gu M, Zhang Q, Morsy MA, Caskey CT. Uncoupling protein-3: a muscle-specific gene upregulated by leptin in ob/ob mice. *Gene* 1998; **207**: 1-7 [PMID: 9511737]

218 **Reidy SP**, Weber JM. Accelerated substrate cycling: a new energy-wasting role for leptin in vivo. *Am J Physiol Endocrinol Metab* 2002; **282**: E312-E317 [PMID: 11788362]

219 **Solinas G**, Summermatter S, Mainieri D, Gubler M, Pirola L, Wymann MP, Rusconi S, Montani JP, Seydoux J, Dulloo AG. The direct effect of leptin on skeletal muscle thermogenesis is mediated by substrate cycling between de novo lipogenesis and lipid oxidation. *FEBS Lett* 2004; **577**: 539-544 [PMID: 15556643 DOI: 10.1016/j.febslet.2004.10.066]

220 **Minaker KL**, Rowe JW, Young JB, Sparrow D, Pallotta JA, Landsberg L. Effect of age on insulin stimulation of sympathetic nervous system activity in man. *Metabolism* 1982; **31**: 1181-1184 [PMID: 6755163 DOI: 10.1016/0026-0495(80)90078-5]

221 **Woods SC**, Ramsay DS. Food intake, metabolism and homeostasis. *Physiol Behav* 2011; **104**: 4-7 [PMID: 21530564 DOI: 10.1016/j.physbeh.2011.04.026]

222 **Landsberg L**, Saville ME, Young JB. Sympathoadrenal system and regulation of thermogenesis. *Am J Physiol* 1984; **247**: E181-E189 [PMID: 6380306]

223 **Olefsky JM**, Kobayashi M. Mechanisms of fasting-induced increase in insulin binding to rat adipocytes. *J Clin Invest* 1978; **61**: 329-338 [PMID: 621276 DOI: 10.1172/JCI108943]

224 **Fleig WE**, Enderle D, Steudter S, Nöther-Fleig G, Ditschuneit H. Regulation of basal and insulin-stimulated glycogen synthesis in cultured hepatocytes. Inverse relationship to glycogen content. *J Biol Chem* 1987; **262**: 1155-1160 [PMID: 3100527]

225 **Nielsen JN**, Richter EA. Regulation of glycogen synthase in skeletal muscle during exercise. *Acta Physiol Scand* 2003; **178**: 309-319 [PMID: 12864735 DOI: 10.1046/j.1365-201X.2003.01165.x]

226 **Barzilai N**, She L, Liu BQ, Vuguin P, Cohen P, Wang J, Rossetti L. Surgical removal of visceral fat reverses hepatic insulin resistance. *Diabetes* 1999; **48**: 94-98 [PMID: 9892227 DOI: 10.2337/diabetes.48.1.94]

227 **Agren G**, Wilander O, Jorpes E. Cyclic changes in the glycogen content of the liver and the muscles of rats and mice: Their bearing upon the sensitivity of the animals to insulin, and their influence on the urinary output of nitrogen. *Biochem J* 1931; **25**: 777-785 [PMID: 16744641]

228 **Arner P**. Adrenergic receptor function in fat cells. *Am J Clin Nutr* 1992; **55**: 228S-236S [PMID: 1309480]

229 **Assali AR**, Ganor A, Beigel Y, Shafer Z, Hershcovici T, Fainaru M. Insulin resistance in obesity: body-weight or energy balance? *J Endocrinol* 2001; **171**: 293-298 [PMID: 11691649 DOI: 10.1677/joe.0.1710293]

230 **Bergendahl M**, Evans WS, Pastor C, Patel A, Iranmanesh A, Veldhuis JD. Short-term fasting suppresses leptin and (conversely) activates disorderly growth hormone secretion in midluteal phase women--a clinical research center study. *J Clin Endocrinol Metab* 1999; **84**: 883-894 [PMID: 10084566]

231 **Grinspoon SK**, Askari H, Landt ML, Nathan DM, Schoenfeld DA, Hayden DL, Laposata M, Hubbard J, Klibanski A. Effects of fasting and glucose infusion on basal and overnight leptin concentrations in normal-weight women. *Am J Clin Nutr* 1997; **66**: 1352-1356 [PMID: 9394686]

232 **Young JB**, Rosa RM, Landsberg L. Dissociation of sympathetic nervous system and adrenal medullary responses. *Am J Physiol* 1984; **247**: E35-E40 [PMID: 6742188]

233 **Askari H**, Tykodi G, Liu J, Dagogo-Jack S. Fasting plasma leptin level is a surrogate measure of insulin sensitivity. *J Clin Endocrinol Metab* 2010; **95**: 3836-3843 [PMID: 20484473 DOI: 10.1210/jc.2010-0296]

234 **Owecki M**, Nikisch E, Miczke A, Pupek-Musialik D, Sowiński J. Leptin, soluble leptin receptors, free leptin index, and their relationship with insulin resistance and BMI: high normal BMI is the threshold for serum leptin increase in humans. *Horm Metab Res* 2010; **42**: 585-589 [PMID: 20455195 DOI: 10.1055/s-0030-1253422]

235 **Dube JJ**, Bhatt BA, Dedousis N, Bonen A, O'Doherty RM. Leptin, skeletal muscle lipids, and lipid-induced insulin resistance. *Am J Physiol Regul Integr Comp Physiol* 2007; **293**: R642-R650 [PMID: 17491114]

236 **Mizuno A**, Murakami T, Doi T, Shima K. Effect of leptin on insulin sensitivity in the Otsuka Long-Evans Tokushima Fatty rat. *Regul Pept* 2001; **99**: 41-44 [PMID: 11257313 DOI: 10.1016/S0167-0115(01)00215-4]

237 **Sáinz N**, Rodríguez A, Catalán V, Becerril S, Ramírez B, Lancha A, Burgos-Ramos E, Gómez-Ambrosi J, Frühbeck G. Leptin reduces the expression and increases the phosphorylation of the negative regulators of GLUT4 traffic TBC1D1 and TBC1D4 in muscle of ob/ob mice. *PLoS One* 2012; **7**: e29389 [PMID: 22253718]

238 **Roden M**. Hepatic glucose production and insulin resistance. *Wien Med Wochenschr* 2008; **158**: 558-561 [PMID: 18998072 DOI: 10.1007/s10354-008-0595-y]

239 **Young JB**, Landsberg L. Suppression of sympathetic nervous system during fasting. *Obes Res* 1997; **5**: 646-649 [PMID: 9449153 DOI: 10.1126/science.867049]

240 **Maclean PS**, Bergouignan A, Cornier MA, Jackman MR. Biology's response to dieting: the impetus for weight regain. *Am J Physiol Regul Integr Comp Physiol* 2011; **301**: R581-R600 [PMID: 21677272]

241 **MacLean PS**, Higgins JA, Wyatt HR, Melanson EL, Johnson GC, Jackman MR, Giles ED, Brown IE, Hill JO. Regular exercise attenuates the metabolic drive to regain weight after long-term weight loss. *Am J Physiol Regul Integr Comp Physiol* 2009; **297**: R793-R802 [PMID: 19587114]

242 **Blundell JE**, King NA. Effects of exercise on appetite control: loose coupling between energy expenditure and energy intake. *Int J Obes Relat Metab Disord* 1998; **22** Suppl 2: S22-S29 [PMID: 9778093]

243 **Edholm OG**, Fletcher JG, Widdowson EM, Mccance RA. The energy expenditure and food intake of individual men. *Br J Nutr* 1955; **9**: 286-300 [PMID: 13250128 DOI: 10.1079/BJN19550040]

244 **Edholm OG**, Adam JM, Healy MJ, Wolff HS, Goldsmith R, Best TW. Food intake and energy expenditure of army recruits. *Br J Nutr* 1970; **24**: 1091-1107 [PMID: 5484729 DOI: 10.1079/BJN19700112]

245 **Mayer J**, Roy P, Mitra KP. Relation between caloric intake, body weight, and physical work: studies in an industrial male population in West Bengal. *Am J Clin Nutr* 1956; **4**: 169-175 [PMID: 13302165]

246 **Wang J**, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L. Overfeeding rapidly induces leptin and insulin resistance. *Diabetes* 2001; **50**: 2786-2791 [PMID: 11723062 DOI: 10.2337/diabetes.50.12.2786]

247 **Benomar Y**, Wetzler S, Larue-Achagiotis C, Djiane J, Tomé D, Taouis M. In vivo leptin infusion impairs insulin and leptin signalling in liver and hypothalamus. *Mol Cell Endocrinol* 2005; **242**: 59-66 [PMID: 16150536 DOI: 10.1016/j.mce.2005.07.003]

248 **Jackman MR**, Steig A, Higgins JA, Johnson GC, Fleming-

Elder BK, Bessesen DH, MacLean PS. Weight regain after sustained weight reduction is accompanied by suppressed oxidation of dietary fat and adipocyte hyperplasia. *Am J Physiol Regul Integr Comp Physiol* 2008; **294**: R1117-R1129 [PMID: 18287221]

249 **Gurr MI**, Jung RT, Robinson MP, James WP. Adipose tissue cellularity in man: the relationship between fat cell size and number, the mass and distribution of body fat and the history of weight gain and loss. *Int J Obes* 1982; **6**: 419-436 [PMID: 7174187]

250 **Löfgren P**, Andersson I, Adolfsson B, Leijonhufvud BM, Hertel K, Hoffstedt J, Arner P. Long-term prospective and controlled studies demonstrate adipose tissue hypercellularity and relative leptin deficiency in the postobese state. *J Clin Endocrinol Metab* 2005; **90**: 6207-6213 [PMID: 16131581 DOI: 10.1210/jc.2005-0596]

251 **Hirsch J**, Batchelor B. Adipose tissue cellularity in human obesity. *Clin Endocrinol Metab* 1976; **5**: 299-311 [PMID: 1085232 DOI: 10.1016/S0300-595X(76)80023-0]

252 **Samuel VT**, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell* 2012; **148**: 852-871 [PMID: 22385956 DOI: 10.1016/j.cell.2012.02.017]

253 **Morrison CD**, Huypens P, Stewart LK, Gettys TW. Implications of crosstalk between leptin and insulin signaling during the development of diet-induced obesity. *Biochim Biophys Acta* 2009; **1792**: 409-416 [PMID: 18852044]

254 **Ritchie IR**, Gulli RA, Stefanyk LE, Harasim E, Chabowski A, Dyck DJ. Restoration of skeletal muscle leptin response does not precede the exercise-induced recovery of insulin-stimulated glucose uptake in high-fat-fed rats. *Am J Physiol Regul Integr Comp Physiol* 2011; **300**: R492-R500 [PMID: 21084675]

255 **Schwartz MW**, Prigeon RL, Kahn SE, Nicolson M, Moore J, Morawiecki A, Boyko EJ, Porte D. Evidence that plasma leptin and insulin levels are associated with body adiposity via different mechanisms. *Diabetes Care* 1997; **20**: 1476-1481 [PMID: 9283801 DOI: 10.2337/diacare.20.9.1476]

256 **Saltiel AR**. Insulin resistance in the defense against obesity. *Cell Metab* 2012; **15**: 798-804 [PMID: 22682220 DOI: 10.1016/j.cmet.2012.03.001]

257 **Myers MG**, Leibel RL, Seeley RJ, Schwartz MW. Obesity and leptin resistance: distinguishing cause from effect. *Trends Endocrinol Metab* 2010; **21**: 643-651 [PMID: 20846876 DOI: 10.1016/j.tem.2010.08.002]

258 **Seals DR**, Bell C. Chronic sympathetic activation: consequence and cause of age-associated obesity? *Diabetes* 2004; **53**: 276-284 [PMID: 14747276 DOI: 10.2337/diabetes.53.2.276]

259 **Ihara S**, Shimamoto K, Watanabe H, Sakai R, Kawana M. An alpha1-receptor blocker reduces plasma leptin levels in hypertensive patients with obesity and hyperleptinemia. *Hypertens Res* 2006; **29**: 805-811 [PMID: 17283868 DOI: 10.1291/hypres.29.805]

260 **Patel SB**, Reams GP, Spear RM, Freeman RH, Villarreal D. Leptin: linking obesity, the metabolic syndrome, and cardiovascular disease. *Curr Hypertens Rep* 2008; **10**: 131-137 [PMID: 18474180 DOI: 10.1007/s11906-008-0025-y]

261 **Ferrannini E**, Cushman WC. Diabetes and hypertension: the bad companions. *Lancet* 2012; **380**: 601-610 [PMID: 22883509 DOI: 10.1016/S0140-6736(12)60987-8.]

262 **Cheung BM**, Li C. Diabetes and hypertension: is there a common metabolic pathway? *Curr Atheroscler Rep* 2012; **14**: 160-166 [PMID: 22281657 DOI: 10.1007/s11883-012-0227-2]

263 **Van Heek M**, Compton DS, France CF, Tedesco RP, Fawzi AB, Graziano MP, Sybertz EJ, Strader CD, Davis HR. Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J Clin Invest* 1997; **99**: 385-390 [PMID: 9022070 DOI: 10.1172/JCI119171]

264 **Berridge KC**, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev* 1998; **28**: 309-369 [PMID: 9858756 DOI: 10.1016/S0165-0173(98)00019-8]

265 **Ikemoto S**. Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev* 2007; **56**: 27-78 [PMID: 17574681 DOI: 10.1016/j.brainresrev.2007.05.004]

266 **Figlewicz DP**, Benoit SC. Insulin, leptin, and food reward: update 2008. *Am J Physiol Regul Integr Comp Physiol* 2009; **296**: R9-R19 [PMID: 18945945 DOI: 10.1152/ajpregu.90725.2008]

267 **Figlewicz DP**, Sipols AJ. Energy regulatory signals and food reward. *Pharmacol Biochem Behav* 2010; **97**: 15-24 [PMID: 20230849 DOI: 10.1016/j.pbb.2010.03.002.]

268 **Davis JF**, Choi DL, Schurdak JD, Fitzgerald MF, Clegg DJ, Lipton JW, Figlewicz DP, Benoit SC. Leptin regulates energy balance and motivation through action at distinct neural circuits. *Biol Psychiatry* 2011; **69**: 668-674 [PMID: 21035790 DOI: 10.1016/j.biopsych.2010.08.028]

269 **Hoebel BG**, Teitelbaum P. Hypothalamic control of feeding and self-stimulation. *Science* 1962; **135**: 375-377 [PMID: 13907995 DOI: 10.1126/science.135.3501.375]

270 **Berthoud HR**, Münzberg H. The lateral hypothalamus as integrator of metabolic and environmental needs: from electrical self-stimulation to opto-genetics. *Physiol Behav* 2011; **104**: 29-39 [PMID: 21549732 DOI: 10.1016/j.physbeh.2011.04.051]

271 **Blum ID**, Patterson Z, Khazall R, Lamont EW, Sleeman MW, Horvath TL, Abizaid A. Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. *Neuroscience* 2009; **164**: 351-359 [PMID: 19666088 DOI: 10.1016/j.neuroscience.2009.08.009]

272 **Adams AC**, Domouzoglou EM, Chee MJ, Segal-Lieberman G, Pissios P, Maratos-Flier E. Ablation of the hypothalamic neuropeptide melanin concentrating hormone is associated with behavioral abnormalities that reflect impaired olfactory integration. *Behav Brain Res* 2011; **224**: 195-200 [PMID: 21669232 DOI: 10.1016/j.bbr.2011.05.039]

273 **Boutrel B**, de Lecea L. Addiction and arousal: the hypocretin connection. *Physiol Behav* 2008; **93**: 947-951 [PMID: 18262574 DOI: 10.1016/j.physbeh.2007.11.022]

274 **Ludwig DS**, Mountjoy KG, Tatro JB, Gillette JA, Frederich RC, Flier JS, Maratos-Flier E. Melanin-concentrating hormone: a functional melanocortin antagonist in the hypothalamus. *Am J Physiol* 1998; **274**: E627-E633 [PMID: 9575823]

275 **Shimada M**, Tritos NA, Lowell BB, Flier JS, Maratos-Flier E. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* 1998; **396**: 670-674 [PMID: 9872314 DOI: 10.1038/25341]

276 **Tritos NA**, Vicent D, Gillette J, Ludwig DS, Flier ES, Maratos-Flier E. Functional interactions between melanin-concentrating hormone, neuropeptide Y, and anorectic neuropeptides in the rat hypothalamus. *Diabetes* 1998; **47**: 1687-1692 [PMID: 9792536 DOI: 10.2337/diabetes.47.11.1687]

277 **Segal-Lieberman G**, Trombly DJ, Juthani V, Wang X, Maratos-Flier E. NPY ablation in C57BL/6 mice leads to mild obesity and to an impaired refeeding response to fasting. *Am J Physiol Endocrinol Metab* 2003; **284**: E1131-E1139 [PMID: 12582011 DOI: 10.1073/pnas.1633636100]

278 **Mantzoros CS**, Qu D, Frederich RC, Susulic VS, Lowell BB, Maratos-Flier E, Flier JS. Activation of beta(3) adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. *Diabetes* 1996; **45**: 909-914 [PMID: 8666142 DOI: 10.2337/diab.45.7.909]

279 **Kerman IA**, Bernard R, Rosenthal D, Beals J, Akil H, Watson SJ. Distinct populations of presympathetic-premotor neurons express orexin or melanin-concentrating hormone in the rat lateral hypothalamus. *J Comp Neurol* 2007; **505**: 586-601 [PMID: 17924541 DOI: 10.1002/cne.21511]

280 **España RA**, Plahn S, Berridge CW. Circadian-dependent and circadian-independent behavioral actions of hypocretin/orexin. *Brain Res* 2002; **943**: 224-236 [PMID: 12101045 DOI: 10.1016/S0006-8993(02)02653-7]

281 **Rodgers RJ**, Halford JC, Nunes de Souza RL, Canto de Souza AL, Piper DC, Arch JR, Blundell JE. Dose-response ef-

fects of orexin-A on food intake and the behavioural satiety sequence in rats. *Regul Pept* 2000; **96**: 71-84 [PMID: 11102655 DOI: 10.1016/S0167-0115(00)00203-2]
282 **Nakamura T**, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, Sakurai T. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res* 2000; **873**: 181-187 [PMID: 10915829 DOI: 10.1016/S0006-8993(00)02555-5]
283 **Adan RA**, Hillebrand JJ, Danner UN, Cardona Cano S, Kas MJ, Verhagen LA. Neurobiology driving hyperactivity in activity-based anorexia. *Curr Top Behav Neurosci* 2011; **6**: 229-250 [PMID: 21243479 DOI: 10.1007/7854_2010_77]
284 **Borer KT**. Nonhomeostatic control of human appetite and physical activity in regulation of energy balance. *Exerc Sport Sci Rev* 2010; **38**: 114-121 [PMID: 20577059 DOI: 10.1097/JES.0b013e3181e3728f]
285 **Rising R**, Harper IT, Fontvielle AM, Ferraro RT, Spraul M, Ravussin E. Determinants of total daily energy expenditure: variability in physical activity. *Am J Clin Nutr* 1994; **59**: 800-804 [PMID: 8147322]
286 **Schulz LO**, Schoeller DA. A compilation of total daily energy expenditures and body weights in healthy adults. *Am J Clin Nutr* 1994; **60**: 676-681 [PMID: 7942572]
287 **Vanhecke TE**, Franklin BA, Miller WM, deJong AT, Coleman CJ, McCullough PA. Cardiorespiratory fitness and sedentary lifestyle in the morbidly obese. *Clin Cardiol* 2009; **32**: 121-124 [PMID: 19301295 DOI: 10.1002/clc.20458]
288 **Pirke KM**, Trimborn P, Platte P, Fichter M. Average total energy expenditure in anorexia nervosa, bulimia nervosa, and healthy young women. *Biol Psychiatry* 1991; **30**: 711-718 [PMID: 1958768 DOI: 10.1016/0006-3223(91)90016-F]
289 **Casper RC**. The 'drive for activity' and "restlessness" in anorexia nervosa: potential pathways. *J Affect Disord* 2006; **92**: 99-107 [PMID: 16448703 DOI: 10.1016/j.jad.2005.12.039]
290 **Borer KT**, Kelch RP, White MP, Dolson L, Kuhns LR. The role of the septal area in the neuroendocrine control of growth in the adult golden hamster. *Neuroendocrinology* 1977; **23**: 133-150 [PMID: 331134 DOI: 10.1159/000122662]
291 **Borer KT**, Kelch RP, Peugh J, Huseman C. Increased serum growth hormone and somatic growth in adult hamsters with hippocampal transections. *Neuroendocrinology* 1979; **29**: 22-33 [PMID: 471195 DOI: 10.1159/000122901]
292 **Borer KT**, Peters NL, Kelch RP, Tsai AC, Holder S. Contribution of growth, fatness, and activity to weight disturbance after septohypothalamic cuts in adult hamsters. *J Comp Physiol Psychol* 1979; **93**: 907-918 [PMID: 512100 DOI: 10.1037/h0077624]
293 **Borer KT**, Bonna R, Kielb M. Hippocampal serotonin mediates hypoactivity in dietarily obese hamsters: a possible manifestation of aging? *Pharmacol Biochem Behav* 1988; **31**: 885-892 [PMID: 3252281 DOI: 10.1016/0091-3057(88)90400-5]
294 **Tou JC**, Wade CE. Determinants affecting physical activity levels in animal models. *Exp Biol Med* (Maywood) 2002; **227**: 587-600 [PMID: 12192100]
295 **Routtenberg A**, Kuznesof AW. Self-starvation of rats living in activity wheels on a restricted feeding schedule. *J Comp Physiol Psychol* 1967; **64**: 414-421 [PMID: 6082873 DOI: 10.1037/h0025205]
296 **Kavanau JL**. Wheel-running preferences of mice. *Z Tierpsychol* 1966; **23**: 858-866 [PMID: 5996819 DOI: 10.1111/j.1439-0310.1966.tb01715.x]
297 **Potter CD**, Borer KT, Katz RJ. Opiate-receptor blockade reduces voluntary running but not self-stimulation in hamsters. *Pharmacol Biochem Behav* 1983; **18**: 217-223 [PMID: 6300933]
298 **Borer KT**, Potter CD, Fileccia N. Basis for the hypoactivity that accompanies rapid weight gain in hamsters. *Physiol Behav* 1983; **30**: 389-397 [PMID: 6867135 DOI: 10.1016/0031-9384(83)90142-7]
299 **Unger JW**, Livingston JN, Moss AM. Insulin receptors in the central nervous system: localization, signalling mechanisms and functional aspects. *Prog Neurobiol* 1991; **36**: 343-362 [PMID: 1887067 DOI: 10.1016/0301-0082(91)90015-S]
300 **Scott MM**, Lachey JL, Sternson SM, Lee CE, Elias CF, Friedman JM, Elmquist JK. Leptin targets in the mouse brain. *J Comp Neurol* 2009; **514**: 518-532 [PMID: 19350671 DOI: 10.1002/cne.22025]
301 **De Luca MA**, Solinas M, Bimpisidis Z, Goldberg SR, Di Chiara G. Cannabinoid facilitation of behavioral and biochemical hedonic taste responses. *Neuropharmacology* 2012; **63**: 161-168 [PMID: 22063718 DOI: 10.1016/j.neuropharm.2011.10.018]
302 **El Khoury MA**, Gorgievski V, Moutsimilli L, Giros B, Tzavara ET. Interactions between the cannabinoid and dopaminergic systems: evidence from animal studies. *Prog Neuropsychopharmacol Biol Psychiatry* 2012; **38**: 36-50 [PMID: 22300746 DOI: 10.1016/j.pnpbp.2011.12.005]
303 **Peciña S**, Berridge KC. Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? *J Neurosci* 2005; **25**: 11777-11786 [PMID: 16354936 DOI: 10.1523/JNEUROSCI.2329-05.2005]
304 **Barbano MF**, Cador M. Opioids for hedonic experience and dopamine to get ready for it. *Psychopharmacology* (Berl) 2007; **191**: 497-506 [PMID: 17031710 DOI: 10.1007/s00213-008-1390-6]
305 **Rosenbaum M**, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S, Gallagher D, Mayer L, Murphy E, Leibel RL. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *J Clin Invest* 2005; **115**: 3579-3586 [PMID: 16322796 DOI: 10.1172/JCI25977]
306 **Ehrlich S**, Burghardt R, Schneider N, Broecker-Preuss M, Weiss D, Merle JV, Craciun EM, Pfeiffer E, Mann K, Lehmkuhl U, Hebebrand J. The role of leptin and cortisol in hyperactivity in patients with acute and weight-recovered anorexia nervosa. *Prog Neuropsychopharmacol Biol Psychiatry* 2009; **33**: 658-662 [PMID: 19296912 DOI: 10.1016/j.pnpbp.2009.03.007]
307 **Exner C**, Hebebrand J, Remschmidt H, Wewetzer C, Ziegler A, Herpertz S, Schweiger U, Blum WF, Preibisch G, Heldmaier G, Klingenspor M. Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. *Mol Psychiatry* 2000; **5**: 476-481 [PMID: 11032380 DOI: 10.1038/sj.mp.4000771]
308 **Hebebrand J**, Exner C, Hebebrand K, Holtkamp C, Casper RC, Remschmidt H, Herpertz-Dahlmann B, Klingenspor M. Hyperactivity in patients with anorexia nervosa and in semi-starved rats: evidence for a pivotal role of hypoleptinemia. *Physiol Behav* 2003; **79**: 25-37 [PMID: 12818707 DOI: 10.1016/S0031-9384(03)00102-1]
309 **Hebebrand J**, Albayrak Ö. Leptin treatment of patients with anorexia nervosa? The urgent need for initiation of clinical studies. *Eur Child Adolesc Psychiatry* 2012; **21**: 63-66 [PMID: 22290117 DOI: 10.1007/s00787-012-0243-3]
310 **Kaiyala KJ**, Prigeon RL, Kahn SE, Woods SC, Schwartz MW. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. *Diabetes* 2000; **49**: 1525-1533 [PMID: 10969837 DOI: 10.2337/diabetes.49.9.1525]
311 **Schwartz MW**, Peskind E, Raskind M, Boyko EJ, Porte D. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* 1996; **2**: 589-593 [PMID: 8616722 DOI: 10.1038/nm0596-589]
312 **Chakravarthy MV**, Booth FW. Eating, exercise, and "thrifty" genotypes: connecting the dots toward an evolutionary understanding of modern chronic diseases. *J Appl Physiol* (1985) 2004; **96**: 3-10 [PMID: 14660491 DOI: 10.1152/japplphysiol.00757.2003]
313 **Campbell BA**, Fibiger HC. Effects of insulin on spontaneous activity during food deprivation. *J Comp Physiol Psychol* 1970; **71**: 341-346 [PMID: 5480866 DOI: 10.1037/h0029132]
314 **Brooks CM**, Lambert HF. A study of the effect of limitation of food intake and the method of feeding on the rate of

weight gain during hypothalamic obesity in the albino rat. *Am J Physiol* 1946; **147**: 695-707 [PMID: 20277062]
315 **Ferguson NB**, Keesey RE. Comparison of ventromedial hypothalamic lesion effects upon feeding and lateral hypothalamic self-stimulation in the female rat. *J Comp Physiol Psychol* 1971; **74**: 263-271 [PMID: 5541552 DOI: 10.1037/h0030315]
316 **Hoebel BG**, Teitelbaum P. Weight regulation in normal and hypothalamic hyperphagic rats. *J Comp Physiol Psychol* 1966; **61**: 189-193 [PMID: 5909295 DOI: 10.1037/h0023126]
317 **Jensen LB**, Quaade F, Sørensen OH. Bone loss accompanying voluntary weight loss in obese humans. *J Bone Miner Res* 1994; **9**: 459-463 [PMID: 8030433 DOI: 10.1002/jbmr.5650090404]
318 **Borer KT**. Absence of weight regulation in exercising hamsters. *Physiol Behav* 1974; **12**: 589-597 [PMID: 4856809 DOI: 10.1016/0031-9384(74)90207-8]
319 **Kennedy GC**, Mitra J. Body weight and food intake as initiating factors for puberty in the rat. *J Physiol* 1963; **166**: 408-418 [PMID: 14031944]
320 **Kennedy GC**. The development with age of hypothalamic restraint upon the appetite of the rat. *J Endocrinol* 1957; **16**: 9-17 [PMID: 13491729 DOI: 10.1677/joe.0.0160009]
321 **Kennedy GC**, Mitra J. Hypothalamic control of energy balance and the reproductive cycle in the rat. *J Physiol* 1963; **166**: 395-407 [PMID: 14031945]
322 **Borer KT**, Kooi AA. Regulatory defense of the exercise-induced weight elevation in hamsters. *Behav Biol* 1975; **13**: 301-310 [PMID: 1120043 DOI: 10.1016/S0091-6773(75)91332-2]
323 **Borer KT**, Kaplan LR. Exercise-induced growth in golden hamsters: effects of age, weight, and activity level. *Physiol Behav* 1977; **18**: 29-34 [PMID: 905378 DOI: 10.1016/0031-9384(77)90089-0]
324 **Browne SA**, Borer KT. Basis for the exercise-induced hyperphagia in adult hamsters. *Physiol Behav* 1978; **20**: 553-557 [PMID: 684089 DOI: 10.1016/0031-9384(78)90246-9]
325 **Borer KT**, Kelch RP. Increased serum growth hormone and somatic growth in exercising adult hamsters. *Am J Physiol* 1978; **234**: E611-E616 [PMID: 665764]
326 **Borer KT**, Nicoski DR, Owens V. Alteration of pulsatile growth hormone secretion by growth-inducing exercise: involvement of endogenous opiates and somatostatin. *Endocrinology* 1986; **118**: 844-850 [PMID: 2867892 DOI: 10.1210/endo-118-2-844]
327 **Tomljenović Borer K**, Kuhns LR. Radiographic evidence for acceleration of skeletal growth in adult hamsters by exercise. *Growth* 1977; **41**: 1-13 [PMID: 870392]
328 **Karsenty G**, Oury F. The central regulation of bone mass, the first link between bone remodeling and energy metabolism. *J Clin Endocrinol Metab* 2010; **95**: 4795-4801 [PMID: 21051575 DOI: 10.1210/jc.2010-1030]

P- Reviewer: Lehtonen SH, Liu EQ, Pirola L, Zhao D
S- Editor: Wen LL **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.630

World J Diabetes 2014 October 15; 5(5): 630-635
ISSN 1948-9358 (online)

REVIEW

Challenges of emerging adulthood-transition from paediatric to adult diabetes

Gurmit Gill, Ananth U Nayak, Julie Wilkins, Jo Hankey, Parakkal Raffeeq, George I Varughese, Lakshminarayanan Varadhan

Gurmit Gill, Ananth U Nayak, Julie Wilkins, George I Varughese, Lakshminarayanan Varadhan, Department of Diabetes and Endocrinology, University Hospital of North Staffordshire NHS Trust, Stoke on Trent, ST4 6QG, United Kingdom
Jo Hankey, Parakkal Raffeeq, Department of Paediatrics, University Hospital of North Staffordshire NHS Trust, Stoke on Trent, ST4 6QG, United Kingdom
Author contributions: Gill G wrote the first draft; all other authors equally contributed to the work, reviewed and edited the manuscript and approved the final version.
Correspondence to: Dr. Ananth U Nayak, MRCP, MRCP, Consultant Physician in Diabetes and Endocrinology, Department of Diabetes and Endocrinology, University Hospital of North Staffordshire NHS Trust, City General, Newcastle Road, Stoke on Trent, ST4 6QG, United Kingdom. ananth.nayak@nhs.net
Telephone: +44-1782-679997 Fax: +44-8436-365428
Received: January 5, 2014 Revised: March 29, 2014
Accepted: June 18, 2014
Published online: October 15, 2014

Abstract

Diabetes mellitus is a complex condition with far reaching physical, psychological and psychosocial effects. These outcomes can be significant when considering the care of a youth transferring from paediatric through to adult diabetes services. The art of mastering a smooth care transfer is crucial if not pivotal to optimising overall diabetic control. Quite often the nature of consultation varies between the two service providers and the objectives and outcomes will mirror this. The purpose of this review is to analyse the particular challenges and barriers one might expect to encounter when transferring these services over to an adult care provider. Particular emphasis is paid towards the psychological aspects of this delicate period, which needs to be recognised and appreciated appropriately in order to understand the particular plights a young diabetic child will be challenged with. We explore the approaches that can be positively adopted in order to improve the experience for child, parents and also the multi- disciplinary team concerned with the overall delivery of this care. Finally we will close with reflection on the potential areas for future development that will ultimately aim to improve long-term outcomes and experiences of the young adolescent confronted with diabetes as well as the burden of disease and burden of cost of disease.

Key words: Transition; Adolescent; Young adult; Diabetes

Core tip: This manuscript is a comprehensive review of the challenges encountered during the transition of diabetes care from paediatric to adult diabetes services. Further we explore the structured transitional programs that could help in the smooth transition of diabetes care from the youth to early adulthood.

Gill G, Nayak AU, Wilkins J, Hankey J, Raffeeq P, Varughese GI, Varadhan L. Challenges of emerging adulthood-transition from paediatric to adult diabetes. *World J Diabetes* 2014; 5(5): 630-635 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/630.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.630

INTRODUCTION

Adolescence is a term derived from the Latin word, "to grow up"[1]. Not only does it denote a transitional phase of physical change and maturation, but also an immense modification of patient psychology. The presence of chronic long term illnesses such as type 1 diabetes may make this vulnerable group even more prone to fall out of the healthcare system and be prone for development of acute or chronic complications.

Diabetes is a complex condition that patients of all

ages often struggle to manage, as it requires many adaptations and modifications to lifestyle. It is often a great challenge when we take into consideration the physical, social and psychological interactions that a young adolescent is often faced with[2]. The scope for development in this area remains vast and the need for a structured framework paramount[3]. The diabetic consultation also changes in a way, from being initially a complex, dynamic parent-led interaction to being a physician-led shortened purpose driven appointment. The lack of this assumed comfortable niche may often leave the adolescent with diabetes feeling abandoned and thus susceptible to poor diabetic control and its complications. The purpose of this article is to highlight the importance of a structured transitional program that could help to alleviate some of the challenges of this turbulent process and help to enable a swift transition from early youth to emerging adulthood.

The key aspects to focus in this review are assessing risks of developing poor glycaemic control during this period, risk of potential complications, acute *vs* chronic as well as possible ways of engaging this at-risk vulnerable cohort of patients. We explore the potential implications of transition from paediatric to adult services and the potential processes that could be considered for service development and also to enhance the patient journey.

THE SCOPE OF THE PROBLEM

The proportion of young adults under the age of 20 years affected by diabetes in 2010 was 0.26%[4]. The "search for diabetes in Youth" study estimated that on average approximately 15000 youth are diagnosed with type 1 diabetes and 3700 with type 2 diabetes annually in the United States[5]. Given the changes in demographics and society, these numbers are projected to increase year on year and henceforth highlighting the need to be vigilant of the problems young teens face and being able to provide a framework to forward the development of this specific aspect of adolescent care[6,7].

The other key feature to be alerted to when accounting for this cohort of patients is the projected increase in the childhood onset of type 2 diabetes[8]. As sedentary lifestyles become more common and fast food more readily available thereby propelling obesity incidence, the emerging numbers of type 2 diabetics is ever more a problem we will encounter in clinical practice[9]. This would therefore cascade down to increasing numbers of adolescents with diabetes who will eventually transition from paediatric to adult care ultimately. The important aspect to be aware of in provision of care for this cohort of patients is to be alert to the changes they will be facing. Thus as any other adolescent will be assuming new roles and changing identities, this is no less apparent in adolescents with diabetes mellitus. The transition services therefore needs to be adapted accordingly and the clinician needs an appreciation of the complexities the youth will be challenged with in general, but more so particularly in the setting of diabetes mellitus.

MODELS OF DEVELOPMENT

There are various psychological models of development that have been put forward to explain the key stages in a young adolescent's life[10]. It is pertinent to be aware of these theories in order to tailor our approach and modify these according to the stages of development. Ignorance of these changes of roles may lead to the provision of sub-optimal care and hence ultimately compromise diabetic control and leave the youth prone to complications, both in the immediate and longer term.

A model of impact of personal change that reflects the changes the young adolescent individual experiences has been previously proposed[10]. The model has a useful analogy to the changes a young adolescent would experience when transitioning of their diabetic care[11]. There is an initial excitement and almost "honeymoon" phase where the young youth is coming of adulthood and excited to be leaving paediatric services, only to gain autonomy of their own care. However this is later followed by a sense of confusion and lack of confidence and almost a crisis stage. Alongside understanding theories of development and change it is also critical to appreciate and understand the corresponding psychosocial changes the youth will be greeted with. This period of emerging adulthood is often the period of most change where young children are assuming new roles of education, moving out of the parental home and progressing towards seeking employment and independence. It is of paramount importance to understand this psychological metamorphosis of a teenager, as it is during this process that the adolescents are most susceptible to run into problems and lack of understanding of this process by the adult care providers acts as a confounding barrier to effective care provision[12]. Care providers and service managers need to acknowledge this and incorporate necessary amendments in their model of care delivery, without which the care could be disruptive, disjointed and not tailored leading to high fall-out rates[13].

In many countries, suboptimal outcomes in the management of diabetes in young adults have lead to centralization of diabetes care. With this the optimization of treatment and outcomes is concentrated in such regional centres and centres of excellence, and subsequently used to reach out to comprehensively improve care in all regions. The need for a multi-disciplinary team, the central role of education and the overlying need for better metabolic control depend on such centres. In developing countries, such centres may develop spontaneously based on perceived need for centralized policies and action. In more comprehensive care systems such as in Europe, marginal outcome data force health care providers to redesign diabetes care, which in some countries is resulting in an orchestrated centre development.

Table 1 Changes in the HbA1c during the transition through the joint transition clinics and the young adult clinics (*n* = 65)

	Joint transition clinic	Young adult clinic
Mean entry age (yr)	17.1	18.5
Number of patients per clinic	2.9	2.7
Mean change HbA1c (DCCT HbA1c %)	0.1	0.2
Mean HbA1c entry (DCCT HbA1c %)	9.8	9.7
Mean HbA1c exit (DCCT HbA1c %)	9.7	9.8
Proportion with > 1% HbA1C (DCCT) improvement	25%	19%
Proportion with improvement in HbA1c	49%	50%

DCCT: Diabetes control and complications trial.

CHALLENGES AND BARRIERS IN THE TRANSITION PROCESS

Delivery of care

Perhaps the biggest change in transition of care is mode of delivery[14]. Initially the child will encounter an aspect of their diabetes care being provided in a very family centered manner, in converse to adult care which is very much assumed and based on the young adult gaining autonomy and identity of their own care, without the parental guidance and support. Different roles and methods have been adopted in the clinical setting to help face and tackle these challenging times[15].

Emerging evidence is gaining credibility that by providing transitional care based on gradual transition is far more successful and advantageous in terms of outcomes, as opposed to offering a simple transfer to care to services[16]. This allows the individual to experience a smooth healthcare experience that is free of plentiful turmoil and change. Certain centers have set up a joint transition clinic whereby paediatricians, adult clinicians and specialist diabetic nurses (DSN) from Paediatric and Adolescent services are directly involved in the delivery of care in this potentially vulnerable period.

We report the model of transition diabetes care in our regional tertiary center where the transition process pans out over 6-8 clinic appointments over a typical 24 mo period, staged through Joint Transition clinic and Young adult clinic. Children with diabetes ready for transition are identified by the paediatric diabetes team and reviewed in the Joint Transition clinics. Majority of these children are between 16 and 18 years of age. During the first two reviews the clinic is led by the paediatric team with the adult diabetes Consultant and a DSN from the adult diabetes team sitting in the joint clinics. The adult team leads the clinic in the subsequent 2 visits after which the care is transferred to a young adult diabetes clinic. Young Adult diabetes clinics are run by the same adult diabetes consultant and adult DSN, provide longer duration of consultation for each appointment, and provide open access to diabetes services through the same named DSN. A telephone reminder service is provided through secretarial staff, to improve attendance rates at these clinics. Each young adult is reviewed 2 to 4 times a year in the Young Adult clinic, for up to 3 years based on clinical needs, before being provisionally transferred to general adult diabetes clinic.

By delivering such a model for transition of care there was an overall significant improvement in attendance rates: 72% attendance rates (of 266 appointments) in the joint transition clinics and 75% attendance rates (of 254 patients appointments) in the young adult diabetes clinic compared to the 45% attendance rate prior to the introduction of this robust pathway.

HbA$_{1c}$ and glycaemic control

The success of a holistic diabetes care can be objectively measured and monitored using glycaemic control as a service indicator. Achievement of target glycaemia in the young adolescent group can be challenging with large studies reporting less than one-third achieving the recommended glycaemic targets[17].

The glycaemic control in the two clinical settings was assessed as part of an internal audit done at our centre. Table 1 shows the changes in the glycaemia as assessed by the HbA$_{1c}$ with the implementation of the transition model at our centre. The HbA$_{1c}$ improved in half of the cohort in the transition model with an significant portion achieving the glycaemic targets of HbA$_{1c}$ < 7.5%.

Loss to follow up

The competing interest of adolescent life along with it inherent psychological changes lead to non-adherence to the service, which can be easily assessed by non-attendance rates to the clinics. There are inevitably adverse short and long term outcomes of patients that are lost to follow up of care[18,19], such as increased risk of acute glycaemia related complications like DKA and severe hypoglycaemia, long term damage to end organs by way of diabetic retinopathy, nephropathy and longer term cardiovascular damage[20]. Patients who are lost to follow up have higher risk of hospitalisation with its huge health care cost implications and increased risk of all-cause mortality[21,22]. Henceforth, various strategies of improving attendance need to be put forward and implemented to improve adherence to the service.

Various centers have devised methods to tackle and approach the above obstacle and have found that use of a simple text reminder service to remind patients of clinic appointments will help them engage with services better thereby helping with improving long term outcomes[23].

At our centre, introduction of a simple telephone service made a significant impact on attendance rates at these clinics. A non-medical staff member of the team (medical secretary) made a phone call 2-3 d prior to the appointment, with the sole purpose of establishing con-

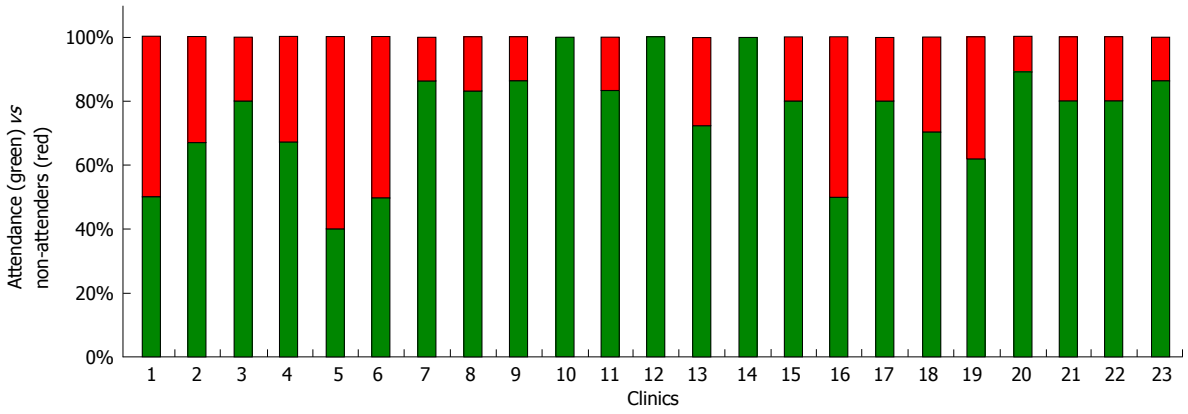

Figure 1 Attendance and non-attendance rates before and after introduction of the telephone service in the young adult clinics[1]. The telephone reminder was 2 d prior to the appointment (clinic 7 onwards, except clinic 16 and 19) significantly improved attendance rates compared to clinics without it.

Table 2 Impact of the telephonic service on the non-attendance rates in the young adult diabetes clinics

Non-attendance rates	Before telephonic intervention (6 clinics)	With telephonic intervention (15 clinics)
Overall non-attendance rate (%)	41	15
New patient (%)	47	8
Follow up patients (%)	30	19

tact and providing a reminder of the forthcoming appointment. The non-attendance rates were reviewed in 23 clinics-6 clinics pre introduction of telephone service and 17 clinics post introduction of the service of which in 2 clinics the service was not used (due to leave of the staff involved and this was effectively a reality check *per se*). There was a significant reduction in non-attendance rate with the introduction of the telephone reminder, both for new and follow-up patients (Table 2). The two of the 17 clinics which did not have this service since the introduction of the process, showed significantly higher non-attendance rates (50% and 38%) thereby internally proving the value of the appointment reminder service and emphasizing how prudent it can be in enhancing attendance to the young adult diabetes clinic (Figure 1). The introduction of a simple telephone service to remind patients of their clinic appointments therefore proved to be a simple addition to improve efficient utilisation of clinic time and in the longer run could demonstrate to be significantly cost effective.

Psychosocial stressors as barriers

Young adults with diabetes are also more likely to face psychological issues hindering their care and management, as evidenced by any patients challenging chronic long-term conditions[24,25]. Thus efficient delivery of care is crucial to allow for this vulnerable patient group in a susceptible period where their lives are simultaneously changing.

Depression in diabetes is a recognised co-morbid factor and will increases mortality and leads to poorer glycaemic control[26]. Up to 33% of adolescent's aged 18-30 years will report depressive symptoms[27]. It is also important to be vigilant of the high risk of eating disorders and substance misuse and insulin misuse, with the risk of misusing insulin for unhealthy weight control measures being quoted to be as high as 57%[28,29].

In our transition clinic setting all patients have access to psychological support from the clinical psychologist embedded in the diabetes team. Some patients are specifically referred to psychology if the teams have any concerns. Authors believe such a model is efficient way of utilization of resources and can be easily replicated across the globe.

Sexual and reproductive health

Unplanned pregnancy remains a major problem in teenagers with co-existing diabetes. The use of contraception has been found to be lower in patients with diabetes (39%) as compared to those without (27%)[30]. Issues around contraception need to be proactively addressed at the young adult diabetes clinics, with emphasis on pre-conception counseling and optimising diabetes care to improve fetal and maternal outcomes[31]. This again highlights the multitude of dimensions that the consultation at the young adult clinic needs to take and address numerous additional challenging issues that young teens will now face.

RECOMMENDATIONS TO IMPROVE MODELS OF CARE

It is therefore prudent that the transition care for children with diabetes should be structured, coordinated with a multi-disciplinary approach with collaboration and communication between the paediatric and adult diabetes teams and making sure the young adult's care is effectively taken over by the adult diabetes team with prior engagement in conjunction with the paediatric team. Despite the clear need for such systematic transition there appears to be lack of a structured approach to this provision and delivery of successful care to provide a service

that is multifaceted and enables the interactions to occur in a step wise fashion allowing the gentle introduction of adult services and gradually stepping away from paediatric input.

Young adults with diabetes, as with any teenage child facing a chronic long term condition, are more vulnerable to the changes of adaptation in care and hence there is greater risk of this care being compromised at a time where they need it most and at a time where the longer term complications (as well as acute) need to be screened and monitored for[32]. One key obstacle identified here is the loss to follow up of these patients. There is evidence to support the use of a simple telephonic calling system in order to aid compliance and concordance with the adult services and ultimately improve outcomes, reduce long-term complications and reduction of end point mortality.

There is evidence that structured transition processes improve health outcomes and quality of life. International organizations including American Diabetes Association, International society for pediatric and adolescent diabetes, Diabetes United Kingdom recommend a structured framework of goals to be outlined and met when transition care of young diabetics to adult services[33-35]. There are no proven uniform strategies to achieve all these goals, although programs that particularly target the young adult with diabetes through education, skills training, specialty transition clinics, or addition of transition coordinators may help towards achieving such goals, for this rising global challenge[36]. It is therefore pivotal that every effort is made to encompass all aspects of their care which will be instrumental in designing and developing a joint care pathway for young adults emerging into adulthood for a well-recognized but less commonly perceived problem in routine clinical practice in the world of diabetes.

REFERENCES

1 The American Heritage Dictionary of the English Language, Fourth Edition. Houghton Mifflin Company, 2000

2 **Laffel LM**, Vangsness L, Connell A, Goebel-Fabbri A, Butler D, Anderson BJ. Impact of ambulatory, family-focused teamwork intervention on glycemic control in youth with type 1 diabetes. *J Pediatr* 2003; **142**: 409-416 [PMID: 12712059 DOI: 10.1067/mpd.2003.138]

3 **Johnson G**. Diabetes transition: Assessment of current best practice and development of a future work programme to improve transition processes for young people with diabetes. 2001. Available from: URL: https: //www.diabetes.org.uk/Documents/nhs-diabetes/paediatrics/diabetes-transition-report.pdf

4 **Centers for Disease Control and Prevention**. National Diabetes Fact Sheet: National Estimates and General Information on Diabetes and Prediabetes in the United States, 2011. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2011. Available from: URL: http://www.cdc.gov/diabetes/pubs/factsheet11.htm

5 **Dabelea D**, Bell RA, D'Agostino RB, Imperatore G, Johansen JM, Linder B, Liu LL, Loots B, Marcovina S, Mayer-Davis EJ, Pettitt DJ, Waitzfelder B. Incidence of diabetes in youth in the United States. *JAMA* 2007; **297**: 2716-2724 [PMID: 17595272 DOI: 10.1001/jama.297.24.2716]

6 **Harjutsalo V**, Sjöberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *Lancet* 2008; **371**: 1777-1782 [PMID: 18502302 DOI: 10.1016/S0140-6736(08)60765-5]

7 **Patterson CC**, Dahlquist GG, Gyürüs E, Green A, Soltész G. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. *Lancet* 2009; **373**: 2027-2033 [PMID: 19481249 DOI: 10.1016/S0140-6736(09)60568-7]

8 Type 2 diabetes in children and adolescents. American Diabetes Association. *Diabetes Care* 2000; **23**: 381-389 [PMID: 10868870 DOI: 10.2337/diacare.23.3.381]

9 **Pinhas-Hamiel O**, Zeitler P. The global spread of type 2 diabetes mellitus in children and adolescents. *J Pediatr* 2005; **146**: 693-700 [PMID: 15870677 DOI: 10.1016/j.jpeds.2004.12.042]

10 **Hopson B,** Adams J. Towards an under-standing: defining some boundaries of transition dynamics. In Transition: Understanding and Managing Personal Change. Adams J, Hayes J, Hopson B, Eds. London, Martin Robertson, 1976: 3-25

11 **Salmi J**, Huupponen T, Oksa H, Oksala H, Koivula T, Raita P. Metabolic control in adolescent insulin-dependent diabetics referred from pediatric to adult clinic. *Ann Clin Res* 1986; **18**: 84-87 [PMID: 3717880]

12 **Nakhla M**, Daneman D, To T, Paradis G, Guttmann A. Transition to adult care for youths with diabetes mellitus: findings from a Universal Health Care System. *Pediatrics* 2009; **124**: e1134-e1141 [PMID: 19933731 DOI: 10.1542/peds.2009-0041]

13 **Frank M**. Evaluation of a transition from pediatric to adult diabetes care program. *Can J Diabetes Care* 2002; **26** (Suppl 1): 253

14 **Logan J**, Peralta E, Brown K, Moffett M, Advani A. Leech A: Smoothing the transition from paediatric to adult services in type 1 diabetes. *J Diabetes Nurs* 2008; **12**: 328-333

15 **Van Walleghem N**, Macdonald CA, Dean HJ. Evaluation of a systems navigator model for transition from pediatric to adult care for young adults with type 1 diabetes. *Diabetes Care* 2008; **31**: 1529-1530 [PMID: 18458141 DOI: 10.2337/dc07-2247]

16 **American Academy of Pediatrics**; American Academy of Family Physicians; American College of Physicians-American Society of Internal Medicine. A consensus statement on health care transitions for young adults with special health care needs. *Pediatrics* 2002; **110**: 1304-1306 [PMID: 12456949]

17 **Petitti DB**, Klingensmith GJ, Bell RA, Andrews JS, Dabelea D, Imperatore G, Marcovina S, Pihoker C, Standiford D, Waitzfelder B, Mayer-Davis E. Glycemic control in youth with diabetes: the SEARCH for diabetes in Youth Study. *J Pediatr* 2009; **155**: 668-72.e1-3 [PMID: 19643434]

18 **Rewers A**, Chase HP, Mackenzie T, Walravens P, Roback M, Rewers M, Hamman RF, Klingensmith G. Predictors of acute complications in children with type 1 diabetes. *JAMA* 2002; **287**: 2511-2518 [PMID: 12020331 DOI: 10.1001/jama.287.19.2511]

19 **Kershnar AK**, Daniels SR, Imperatore G, Palla SL, Petitti DB, Pettitt DJ, Marcovina S, Dolan LM, Hamman RF, Liese AD, Pihoker C, Rodriguez BL. Lipid abnormalities are prevalent in youth with type 1 and type 2 diabetes: the SEARCH for Diabetes in Youth Study. *J Pediatr* 2006; **149**: 314-319 [PMID: 16939739 DOI: 10.1016/j.jpeds.2006.04.065]

20 **Rodriguez BL**, Fujimoto WY, Mayer-Davis EJ, Imperatore G, Williams DE, Bell RA, Wadwa RP, Palla SL, Liu LL, Kershnar A, Daniels SR, Linder B. Prevalence of cardiovascular disease risk factors in U.S. children and adolescents with diabetes: the SEARCH for diabetes in youth study. *Diabetes Care* 2006; **29**: 1891-1896 [PMID: 16873798 DOI: 10.2337/dc06-0310]

21 **Laing SP**, Swerdlow AJ, Slater SD, Botha JL, Burden AC, Waugh NR, Smith AW, Hill RD, Bingley PJ, Patterson CC,

Qiao Z, Keen H. The British Diabetic Association Cohort Study, I: all-cause mortality in patients with insulin-treated diabetes mellitus. *Diabet Med* 1999; **16**: 459-465 [PMID: 10391392 DOI: 10.1046/j.1464-5491.1999.00075.x]

22 **Jacobson AM**, Hauser ST, Willett J, Wolfsdorf JI, Herman L. Consequences of irregular versus continuous medical follow-up in children and adolescents with insulin-dependent diabetes mellitus. *J Pediatr* 1997; **131**: 727-733 [PMID: 9403654 DOI: 10.1016/S0022-3476(97)70101-X]

23 **Franklin VL**, Waller A, Pagliari C, Greene SA. A randomized controlled trial of Sweet Talk, a text-messaging system to support young people with diabetes. *Diabet Med* 2006; **23**: 1332-1338 [PMID: 17116184 DOI: 10.1111/j.1464-5491.2006.01989.x]

24 **Hislop AL**, Fegan PG, Schlaeppi MJ, Duck M, Yeap BB. Prevalence and associations of psychological distress in young adults with Type 1 diabetes. *Diabet Med* 2008; **25**: 91-96 [PMID: 18199136 DOI: 10.1111/j.1464-5491.2007.02310.x]

25 **Hood KK**, Huestis S, Maher A, Butler D, Volkening L, Laffel LM. Depressive symptoms in children and adolescents with type 1 diabetes: association with diabetes-specific characteristics. *Diabetes Care* 2006; **29**: 1389-1391 [PMID: 16732028 DOI: 10.2337/dc06-0087]

26 **Lustman PJ**, Anderson RJ, Freedland KE, de Groot M, Carney RM, Clouse RE. Depression and poor glycemic control: a meta-analytic review of the literature. *Diabetes Care* 2000; **23**: 934-942 [PMID: 10895843 DOI: 10.2337/diacare.23.7.934]

27 **Lawrence JM**, Standiford DA, Loots B, Klingensmith GJ, Williams DE, Ruggiero A, Liese AD, Bell RA, Waitzfelder BE, McKeown RE. Prevalence and correlates of depressed mood among youth with diabetes: the SEARCH for Diabetes in Youth study. *Pediatrics* 2006; **117**: 1348-1358 [PMID: 16585333 DOI: 10.1542/peds.2005-1398]

28 **Neumark-Sztainer D**, Patterson J, Mellin A, Ackard DM, Utter J, Story M, Sockalosky J. Weight control practices and disordered eating behaviors among adolescent females and males with type 1 diabetes: associations with sociodemographics, weight concerns, familial factors, and metabolic outcomes. *Diabetes Care* 2002; **25**: 1289-1296 [PMID: 12145223 DOI: 10.2337/diacare.25.8.1289]

29 **Rydall AC**, Rodin GM, Olmsted MP, Devenyi RG, Daneman D. Disordered eating behavior and microvascular complications in young women with insulin-dependent diabetes mellitus. *N Engl J Med* 1997; **336**: 1849-1854 [PMID: 9197212 DOI: 10.1056/NEJM199706263362601]

30 **Vahratian A**, Barber JS, Lawrence JM, Kim C. Family-planning practices among women with diabetes and overweight and obese women in the 2002 National Survey For Family Growth. *Diabetes Care* 2009; **32**: 1026-1031 [PMID: 19279299 DOI: 10.2337/dc08-2105]

31 **Charron-Prochownik D**, Sereika SM, Wang SL, Hannan MF, Fischl AR, Stewart SH, Dean-McElhinny T. Reproductive health and preconception counseling awareness in adolescents with diabetes: what they don't know can hurt them. *Diabetes Educ* 2006; **32**: 235-242 [PMID: 16554427 DOI: 10.1177/0145721706286895]

32 **Pacaud D**, Yale JF, Stephure D, Dele-Davies H. Problems in transition from pediatric care to adult care for individuals with diabetes. *Can J Diabetes* 2005; **40**: 29-35

33 **Peters A**, Laffel L. Diabetes care for emerging adults: recommendations for transition from pediatric to adult diabetes care systems: a position statement of the American Diabetes Association, with representation by the American College of Osteopathic Family Physicians, the American Academy of Pediatrics, the American Association of Clinical Endocrinologists, the American Osteopathic Association, the Centers for Disease Control and Prevention, Children with Diabetes, The Endocrine Society, the International Society for Pediatric and Adolescent Diabetes, Juvenile Diabetes Research Foundation International, the National Diabetes Education Program, and the Pediatric Endocrine Society (formerly Lawson Wilkins Pediatric Endocrine Society). *Diabetes Care* 2011; **34**: 2477-2485 [PMID: 22025785 DOI: 10.2337/dc11-1723]

34 **Diabetes UK**. Care recommendations July 2008: Transition from paediatric to adult services. Available from: URL: http:// www.diabetes.org.uk

35 **Court JM**, Cameron FJ, Berg-Kelly K, Swift PG. Diabetes in adolescence. *Pediatr Diabetes* 2009; **10** Suppl 12: 185-194 [PMID: 19754629 DOI: 10.1111/j.1399-5448.2009.00586.x]

36 **Patterson C**, Guariguata L, Dahlquist G, Soltész G, Ogle G, Silink M. Diabetes in the young - a global view and worldwide estimates of numbers of children with type 1 diabetes. *Diabetes Res Clin Pract* 2014; **103**: 161-175 [PMID: 24331235 DOI: 10.1016/j.diabres.2013.11.005]

P- Reviewer: Haidara M, Kesavadev J, Masaki T **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.636

World J Diabetes 2014 October 15; 5(5): 636-650
ISSN 1948-9358 (online)

REVIEW

Treatment of type 2 diabetes, lifestyle, GLP1 agonists and DPP4 inhibitors

Gerald H Tomkin

Gerald H Tomkin, Diabetes Institute of Ireland, Beacon Hospital, Sandyford, Dublin 18, Ireland
Gerald H Tomkin, Department of Diabetes and Endocrinology, Trinity College, Dublin 2, Ireland
Author contributions: Tomkin GH solely contributed to this paper.
Correspondence to: Gerald H Tomkin, Professor, Diabetes Institute of Ireland, Beacon Hospital, Sandyford, Clontra, Quinns Road, Shankill, Dublin 18, Ireland. gerald.tomkin@tcd.ie
Telephone: +353-1-2390658 Fax: +353-1-2721395
Received: January 26, 2014 Revised: July 23, 2014
Accepted: July 27, 2014
Published online: October 15, 2014

Abstract

In recent years the treatment focus for type 2 diabetes has shifted to prevention by lifestyle change and to more aggressive reduction of blood sugars during the early stage of treatment. Weight reduction is an important goal for many people with type 2 diabetes. Bariatric surgery is no longer considered a last resort treatment. Glucagon-like peptide-1 agonists given by injection are emerging as a useful treatment since they not only lower blood sugar but are associated with a modest weight reduction. The role of the oral dipeptidyl peptidase 4 inhibitors is emerging as second line treatment ahead of sulphonylureas due to a possible beneficial effect on the beta cell and weight neutrality. Drugs which inhibit glucose re-absorption in the kidney, sodium/glucose co-transport 2 inhibitors, may have a role in the treatment of diabetes. Insulin treatment still remains the cornerstone of treatment in many patients with type 2 diabetes.

Key words: Type 2 diabetes; Lifestyle modification; Dipeptidyl peptidase 4 inhibitors; Glucagon-like peptide-1 agonists; Insulin

Core tip: Treatment of diabetes is difficult. Initial success in achieving treatment goals is followed by deterioration and the necessity for additional treatments. Exciting new drugs with new modes of action, have stimulated diabetologists to strive for improved control in the knowledge that complications will be reduced or prevented. Obese patients, who loose weight on glucagon-like peptide-1 agonists are usually delighted with these drugs but for those who fail to loose weight changing to oral dipeptidyl peptidase-4 inhibitors would seem a good choice. sodium-glucose transporter-2 inhibitors have the added benefit of being effective even if blood sugar is near to target but uro-genital infection is a concern.

Tomkin GH. Treatment of type 2 diabetes, lifestyle, GLP1 agonists and DPP4 inhibitors. *World J Diabetes* 2014; 5(5): 636-650 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/636.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.636

INTRODUCTION

Readers interested in diabetes must be sick and tired reading that diabetes is a global problem of immense size and getting worse by the day with predictions that we will all have the disease one day! I exaggerate of course but it is sad to realise that although we know so much more about the condition we have made little progress in reducing or conquering the disease. A recent history of diabetes in the past 200 years by Polonsky[1] gives an excellent review of the history of discovery of so many mechanisms that are faulty in diabetes and the number of Nobel prize winners who have contributed to such wonderful success, yet more and more people are being diagnosed with the condition/disease and the consequences are immense in terms of suffering and financial cost. One should not forget that before the discovery of insulin 90 years ago

diabetes was a rapidly fatal disease and there was little interest in what we now term type 2 diabetes. Type 2 diabetes now makes up 90% of all diabetes. Insulin resistance rather than insulin deficiency is the major player in the vast majority of type 2 diabetes and type 2 diabetes can be reversed, at least in many patients, with exercise and weight reduction. This is not new information but was highlighted by Taylor's group in Newcastle in 2011[2] when they did a very simple experiment on patients who had diabetes, were obese and managed with tablets. They got 13 patients to do what was common practice and fashionable 40 years ago. They put the patients on an 800 kcal diet, a diet that has been proven beyond doubt to cause weight loss. Indeed there has never been a report of anyone who can maintain their weight on an 800 kcal diet. Compliance was checked by urinary ketones and weight loss. Eleven of the patients succeeded in finishing the eight week diet and lost as much weight as would be expected from bariatric surgery. Just like what happens following bariatric surgery in patients with type 2 diabetes, the diabetes disappeared and blood pressure and lipids improved. Nothing spectacular so far and the study would not have been worthy of reporting since all this is well known and has been done many times before, as Professor Yki-Jarvinen in her leading article in Diabetologia[3] wrote "the only problem is that in medical school and when I was training as an endocrinologist nobody told me how to get patients to follow such a diet". Only 10% of patients are able to follow dietary restriction advice and only the minority take the exercise treatment. Worse, of those who do succeed 90% relapse. Indeed this is why low calorie diets became unfashionable and large type 2 diabetic trials such as the Steino Hospital trial[4] did not include weight reduction as part of their protocol. The Newcastle group[2] converted an unoriginal and mundane study into a really exciting study by demonstrating that liver fat almost disappeared completely within a week and this was associated with a very large improvement in blood sugar and insulin resistance. The rapidity of improvement was interesting and the significance of the reduction of fat around the beta cell, a new finding of uncertain importance. However a plausible theory is that fat in the vicinity of the beta cell and in particular cholesterol, may be easily oxidised and the release of free radicals contributes to damage to the beta cell. In this regard a gene variant Ckal1, a gene associated with protein translation, has been shown to be very sensitive to oxidation and it is associated with a feeble insulin response[5]. Beta cells have the ability to regenerate and early and intensive reduction in blood sugar has been shown to improve beta cell function. Hyperglycaemia creates a vicious circle-the higher the blood sugar the greater the damage to the beta cell and the greater the damage to the beta cell the higher goes the sugar. Hence the drive to prevent hyperglycaemia by intervention in the pre-diabetes phase and to normalise blood sugar in the early stages of diabetes. The final result of the Newcastle group study that made me and many others sit up and take notice was the demonstration that the beta cell recovered, not partially but completely, and even the first phase insulin release returned to normal so the patients really did reverse their diabetes. This article was of such interest that it made headlines in daily newspapers around the world. Patients and their relatives, perhaps for the first time, really understood the damage diabetes does and gained new hope seeing a goal of reversal of diabetes and the possibility of discontinuation of diabetes medications. Beta trophin has been discovered-a hormone expressed mostly in liver and fat that stimulates beta cell proliferation, expands beta cell mass and improves glucose tolerance in a mouse model[6]. Perhaps an exciting new way to help to reverse diabetes in the future?

The July 2012 edition of the Lancet[7] carried on its cover "Physical inactivity: Worldwide", we estimated that physical inactivity causes 6%-10% of the major non-communicable diseases. Physical inactivity seems to have an effect similar to that of smoking or obesity. Min Lee *et al*[8] examined how much disease could be averted if inactivity were eliminated. Diabetes, as expected, is one of the major diseases the authors looked at. They concluded that not only did physical inactivity account for 6%-10% of the major non communicable diseases but this unhealthy behaviour causes 9% of premature mortality. There is good evidence to demonstrate that overweight or obese children who become obese as adults are at increased risk of diabetes whereas overweight or obese children who became non-obese by adulthood are not[9]. More importantly many studies have shown that educational interventions in physical activity have actually been successful and indeed more successful than interventions for obesity. Heath *et al*[10] in the same issue of the Lancet, examined interventions from around the world and demonstrate that the literature is convincing in demonstrating that behavioural and social approaches are effective. The improvements are seen among people of various ages and from different social groups, countries and communities. The authors make the point that although individuals need to be informed and motivated to adopt physical activity, the public health priority should be to ensure that environments are safe and supportive of health and wellbeing.

Since we know so much about the risk of developing diabetes, it should be possible to have treatment to prevent diabetes in many patients. The diabetes prevention program outcome study[11] has been recently published. This ongoing study demonstrated a clear reduction in diabetes incidence in participants randomly assigned to a lifestyle intervention or metformin during the intervention period. The authors end by stating that their data "support early and aggressive measures for long term prevention of diabetes in people at risk". Intensive lifestyle intervention has been shown to slow the decline in mobility in overweight adults with diabetes[12]. A disappointing result has recently come from the Look AHEAD study[13]. The study was designed to test the hypothesis that an intensive life style intervention for weight loss would decrease cardiovascular morbidity and

mortality in over weight patients with type 2 diabetes. More than 5000 patients took part in the study and the median follow-up of the study was for 9.5 years, weight loss was modest in the intervention group (6% *vs* 3.5% at the end of the study). Alas there was no reduction in the rate of cardiovascular events. The study results are perhaps not surprising in that significant weight reduction is unachievable in most patients but does suggest that we as physicians should accept that most patients are unable to loose weight and should not be made to feel guilty about this. On the other hand to continue to engage the patient in meticulous control of blood pressure, lipids and blood sugar, together with cessation of cigarette smoking, a healthy diet and exercise, are of proven benefit.

Casazza *et al*[14] have written an excellent article entitled "myths, presumptions and facts about obesity". The definition of a presumption was a belief in the absence of supporting scientific evidence; a Myth was defined as a belief persisting despite contradictory evidence. Facts were suppositions backed by sufficient evidence to consider them proven for practical purposes. The authors note that sometimes action is taken by policy makers in the absence of strong scientific evidence "This principle of action should not be mistaken as justification for drawing conclusions". The myths examined were: (1) that small sustained changes in energy intake or expenditure will produce large long term weight changes; (2) Setting realistic goals for weight loss is important otherwise patients will become frustrated and loose less weight; (3) Large rapid weight loss is associated with poor long term weight outcomes as compared with slow gradual weight loss; (4) It is important to assess the stage of diet readiness in order to help patients who request weight loss treatment; (5) Physical education courses in their present form play a part in reducing childhood obesity; (6) Breast feeding is protective against obesity; and (7) A bout of sexual activity burns 100-300 cal for each participant.

A stepwise approach to the management of diabetes has become a fashionable concept in recent years with many published paradigms of the steps which are variable and often contradictory or display so many different stairways that they become very confusing. The first step depends on getting the patient at the very beginning of their path, that is in the pre-diabetes stage but even then they may have already suffered from macrovascular and microvascular damage[15-18]. There is little dissention in advising the lifestyle changes but, should metformin also be used or should one wait and see the effect first of the lifestyle changes? Information on this point is available, for example in the trial by Snehalatha *et al*[19] 2009. There seemed to be no advantage to add metformin to life style changes so perhaps metformin should be reserved for those patients who are unable to adhere to life style changes?

Once diabetes has been diagnosed can one wait and see the result of life style changes or should one aggressively control blood sugar? High glucose is toxic to the beta cell. Exciting new information suggests that the beta cell may dedifferentiate under high glucose attack by causing reduction in a key transcription factor, Fox 01. This dedifferentiation results in the production of inactive proinsulin and an increase in glucagon[20]. Intensive insulin therapy at diagnosis of type 2 diabetes has been shown to reverse diabetes. Weng *et al*[21] studied 382 patients and had divided them into 3 groups. Continuous insulin infusion, multiple injections or oral agents were used to achieve rapid normalisation of hyperglycaemia. Treatment was stopped after normoglycemia was maintained for two weeks. After a year 51% and 44% of the insulin treated patients were in remission where as only 26% of the patients in the oral agent group had gone into remission. The evidence to support early and aggressive treatment for type 2 diabetes has not been widely accepted. The reasons are probably due to a shortage of personnel to manage patients. In my country there is a long waiting list to be seen in a diabetic clinic and general practitioners are usually unhappy about starting insulin. The better understanding of the beta cell pathology of diabetes should persuade physicians to adopt a more urgent approach to diabetes management in the future. A systematic review and meta-analysis on short term intensive insulin therapy in type 2 diabetes gives further support for the ability of this treatment to modify disease progression[22].

BARIATRIC SURGERY

Bariatric surgery for obese type 2 diabetes has been refined over the last few years. Laparoscopic surgery has made operation on morbidly obese patients who have diabetes, and indeed those who do not have diabetes, much safer and very often will reverse the diabetes. The operation has been shown to reduce cardiovascular risk. As with all operations the experience of the surgeon and indeed the surgical unit plays a very important part in outcome. A Cochrane review[23] in 2009 concluded that bariatric surgery is more effective than conventional treatment in achieving and in sustaining weight loss in people with obesity. Improvements in health related quality of life and obesity related co morbidities including type 2 diabetes, dyslipidaemia and sleep apnoea are further benefits. A very good review of the subject has recently been written by Dixon *et al*[24].

Mingrone *et al*[25] in 2012 published a single centre non-blinded randomised controlled trial to examine the difference in outcome between surgery as compared to usual medical therapy. Surgery was either gastric bypass or bilio-pancreatic diversion. At the end of 2 years HbA1c was 6.35% in the gastric bypass group and 4.95% in the bilio-pancreatic-diversion group as compared to 7.69% in the medically treated group. Diabetes remission had occurred in 75% of the gastric bypass group and 95% in the bilio-pancreatic diversion group. No patient in the medical group had reversed their diabetes. There were no deaths and almost no complications in the surgical group[25].

In the same edition of the journal Schauer *et al*[26] evaluated the efficacy of intensive medical therapy as compared to medical therapy plus Roux en Y gastric bypass or sleeve gastrectomy in 150 obese patients with uncontrolled type 2 diabetes. The primary end point was the proportion of patients with a glycated haemoglobin level of 6.0% or less, 12 mo after treatment. Twelve percent of the medical group, 42% in the gastric bypass group and 37% in the sleeve gastrectomy group achieved the primary end point. HbA1c was 7.5% in the medical group 6.4% in the gastric bypass group and 6.6% in the sleeve gastrectomy group. No deaths or life threatening complications occurred[26]. An editorial in the same edition by Zimmet *et al*[27] suggests that the bariatric surgery should not be seen as a last resort. More recently Arterburn *et al*[28] did a retrospective analysis to compare rates of diabetes remission, relapse and all cause mortality amongst severely obese adults with diabetes who underwent bariatric surgery *vs* non-surgical treated individuals. At 2 years the surgery subjects had significantly higher diabetes remission rates 73.7% compared to non surgical subjects with 6.9%. The surgical subjects also experienced lower relapse rates with no higher risk of death[28].

NEW INSULINS FOR TREATMENT OF TYPE 2 DIABETES

Many different regimes have been proposed and indeed are in use for the treatment of type 2 diabetes when life style and metformin have failed to control hyperglycaemia. A three year efficacy of complex insulins in type 2 diabetes demonstrated that the addition of a basal or prandial insulin based regimen to oral therapy had better diabetic control than those who added a biphasic insulin regimen[29]. My own feeling is that, as so many patients with type 2 diabetes don't increase their blood sugars overnight, attention should be paid to controlling the post evening meal rise in blood sugar so that the patient goes to bed with a normal blood sugar, long acting insulins being reserved for those patients in whom blood sugars rise overnight. To me it doesn't make sense to give a basal dose of a long acting insulin pre bed with the risk of overnight hypoglycaemia to a patient whose blood sugar has not been shown to rise overnight. Insulin degludec is almost identical to human insulin but with the last amino acid deleted from the B chain and addition of a glutamyl link from LysB29 to a hexadecanoic fatty acid[30]. Two phase 3 studies were reported recently[31,32]. In the first study type 1 diabetic patients (472 subjects) were subjected to insulin degludec and 157 to glargine insulin[31]. Although there was no difference in HbA1c at the end of the study and no difference in overall, confirmed hypoglycaemia; overnight hypoglycaemia was 25% less in the insulin degludec and of course nocturnal hypoglycaemia is what many patients fear most. The second study Garber *et al*[32] reported the effect of the new insulin in type 2 diabetic patients *vs* insulin glargine. Again after 1 year there was no difference between the 2 groups in HbA1c. Overall hypoglycaemia was a little less in the insulin degludec group and nocturnal hypoglycaemia was also a little lower (1.4 *vs* 1.8 episodes per patient-year exposure). The authors conclude that the newer basal insulins with lower hypoglycaemia events may allow more intensive blood sugar lowering treatment. From the results presented in their paper, insulin degludec does not seem to be the answer. An editorial by Tahrani *et al*[33] in the same edition, ends by saying that insulin degludec is not a revolution but an evolution of insulin therapy for patients with both type 1 and type 2 diabetes.

SODIUM GLUCOSE CO-TRANSPORT-2 INHIBITORS

Glycosuria occurs when the blood glucose reaches a threshold of about 10 mmol/L. However some people will excrete glucose at much lower levels of blood glucose (renal glycosuria). The discovery that glucose is transported across the proximal tubule membrane by sodium/glucose co-transport 2 (SGLT2) and that a naturally occurring polymorphism of the gene causes renal glycosuria, paved the way for the development of SGLT2 receptor inhibitors as a way of promoting renal glucose excretion and therefore calorie loss and reduction of blood sugar. Two drugs have undergone clinical trials dapaglifozin and canaglifozin and have been the subject of a meta analysis by Clar *et al*[34]. The drugs both result in blood glucose reduction of about 0.5%-1% with some weight loss. Urinary and genital infections were more common. Hypoglycaemia did not occur any more frequently that placebo. The results of the Cantata-SU trial have recently been published[35]. The trial was a 52 wk study in type 2 diabetes with patients who were inadequately controlled with metformin. Canagliflozin was compared to Glimepiride. 1452 patients were randomised in a phase 3 non-inferiority, double blind, randomised trial. Three hundred mg of Canagliflozin reduced HbA1c from a mean of 7.8% to 6.9% (mmol/L) a reduction of 0.9%. Hypoglycaemia was less common on Canagliflozin and there was a 4 kg reduction in weight with a small reduction in blood pressure. There was a 0.25 increase in LDL cholesterol but also a slight, 0.1% increase in HDL cholesterol and a very slight reduction in triglycerides also of 0.1%. Genital mycotic infections occurred in 8% in men and 14% in women on the 300 mg dose. The study suggests that the benefit of the drug is a useful reduction in HbA1c and weight reduction. The blood pressure reduction is also of benefit but the rise in LDL might be a worry and the mycotic genital infections and urinary tract infections might make the drug unacceptable to many patients who may have presented with these problems when first diagnosed. An editorial in the Lancet where the results were published is entitled "SGLT2 inhibitors for diabetes: turning symptoms into therapy" and makes the point that the place of this class of drugs in the treatment of type diabetes is still to be decided[36]. There has been concern about breast and bladder cancer as well

as long-term cardiovascular adverse effects also making surveillance mandatory. Another recently published study comparing canagliflozin with placebo and sitagliptin produced similar results[37]. A randomised, blinded, prospective Phase 111 study on dapagliflozin as monotherapy in drug naive Asian patients with type 2 diabetes found that with the 10 mg dose HbA1c had fallen from a mean of 8.26% to 7.15% as compared to a fall of only 0.29% for placebo(a difference of 0.82%) Genital infections occurred in 4.5% of patients and Urinary tract infections in 5.3%[38].

The role of these drugs in the treatment of type 2 diabetes is not clear at present but the lack of risk of hypoglycaemia and the weight reduction suggest that there is a place for them in certain patients who are inadequately controlled and in whom an extra 0.5% or more reduction in blood sugar would be of benefit in bringing the patient into the acceptable blood sugar range.

METFORMIN

The reason for metformin as first line pharmacological treatment is based on many studies suggesting that metformin is weight neutral or associated with very modest weight loss as compared with sulphonylureas which cause slight weight gain initially. Also, in experimental conditions reperfusion after myocardial infarction is reduced by sulphonylureas. As long ago as 1971 the University Group Diabetes Program[39] showed that tolbutamide, a first generation sulphonylurea, was associated with an increased cardiovascular risk in diabetes. The UKPDS trial[40] suggested that metformin has a protective effect on mortality. Roumie *et al*[41] examined the comparative effectiveness of sulphonylurea and metformin monotherapy on cardiovascular events in type 2 diabetes mellitius. This was a very large retrospective cohort study examining cardiovascular outcomes. The crude rates of composite outcome were 18.2 per 1000 person years in the sulphonylurea users and 10.4 per 1000 person years in the metformin group. A wonderful editorial in the same edition of the Annals of Internal Medicine by Nissen[42] entitled "Cardiovascular effects of Diabetes Drugs; Emerging from the dark ages", likens the dark ages after the fall of the Roman Empire to the time between the University Group Diabetes Program in 1972[39] which showed that treatment for diabetes with phenformin or tolbutamide was associated with increased cardiovascular risk, and 2012. The article explains why there is still uncertainty about the effect of sulphonylureas and cardiovascular events. Nissen[42] suggests that the study is hypothesis generating rather than definitive and that high quality evidence is still missing "Continued darkness is not an acceptable option" he concludes.

INCRETINS

It has been known for many years that intravenous glucose will not stimulate insulin secretion to the same extent as a similar glucose load given orally. It was discovered that hormones secreted from the intestine in response to a glucose load had the ability to release glucose from the pancreas. These hormones were called incretins and they are responsible for at least 50% of insulin secretion following a meal. In 1971 a peptide was isolated from the intestine which had the ability to inhibit gastric acid secretion and was therefore called gastric inhibitory polypeptide (GIP)[43]. GIP was later found to stimulate insulin secretion. What was very interesting was that GIP would only stimulate insulin secretion in the presence of high blood sugar. This finding has implications in treatment terms since drug that only works with high blood sugar would be much less likely to cause hypoglycaemia. Patients, their families and of course doctors and other health care professionals all fear hypoglycaemia. Garber[44] refers to the many hospital visits caused by hypoglycaemia and suggests that minimisation of hypoglycaemia should be a goal for treatment of type 2 diabetes. I would certainly agree. In a survey insulin accounted for 13.9% of overall admissions to hospital from adverse drug reactions and oral anti-diabetic drugs 10.7%[45].

Another incretin was discovered in 1985 and called glucagon-like peptide-1 (GLP-1)[46]. This hormone was also dependent on high blood sugar level for full action. Both GIP and GLP-1 act by binding to specific receptors and so release insulin. GLP-1 has another action, it inhibits gastric emptying and this has been of benefit in the treatment of diabetic patients because the feeling of satiety leads to weight reduction. Another beneficial effect of the reduction in rate of gastric emptying is to delay absorption of food, a mechanism which improves blood sugar excursion. GLP-1 also regulates appetite and food intake through its effect the hypothalamus. A recent review of the effects of GLP1 on appetite and body weight with a focus on the central nervous system has been published[47].

GLP-1 agonists have been shown to stimulate B cell growth in animals and cell cultures. In humans it is less clear if these drugs can improve insulin output by regenerating the B cell. It seems less likely that the dipeptidyl peptidase (DPP)-4 inhibitors could also have an effect on B-cell re-growth. However an abstract presented at the Annual American Diabetes Association meeting in 2010 suggested that linagliptin was able to restore beta cell function in human isolated islets[48]. Vildagliptin has also been shown to improve beta cell function and glucose tolerance but also to improve the extensive peri-insulitis found in the mouse model examined[49].

A very interesting effect of GLP-1 analogue therapy has been described in obese type 2 diabetic patients. The investigators found a reduction in inflammatory macrophages and a reduction in inflammatory cytokines together with an increase in the adipokine adiponectin. The researchers had previously described a case of psoriasis that was greatly improved by GLP-1 agonist therapy[50]. The new study does suggest an important beneficial effect of GLP-1 analogue therapy that needs further inves-

tigation[51]. A good review on the extrahepatic effects of GLP-1 receptor Agonists has just been published[52].

DEVELOPMENT OF GLP-1 FOR THE TREATMENT OF DIABETES

Exenatide is a GLP-1 receptor agonist. It is a 39 amino acid peptide produced in the saliva glands of the Gila monster lizard[53] it has 53% amino acid homology to full length GLP-1 and it binds with greater affinity than GLP-1 to the GLP-I receptor in GLP-1 receptor expressing cells[54]. DPP-4 cleaves peptides and is responsible for the rapid breakdown of GLP-1. DPP-4 does not denature exenatide because of the slight amino acid differences and in human studies the half life ranges from 3.3 to 4 h[55]. Exenatide (Eli Lilly) is now in clinical use in many countries for the treatment of diabetes. It must be given an hour before meals on a twice a day basis. Many trials have reported that the drugs cause about a 1% reduction in HbA1c and reduction in body weight of 5.3 kg at the end of 3 years of treatment[56]. The dropout rate is about 20%, many patients refusing treatment because of nausea.

EXENITIDE

Attempts have been made to prolong the action of exenatide using a polylactide glycolide microsphere suspension so that the drug can be given weekly. Kim *et al*[57], in a randomised placebo-controlled phase 2 study examined the effect of exenatide long acting release, a long acting release exenatide formulation, found that a weekly dose for 15 wk in patients with type Ⅱ diabetes resulted in a 1.4% reduction in HbA1c, suggesting that once a week formulation may be as good as, if not better than, twice daily injections of exenatide. In particular there were no dropouts in the trial due to adverse events. Liraglutide is a long acting GLP-1 analogue with attachment of a C-16 free fatty acid derivative. The free fatty acid derivative promotes non-covalent binding of liraglutide to albumen thereby increasing plasma half life. A recent study comparing liraglutide once a day with exenatide twice a day found that liraglutide improved HbA1c significantly more (-1.12% *viz* -0.79%) and was generally better tolerated[58]. The study has demonstrated that glycaemic improvement and weight reduction are independent of each other. This fits in with other studies which suggest that the weight loss is not, in itself, the cause of the improved blood sugar control[59].

In a recent paper Derosa *et al*[60] examined the effect of exenatide on beta cell function. The authors used the homeostasis model assessment beta cell function index as well as assessing pro-insulin and insulin with arginine stimulation under clamp conditions. The results suggested that beta cell function was improved by exenatide. However a caveat, HbA1c was significantly better after the 12 mo of exenatide as compared to placebo. It is well known that hyperglycaemia is toxic to the beta cell hence the improved glucose might have been responsible for the beta cell improvement rather than the drug itself. Bunck *et al*[61] showed similar results compared to glargine. In their study combined glucose and arginine stimulated C peptide secretion was 2.46 fold greater after 52 wk of exenatide treatment compared with insulin glargine treatment with a non significant (P = 0.55) 0.8% reduction in HbA1c as compared to a -0.7% reduction in the glargine group. Four weeks after cessation, the beta cell function returned to pre treatment levels.

Exenatide, was compared to glimepiride in patients who were not controlled on metformin alone[62]. About 1000 patients were divided into 2 groups and studied on average for 2 years although some went on for 42 mo. At the end of 3 mo both groups had decreased HbA1c from around 7.4% to 6.8% but by 36 mo the glimperamide group had gone back to a HbA1c of more than 7.3% whereas the exenatide group, although increasing their HbA1c slowly over the 3 years, was significantly lower at a level of just over 7.2%. Body weight fell in the exenatide group by 3.32 kg and rose in the glimperamide group by 1.15 kg. Systolic blood pressure (BP) decreased in the exenatide group by 1.9 mmHg with no change in the Glimpereride group. Less patients in the exenatide group experienced a hypoglycaemic episode. In the first 6 mo 49 patients in the exenatide group discontinued mostly due to gastrointestinal side effects as compared to 17 in the glimepiride group (P = 0.001) Buse *et al*[63] examined whether twice daily exenatide injections reduced HbA1c levels more than placebo in patients receiving Glargine insulin. HbA1c decreased by 1.74% in the exenatide group as compared to 1.04% in the placebo group over a 30 wk period. Hypoglycaemia was similar in the 2 groups and 13 treatment patients and 1 placebo recipient discontinued the study because of adverse events, nausea and vomiting being the main problems.

LIRAGLUTIDE

At the beginning of 2012 the FDA approved the marketing of extended release exenatide (Bydurion). The drug is given weekly by injection. Liraglutide is a human GLP-1 analog given by once daily injection with a good safety record and HbA1 lowering effect similar to the other GLP-1 agonists. A 2-year report on safety, tolerability and sustained weight loss over 5.2 years with once daily liraglutide has been published[64]. Two hundred and sixty eight of 398 people who entered the extension of the original 20 wk trial completed 2 years. Weight loss was 7.8 kg from screening and was maintained. There were improvements in BP and lipids. Patients with diabetes however were excluded from taking part in this trial. The Duration Trial 6[65] reported on a study comparing daily liraglutide to weekly extended release exenatide. This was a 26 wk trial with more than 400 patients in either arm. Liraglutide was associated with a greater change in HbA1c (-0.48% *viz* 1.28%). Nausea was more common in the liraglutide group (21% *viz* 9%) and also vomiting (11% *viz* 4%) 5% of patients allocated to liraglutide discontinued the treatment as compared to 3% allocated to exenatide because

of adverse events. The results suggest that the patient might be allowed to choose whether to have a drug which is injected daily but with no diluting procedure before the injection or a weekly injection with less blood sugar lowering effect but less side effects. Non-alcoholic steatosis has become a problem in type 2 diabetes. The LEAN study is currently examining whether liraglutide will improve non-alcoholic steatohepatitis outcome[66].

LIXISENATIDE

Lixinitide is another potent, selective, once daily GLP-1 agonist. A randomised placebo controlled double blind trial examined lixisentide daily injection in Asian patients with type 2 diabetes insufficiently controlled on basal insulin with or without sulphonylureas[67]. This was a 24 wk study. These patients were not obese body Mass Index 25.3 kg/m^2. Eighty-two percent of patients reached and stayed on the maintenance dose of lixisenatide (20 μg once a day). There was a significant reduction in HbA1c compared to controls. The difference at the end of the trial was 0.88%. There was no significant change in weight compared to controls. The incidence of serious side effects were similar in both groups. Two patients in the lixisenatide group experienced cerebrovascular infarction. Forty-two percent of study drug patients experienced hypoglycaemia as compared to 24% on placebo. Fonseca *et al*[68] examined efficacy and safety of once daily lixisenatide at different doses. HbA1c was reduced by 0.66% compared to placebo. Postprandial and fasting blood sugars were significantly lower in the treatment group. In a study by Kapitza *et al*[69] lixisenatide once daily was compared to liraglutide once daily in patients with type 2 diabetes insufficiently controlled on metformin. This was only a 28 d study but the results showed that liraglutide controlled fasting blood glucose better than lixisenatide but postprandial blood sugar was better controlled by lixisenatide. A review discussing the place of this GLP-1 agonist as an add on therapy to basal insulin has recently been published[70].

TASPOGLUTIDE

Ipsen Roche had another GLP-1 analogue under review called taspoglutide. This is a GLP-1 analogue which has a prolonged action and is in phase Ⅱ trials. The drug has been shown to improve diabetes control and lowers body weight in subjects with diabetes. In a study involving once a week injections in 306 type 2 diabetic subjects who were already on Metformin, 8 wk treatment was associated with a reduction in HbA1c. The highest dose gave an HbA1c reduction of 0.9% and a weight reduction of 1.9 kg as compared to placebo. Nauck *et al*[71] report on a 24 wk study using a 10 mg or a 20 mg dose of Taspoglutide, comparing once a week dosing to daily glargine insulin. One thousand and forty-nine patients were randomised into 3 groups. Withdrawal rates were 21% for each of the Taspoglutide groups and 9% for the glargine group. HbA1c of < 53% was achieved in 39.47% and 32% receiving Taspoglutide 10 mg, 20 mg and HbA1c < 48 in 18%, 24%, and 14% of patients or glargine insulin respectively. Lower fasting blood sugars were achieved by glargine insulin. Serious hypersensitivity reactions occurred in 2 patients on Taspoglutide. However confirmed hypoglycaemia was less with the study drug (0.3%, 0.9% *viz* 3.1%) and weight loss was greater on Taspoglutide (-3.3 and -4.1 kg). Withdrawals due to adverse effects occurred in 9%, 13% on Taspoglutide and in 1% on the glargine insulin. An addendum to the paper states that Roche has now stopped the development of the drug. Ibsen is currently pursuing further investigations. Rosenstock *et al*[72] examined the fate of Taspoglutide once a week *vs* Exenatide for type 2 diabetes. The doses used were again 10 mg or 20 mg as compared to twice daily exenatide 10 μg. Reduction in HbA1c was -1.24 with 10 mg and -1.31 with the 20 mg as compared to exenatide from a starting HbA1c of 8.1%. Withdrawals were higher in the study drug patients and the authors conclude that even though Taspoglutide caused lower blood sugars the level of side effects was unacceptable.

Albiglutide is a long acting subcutaneous albumen-based fusion of GLP-1[73]. In February 2009 Glaxo SmithKline (GSK) began phase 3 studies in type Ⅱ diabetes. Albiglutide is a GLP-1 mimetic generated by genetic fusion of a DPP-4-resistant GLP-1 dimer to human albumin[74]. The formulation was originally developed by Human Genome Sciences (HGS) and named Albugon, GSK having bought the drug in 2004 for all human therapeutic and prophylactic applications of Albiglutide. In 1999 Centeon (now Aventis Berring) granted Principia (now HGS) world wide rights to its recombinant fusion proteins and its related yeast technologies[75].

ANTIBODIES TO GLP-1 AGONISTS

Therapeutic proteins/peptides with structural similarity to endogenous proteins/peptides often have unwanted immunogenicity. Antibodies to the GLP-1 agonists have been described and may inhibit the action of the agonist. The role of antibody formation to the various agonists on the market at present are uncertain. A study by Buse *et al*[76] in 2011 suggested that antibodies to liraglutide did not inhibit efficacy however antibodies to exenitide, if they were high, was associated with a smaller HbA1c reduction. Antialbiglutide antibodies developed in 2.5% of patients in an 8 wk trial.

GLP-1 AND THE CARDIOVASCULAR SYSTEM

Endothelial dysfunction is a common finding in diabetes and an early marker of atherosclerosis. GLP-1 has been shown to improve endothelial dysfunction[77,78]. GLP-1 exerts a cardio-protective effect against ischaemic damage and heart failure. Diabetes is associated with an increased risk of atherosclerosis and myocardial infarction.

Ischaemic preconditioning is a protective mechanism by which the heart may protect itself from prolonged ischaemia. The University Group Diabetes Programme report[39], more than 40 years ago, suggested that tolbutamide might increase myocardial infarction and mortality. Glibenclamide has been shown to affect ischaemic preconditioning but trials have not shown beyond doubt that it is associated with increased myocardial infarction. However drugs that inhibit the K ATP channel opening, such as glibenclamide, are related to loss of ischaemic preconditioning[79-81]. GLP-1 receptors are found in the heart. Increased glucose uptake by the cardiac myocyte is beneficial in protecting the heart from ischaemia changes[82]. Studies in situ and *ex-vivo* suggest a beneficial effect on the heart muscle when under ischaemic stress. Bao *et al*[83] examined the effect of albiglutide in rats after myocardial ischaemia reperfusion injury. They measured cardiac glucose uptake and cardiac metabolic flux. They found enhanced glucose uptake and reduced myocardial infarct size and improved cardiac function. It has yet to be shown if this effect also occurs in humans and if myocardial infarct size and mortality will be reduced by GLP-1 agonists. DPP-4 inhibitors have been less well studied in cardiac ischaemic preconditioning. In a study by Rahmi *et al*[84] rapaglinide, a sulphonylurea like drug, inhibited ischaemic preconditioning as measured by stress testing in patients with type 2 diabetes who already had evidence of coronary atherosclerosis. Vildagliptin, a DPP-4 inhibitor, did not alter preconditioning in 72% of patients whereas 83% of the repaglinide patients had ischaemia earlier in their stress test.

GLP-1 AND THE PANCREAS

Pancreatitis has been described in patients using GLP-1 agonists. A report in 2010 stated that 8 cases during clinical development and 36 post marketing reports are available[85]. A recent report[86] examined a large United States health insurance claims database and could find no increased risk of acute pancreatitis using twice daily exenatide. However there were several limitations to the study and it was a pity that other GLP-1 agonists were not investigated at the same time but the study was funded by Amylin and Eli Lilly. Stimulation of GLP-1 receptors that are found in the exocrine pancreas might lead to overgrowth of the epithelial cells in the small ducts causing pancreatitis through obstruction. A worry has been raised that GLP-1 agonists may induce metaplasia and premalignant changes[87,88].

GLP1 AND THE THYROID

The thyroid contains GLP1 receptors and Gier *et al*[89] also found coincident immunoreactivity for calcitonin and GLP-1 receptors in both medullary thyroid carcinoma and C cell hyperplasia. C cell carcinoma of the thyroid has been seen in animals dosed with GLP-1 agonists and can be explained by the finding of GLP-1 receptors in the thyroid[89]. GLP-1 receptor immuno-reactivity was also found in 18% of papillary thyroid carcinoma. The authors speculate on the consequences of long term stimulation of these GLP-1 receptors. They suggest that prospective studies need to be done to exclude an increase in papillary and medullary carcinoma in the thyroid.

DPP-4 INHIBITORS

These drugs act by inhibiting the enzyme that breaks down GLP-1, thus increasing the level of GLP-1 in the blood stream. They are however not able to raise the GLP-1 levels to levels found after injection of GLP-1 agonists and therefore their hypoglycaemic efficacy is less than that of GLP-1 agonists. Sitagliptin, vildagliptin, saxagliptin and linagliptin have already been approved in the United States and in Europe. An excellent systematic review and meta-analysis has been published in the British Medical Journal in 2012[90]. Compared with metformin, DPP-4 inhibitors were associated with a smaller decline in HbA1c and a lower chance of attaining a HbA1c goal of less than 7%. As a second line treatment DPP-4 inhibitors achieved a smaller decline in HbA1c than the other hypoglycaemic drugs. There was however, no significant difference in attaining an HbA1c of less than 7% when compared to sulphonylureas. They were less effective in lowering body weight when compared to metformin. When added to metformin they had a favourable weight profile compared to metformin and sulphonylureas or pioglitazone but not when compared to GLP-1 agonists. Hypoglycaemia was less common when a DPP-4 inhibitor was added to metformin as compared to a sulphonylurea added to metformin. There is evidence to suggest that the DPP-4 inhibitors are more effective in lowering glucose in Asians than non Asians[91]. A one year follow up of DPP-4 inhibitors *vs* sulfonylureas on top of metformin has been published recently[92]. Patients with prior metformin therapy received a dual combination of metformin with either DPP-4 inhibitor or sulfonylureas. There was no significant difference in either body weight or HbA1c. Hypoglycaemia was significantly less in the patients taking DPP-4 inhibitors. These patients had significantly less transitory cerebral ischaemic attacks whereas other cardiovascular events were of borderline significance.

There are 6 DPP-4 inhibitors (*e.g.*, Sitigliptin, Linagliptin, Vildagliptin, Alogliptin, Saxagliptin, Teneligliptin) on the market minor variation in their chemical composition have not been translated to particular benefit although it should be noted that linagliptin is mostly excreted in pathways other than the kidney and hence dosage does not have to be reduced in moderate renal failure.

Vildagliptin, a DPP-4 inhibitor which increases circulating GLP-1 levels, has been shown to ameliorate the deposition of amyloid beta and tau phosphorylation in a streptozotosin induced animal model of diabetes[93]. A study by Omar *et al*[94] using a high fat diet induced obesity model in mice of advanced age has demonstrated that Vildagliptin confirms other rodent models of diabetes in preserving beta cell mass mainly through inducing beta cell proliferation and reducing beta cell apoptosis[94-96].

Omar *et al*[94] found that Vildagliptin improved glucose secretion in response to oral glucose. Beta cell area was not significantly altered by Vildagliptin treatment in these mice but peri insulitis was prevented by Vildagliptin. Sitagliptin has also been shown to protect against amyloid associated beta cell loss but its effect was not different to that of Metformin[97].

The binding modes of these drugs has recently been investigated[98]. Based on their binding sites the authors divided the drugs into 3 categorise, Vildagliptin and Saxagliptin, Alogliptin and Linagliptin, Sitagliptin and teneligliptin. It is not clear whether these different binding modes have clinical relevance but may help in the development of better inhibitors in the future. Unlike GLP-1 agonists the DPP-4 inhibitors do not pass the blood brain barrier and have no effect on satiety, nor do they effect gastric emptying. Although the different DPP-4 inhibitors have some differences including potency, half lives and metabolism there does not seem to be any meaningful difference in their ability to lower blood sugar and this is probably why there are virtually no head to head studies (one head to head study showed no difference between saxagliptin and sitagliptin when combined with metformin[99]. A good review of the differences has been written by Capuano *et al*[100]. Most of the DPP-4 inhibitors can be administered once daily but Vildagliptin needs to be given twice daily. Saxagliptin is mainly metabolised by CYP3A4/5 isoforms to a major active metabolite 5-saxahydroxygliptin. It is suggested that the dosage of saxagliptin be modified if co administration with CYP3A4/5 inducers such as rifampicin or inhibitors such as ketoconazole.

SITAGLIPTIN

Insulin glargine *vs* sitagliptin another DPP-4 inhibitor was studied by Aschner *et al*[101]. About 250 patients in each group were studied for more than 6 mo. At the start patients were already on metformin which was continued during the study. HbA1c was significantly lower in the glargine group. There were more hypoglycaemic episodes and slight weight gain in the glargine group where as there was slight weight loss in the Sitagliptin group. A recent study compared the effect of sitagliptin or glibenclamide in addition to metformin and pioglitazone on glycaemic control and beta cell function[102]. Body weight reached was lower with sitagliptin. Fasting plasma insulin and homeostasis model assessment of insulin resistance with glibenclamide were significantly increased with glibenclamide and decreased with sitagliptin. Sitagliptin did not change the homeostasis model assessment of beta cell function but the value was significantly increased by glibenclamide. Both glibenclamide and sitagliptin increased C-peptide.

VILDAGLIPTIN

A 24 wk study in elderly patients was recently published[103]. The study investigated the feasibility of setting and achieving individualised targets over 24 wk for elderly patients (over 70 years of age with type 2 diabetes). The patients who were treated with vildagliptin achieved a 0.6% reduction in HbA1c from a baseline of 7.9% as compared with placebo. There were no tolerability issues as compared to placebo, hypoglycaemic events were 2.2% in the vildagliptin arm and 0.7% in the placebo arm. Individualising goal HbA1c is thought to be appropriate particularly in the frail elderly[104]. The benefit of reducing HbA1c by less than 1% in this age group is uncertain. There seems no doubt that in the frail elderly hypoglycaemia is a very serious threat to health[105,106]. Macrovascular disease/events seem to respond better to blood pressure and lipid interventions than to blood sugar lowering at least in the short term[107] but microvascular damage and retinopathy prevention, particularly in patients who already have significant damage, should make the Physician consider carefully the probable benefit of tighter blood sugar control. Under these circumstances one might not choose a DPP-4 inhibitor since they work better in the higher blood sugar range and are less likely to result in the achievement of a HbA1c of 6.5% (48 mmol/L). The efficacy and safety of vildagliptin in patients with type 2 diabetes inadequately controlled on Metformin and sulphonylurea suggests that a mean HbA1c of 8.75% can be improved by about 0.75% as compared to placebo[108]. It is such a pity that the GLP-1 agonists work best at high HbA1c levels and are less effective in reduction of HbA1c as the HbA1c gets near to target. However in this trial 25% more patients reached a target of 7% as compared to controls(38.6% *vis* 13.9%).

SAXAGLIPTIN

The 4-year safety of saxagliptin has recently been published[109]. No new safety issue findings appeared during the 4 years of treatment alone or with metformin and hypoglycaemia did not increase the risk of hypoglycaemia. The cardiovascular safety of diabetic drugs continues to raise concern[109]. Saxagliptin was examined by Scirica *et al*[110]. They randomised 16492 patients with type 2 diabetes who had a history of or who were at risk for cardiovascular events, to receive Saxagliptin or placebo and followed them for a median of 2.1 years. The HbA1c at the beginning of the study was 8.0% and at the end of the study the HbA1c in the Saxagliptin arm had decreased to 7.5% and the placebo arm to 7.8%. A surprising finding was that more patients in the Saxagliptin group were hospitalised for heart failure but otherwise the cardiovascular end point results were similar between the two groups. Hospitalisation for hypoglycaemia occurred infrequently and was similar in the two groups but significantly more patients in the saxagliptin group reported at least one hypoglycaemic event. Thus this 2-year study gives little support for the use of saxagliptin in these patients.

LINAGLIPTIN

Linagliptin is a once a day oral DPP-4 inhibitor. It is an

orally active small molecule which was licensed in United States in 2011. It is mostly excreted in the faeces and there are no clinically relevant alterations in linagliptin pharmokinetics resulting from renal or liver impairment[111]. A recent study has confirmed that renal impairment has no clinically relevant effect on the long term exposure of linagliptin in patients with type 2 diabetes[112].

A 2-year efficacy and safety study of linagliptin compared to glimepiride in patients inadequately controlled on metformin was reported recently[113]. More than 1400 patients were divided into two groups. HbA1c at the end of the study was similar in the two groups but there was less hypoglycaemia and there were significantly less cardiovascular events (1 *vs* 2). Hypoglycaemia is not usually a problem in the treatment of type 2 diabetes but recently has been suggested to be a therapeutic concern. The efficacy and safety of Linagliptin in subjects with type 2 diabetes was analysed by Del Prato *et al*[114]. Pooled analysis of data from 2258 subjects in 324 wk phase 3 studies. Oral linagliptin or placebo as monotherapy added on to metformin or added on to metformin plus a sulphonylurea were the treatments investigated. Although linagliptin was effective the patients had a mean HbA1c of 9.0% and the level of HbA1c only dropped to 8.3% still unacceptably high for many patients. DPP-4 inhibitors unfortunately work less well the lower the starting HbA1c[102]. A study of linagliptin in patients aged over 70 years found that HbA1c was lowered by 0.64% from 7.8% to 7.2% with a safety profile similar to placebo. Whether long term studies in this age group will show benefit in measurable outcome is speculative at this time.

ALOGLIPTIN

Alogliptin seems to have much the same characteristics as the other DPP-4 inhibitors on the market, A useful review has recently been published[115]. Another large study specifically looking at cardiovascular disease in type 2 diabetic patients has been reported[116]. More than 5000 patients who had type 2 diabetes and either an acute myocardial infarction or unstable angina requiring hospitalisation within the previous 15 to 90 d received allogliptin or placebo in addition to existing antidiabetic and cardiovascular drug treatment. HbA1c at the start of the trial was 8.0% and at the end of the study had come down to 7.7% as compared to 7.97% in the placebo group. Hypoglycaemia was similar in the two groups. Again this large study makes one question the value of the addition of the DPP-4 inhibitor which was associated with such a modest drop in HbA1c.

TENELIGLIPTIN

Teneligliptin is another DPP-4 inhibitor which has been recently reviewed[117].

DPP-4 INHIBITORS AND THE HEART

GLP-1 receptors, which are found in the heart increase glucose uptake by the cardiac myocyte is beneficial in protecting the heart from ischaemia changes[118]. Matsubara *et al*[119] examined 44 patients with coronary artery disease and uncontrolled diabetes (HbA1c > 7.4%). Sitagliptin or aggressive conventional treatment was compared after 6 mo. Endothelial function was significantly improved in the sitagliptin group with no difference in fasting blood sugar at the end of the trial but a reduction in HbA1c of 0.6% in each group. C-reactive protein (CRP) reduced significantly in the sitagliptin group with a significant correlation between the CRP and the vascular reactivity but not with HbA1c.

DPP-4 INHIBITORS AND THE PANCREAS

Butler *et al*[120] examined the pancreata of 7 individuals treated with sitagliptin and 1 with exenatide compared with 12 individuals with type 2 diabetes treated with other agents, and 14 non-diabetics. There was an increase in the number of pre-malignant lesions and marked alpha cell hyperplasia with glucagon expressing micro adenomas and a glucagon expressing neuroendocrine tumour in one of the eight. Because the number of diabetics who were not on treatment with DPP-4 based therapy were so few the evidence is insufficient for alarm but the evidence for caution and vigilance in the next number of years is clear and persuasive.

Sero negative polyarthropathy has been recorded with the use of DPP-4 inhibitors. Three patients were described by Crickx *et al*[121] and one case by Ambrosio *et al*[122]. The acute arthritis is not perhaps surprising since DPP-4, also named CD 26 is expressed on many cells involved in the immune process.

CONCLUSION

New treatments for diabetes are coming on line but prevention and treatment of obesity through increased exercise and reduced calorie intake still seems the best option in most patients with type 2 diabetes. Those with insulin deficiency have new options which are exciting as they demonstrate new approaches to treatment but their glucose lowering effects are modest and mostly most effective when blood sugars are high thus of less use when blood sugars are near to, but not at, target in spite of a combination treatment.

REFERENCES

1 **Polonsky KS**. The past 200 years in diabetes. *N Engl J Med* 2012; **367**: 1332-1340 [PMID: 23034021 DOI: 10.1056/NEJMra1110560]

2 **Lim EL**, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* 2011; **54**: 2506-2514 [PMID: 21656330]

3 **Yki-Järvinen H**. Type 2 diabetes: remission in just a week. *Diabetologia* 2011; **54**: 2477-2479 [PMID: 21826485 DOI: 10.1007/s00125-011-2266-6]

4 **Gaede P**, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 2003; **348**: 383-393 [PMID: 12556541]

5 **Wei FY**, Suzuki T, Watanabe S, Kimura S, Kaitsuka T, Fujimura A, Matsui H, Atta M, Michiue H, Fontecave M, Yamagata K, Suzuki T, Tomizawa K. Deficit of tRNA(Lys) modification by Cdkal1 causes the development of type 2 diabetes in mice. *J Clin Invest* 2011; **121**: 3598-3608 [PMID: 21841312 DOI: 10.1172/JCI58056]

6 **Yi P**, Park JS, Melton DA. Betatrophin: a hormone that controls pancreatic β cell proliferation. *Cell* 2013; **153**: 747-758 [PMID: 23623304 DOI: 10.1016/j.cell]

7 **Kohl HW**, Craig CL, Lambert EV, Inoue S, Alkandari JR, Leetongin G, Kahlmeier S. The pandemic of physical inactivity: global action for public health. *Lancet* 2012; **380**: 294-305 [PMID: 22818941 DOI: 10.1016/S0140-6736(12)60898-8]

8 **Lee IM**, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet* 2012; **380**: 219-229 [PMID: 22818936 DOI: 10.1016/S0140-6736(12)61031-9]

9 **Juonala M**, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, Srinivasan SR, Daniels SR, Davis PH, Chen W, Sun C, Cheung M, Viikari JS, Dwyer T, Raitakari OT. Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med* 2011; **365**: 1876-1885 [PMID: 22087679 DOI: 10.1056/NEJMoa1010112]

10 **Heath GW**, Parra DC, Sarmiento OL, Andersen LB, Owen N, Goenka S, Montes F, Brownson RC. Evidence-based intervention in physical activity: lessons from around the world. *Lancet* 2012; **380**: 272-281 [PMID: 22818939 DOI: 10.1016/S0140-6736(12)60816-2]

11 **Perreault L**, Pan Q, Mather KJ, Watson KE, Hamman RF, Kahn SE. Effect of regression from prediabetes to normal glucose regulation on long-term reduction in diabetes risk: results from the Diabetes Prevention Program Outcomes Study. *Lancet* 2012; **379**: 2243-2251 [PMID: 22683134 DOI: 10.1016/S0140-6736(12)60525-X]

12 **Rejeski WJ**, Ip EH, Bertoni AG, Bray GA, Evans G, Gregg EW, Zhang Q. Lifestyle change and mobility in obese adults with type 2 diabetes. *N Engl J Med* 2012; **366**: 1209-1217 [PMID: 22455415 DOI: 10.1056/NEJMoa1110294]

13 **Wing RR**, Bolin P, Brancati FL, Bray GA, Clark JM, Coday M, Crow RS, Curtis JM, Egan CM, Espeland MA, Evans M, Foreyt JP, Ghazarian S, Gregg EW, Harrison B, Hazuda HP, Hill JO, Horton ES, Hubbard VS, Jakicic JM, Jeffery RW, Johnson KC, Kahn SE, Kitabchi AE, Knowler WC, Lewis CE, Maschak-Carey BJ, Montez MG, Murillo A, Nathan DM, Patricio J, Peters A, Pi-Sunyer X, Pownall H, Reboussin D, Regensteiner JG, Rickman AD, Ryan DH, Safford M, Wadden TA, Wagenknecht LE, West DS, Williamson DF, Yanovski SZ. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *N Engl J Med* 2013; **369**: 145-154 [PMID: 23796131 DOI: 10.1056/NEJM]

14 **Casazza K**, Fontaine KR, Astrup A, Birch LL, Brown AW, Bohan Brown MM, Durant N, Dutton G, Foster EM, Heymsfield SB, McIver K, Mehta T, Menachemi N, Newby PK, Pate R, Rolls BJ, Sen B, Smith DL, Thomas DM, Allison DB. Myths, presumptions, and facts about obesity. *N Engl J Med* 2013; **368**: 446-454 [PMID: 23363498]

15 **Diabetes Prevention Program Research Group**. The prevalence of retinopathy in impaired glucose tolerance and recent-onset diabetes in the Diabetes Prevention Program. *Diabet Med* 2007; **24**: 137-144 [DOI: 10.1111/j.1464-5491.2007.02043.x]

16 **Cheng YJ**, Gregg EW, Geiss LS, Imperatore G, Williams DE, Zhang X, Albright AL, Cowie CC, Klein R, Saaddine JB. Association of A1C and fasting plasma glucose levels with diabetic retinopathy prevalence in the U.S. population: Implications for diabetes diagnostic thresholds. *Diabetes Care* 2009; **32**: 2027-2032 [DOI: 10.2337/dc09-0440]

17 **Ford ES**, Zhao G, Li C. Pre-diabetes and the risk for cardiovascular disease: a systematic review of the evidence. *J Am Coll Cardiol* 2010; **55**: 1310-1317 [PMID: 20338491 DOI: 10.1016/j.jacc.2009.10.060]

18 **Ziegler D**, Zentai CP, Perz S, Rathmann W, Haastert B, Döring A, Meisinger C; KORA Study Group. Prediction of mortality using measures of cardiac autonomic dysfunction in the diabetic and non-diabetic population: the MONICA/KORA Augsburg Cohort Study. *Diabetes Care* 2008; **31**: 556-561 [DOI: 10.2337/dc07-1615]

19 **Snehalatha C**, Mary S, Selvam S, Sathish Kumar CK, Shetty SB, Nanditha A, Ramachandran A. Changes in insulin secretion and insulin sensitivity in relation to the glycemic outcomes in subjects with impaired glucose tolerance in the Indian Diabetes Prevention Programme-1 (IDPP-1). *Diabetes Care* 2009; **32**: 1796-1801 [PMID: 19587369 DOI: 10.2337/dc09-0676]

20 **Dor Y**, Glaser B. β-cell dedifferentiation and type 2 diabetes. *N Engl J Med* 2013; **368**: 572-573 [PMID: 23388011 DOI: 10.1056/NEJMcibr1214034]

21 **Weng J**, Li Y, Xu W, Shi L, Zhang Q, Zhu D, Hu Y, Zhou Z, Yan X, Tian H, Ran X, Luo Z, Xian J, Yan L, Li F, Zeng L, Chen Y, Yang L, Yan S, Liu J, Li M, Fu Z, Cheng H. Effect of intensive insulin therapy on beta-cell function and glycaemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial. *Lancet* 2008; **371**: 1753-1760 [PMID: 18502299 DOI: 10.1016/S0140-6736(08)]

22 **Kramer CK**, Zinman B, Retnakaran R. Short-term intensive insulin therapy in type 2 diabetes mellitus: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* 2013; **1**: 28-34 [PMID: 24622264 DOI: 10.1016/S2213-8587(13)70006-8]

23 **Colquitt JL**, Picot J, Loveman E, Clegg AJ. Surgery for obesity. *Cochrane Database Syst Rev* 2009; (**2**): CD003641 [PMID: 19370590 DOI: 10.1002/14651858.CD003641.pub3]

24 **Dixon JB**, le Roux CW, Rubino F, Zimmet P. Bariatric surgery for type 2 diabetes. *Lancet* 2012; **379**: 2300-2311 [PMID: 22683132]

25 **Mingrone G**, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Leccesi L, Nanni G, Pomp A, Castagneto M, Ghirlanda G, Rubino F. Bariatric surgery versus conventional medical therapy for type 2 diabetes. *N Engl J Med* 2012; **366**: 1577-1585 [PMID: 22449317]

26 **Schauer PR**, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, Thomas S, Abood B, Nissen SE, Bhatt DL. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med* 2012; **366**: 1567-1576 [PMID: 22449319 DOI: 10.1056/NEJMoa1200225]

27 **Zimmet P**, Alberti KG. Surgery or medical therapy for obese patients with type 2 diabetes? *N Engl J Med* 2012; **366**: 1635-1636 [PMID: 22449318 DOI: 10.1056/NEJMe1202443]

28 **Arterburn D**, Bogart A, Coleman KJ, Haneuse S, Selby JV, Sherwood NE, Sidney S, Theis MK, Campos GM, McCulloch D, O' Connor PJ. Comparative effectiveness of bariatric surgery vs. nonsurgical treatment of type 2 diabetes among severely obese adults. *Obes Res Clin Pract* 2013; **7**: e258-e268 [PMID: 24306153 DOI: 10.1016/j.orcp]

29 **Holman RR**, Farmer AJ, Davies MJ, Levy JC, Darbyshire JL, Keenan JF, Paul SK. Three-year efficacy of complex insulin regimens in type 2 diabetes. *N Engl J Med* 2009; **361**: 1736-1747 [PMID: 19850703 DOI: 10.1056/NEJMoa0905479]

30 **Jonassen I**, Havelund S, Hoeg-Jensen T, Steensgaard DB, Wahlund PO, Ribel U. Design of the novel protraction mechanism of insulin degludec, an ultra-long-acting basal insulin. *Pharm Res* 2012; **29**: 2104-2114 [PMID: 22485010 DOI: 10.1007/s11095-012-0739-z]

31 **Heller S**, Buse J, Fisher M, Garg S, Marre M, Merker L, Renard E, Russell-Jones D, Philotheou A, Francisco AM, Pei H, Bode B. Insulin degludec, an ultra-longacting basal in-

sulin, versus insulin glargine in basal-bolus treatment with mealtime insulin aspart in type 1 diabetes (BEGIN Basal-Bolus Type 1): a phase 3, randomised, open-label, treat-to-target non-inferiority trial. *Lancet* 2012; **379**: 1489-1497 [PMID: 22521071 DOI: 10.1016/S0140-6736(12)60204-9]

32 **Garber AJ**, King AB, Del Prato S, Sreenan S, Balci MK, Muñoz-Torres M, Rosenstock J, Endahl LA, Francisco AM, Hollander P. Insulin degludec, an ultra-longacting basal insulin, versus insulin glargine in basal-bolus treatment with mealtime insulin aspart in type 2 diabetes (BEGIN Basal-Bolus Type 2): a phase 3, randomised, open-label, treat-to-target non-inferiority trial. *Lancet* 2012; **379**: 1498-1507 [PMID: 22521072 DOI: 10.1016/S0140-6736(12)60205-0]

33 **Tahrani AA**, Bailey CJ, Barnett AH. Insulin degludec: a new ultra-longacting insulin. *Lancet* 2012; **379**: 1465-1467 [PMID: 22521058 DOI: 10.1016/S0140-6736(12)60527-3]

34 **Clar C**, Gill JA, Court R, Waugh N. Systematic review of SGLT2 receptor inhibitors in dual or triple therapy in type 2 diabetes. *BMJ Open* 2012; **2**: pii: e001007 [PMID: 23087012 DOI: 10.1136/bmjopen-2012-001007]

35 **Cefalu WT**, Leiter LA, Yoon KH, Arias P, Niskanen L, Xie J, Balis DA, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin versus glimepiride in patients with type 2 diabetes inadequately controlled with metformin (CANTA-TA-SU): 52 week results from a randomised, double-blind, phase 3 non-inferiority trial. *Lancet* 2013; **382**: 941-950 [PMID: 23850055 DOI: 10.1016/S0140-6736(13)60683-2]

36 **Diamant M**, Morsink LM. SGLT2 inhibitors for diabetes: turning symptoms into therapy. *Lancet* 2013; **382**: 917-918 [PMID: 23850056 DOI: 10.1016/S0140-6736(13)60902-2]

37 **Lavalle-González FJ**, Januszewicz A, Davidson J, Tong C, Qiu R, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin compared with placebo and sitagliptin in patients with type 2 diabetes on background metformin monotherapy: a randomised trial. *Diabetologia* 2013; **56**: 2582-2592 [PMID: 24026211]

38 **Ji L**, Ma J, Li H, Mansfield TA, T'joen CL, Iqbal N, Ptaszynska A, List JF. Dapagliflozin as monotherapy in drug-naive Asian patients with type 2 diabetes mellitus: a randomized, blinded, prospective phase III study. *Clin Ther* 2014; **36**: 84-100.e9 [PMID: 24378206 DOI: 10.1016/j.clinthera.2013.11.002]

39 **Goldner MG**, Knatterud GL, Prout TE. Effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. 3. Clinical implications of UGDP results. *JAMA* 1971; **218**: 1400-1410 [PMID: 4941698 DOI: 10.1001/jama.1971.03190220020005]

40 Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 854-865 [PMID: 9742977 DOI: 10.1016/S0140-6736(98)07037-8]

41 **Roumie CL**, Hung AM, Greevy RA, Grijalva CG, Liu X, Murff HJ, Elasy TA, Griffin MR. Comparative effectiveness of sulfonylurea and metformin monotherapy on cardiovascular events in type 2 diabetes mellitus: a cohort study. *Ann Intern Med* 2012; **157**: 601-610 [PMID: 23128859 DOI: 10.7326/0003-4819-157-9-201211060-00003]

42 **Nissen SE**. Cardiovascular effects of diabetes drugs: emerging from the dark ages. *Ann Intern Med* 2012; **157**: 671-672 [PMID: 23128866]

43 **Brown JC**, Dryburgh JR. A gastric inhibitory polypeptide. II. The complete amino acid sequence. *Can J Biochem* 1971; **49**: 867-872 [PMID: 5120249 DOI: 10.1139/o71-122]

44 **Garber AJ**. Hypoglycaemia: a therapeutic concern in type 2 diabetes. *Lancet* 2012; **379**: 2215-2216 [PMID: 22704156 DOI: 10.1016/S0140-6736]

45 **Budnitz DS**, Lovegrove MC, Shehab N, Richards CL. Emergency hospitalizations for adverse drug events in older Americans. *N Engl J Med* 2011; **365**: 2002-2012 [PMID: 22111719 DOI: 10.1056/NEJMsa1103053]

46 **Schmidt WE**, Siegel EG, Creutzfeldt W. Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. *Diabetologia* 1985; **28**: 704-707 [PMID: 3905480 DOI: 10.1007/BF00291980]

47 **van Bloemendaal L**, Ten Kulve JS, la Fleur SE, Ijzerman RG, Diamant M. Effects of glucagon-like peptide 1 on appetite and body weight: focus on the CNS. *J Endocrinol* 2014; **221**: T1-16 [PMID: 24323912 DOI: 10.1530/JOE-13-0414]

48 **Shah P**, Ardestani A, Dharmadhikari G, Laue S, Schumann DM, Kerr-Conte J, Pattou F, Klein T, Maedler K. The DPP-4 inhibitor linagliptin restores β-cell function and survival in human isolated islets through GLP-1 stabilization. *J Clin Endocrinol Metab* 2013; **98**: E1163-E1172 [PMID: 23633194]

49 **Miyagawa K**, Kondo T, Goto R, Matsuyama R, Ono K, Kitano S, Kawasaki S, Igata M, Kawashima J, Matsumura T, Motoshima H, Araki E. Effects of combination therapy with vildagliptin and valsartan in a mouse model of type 2 diabetes. *Cardiovasc Diabetol* 2013; **12**: 160 [PMID: 24188631 DOI: 10.1186/1475-2840-12-160]

50 **Buysschaert M**, Tennstedt D, Preumont V. Improvement of psoriasis during exenatide treatment in a patient with diabetes. *Diabetes Metab* 2012; **38**: 86-88 [PMID: 22227407 DOI: 10.1016/j.diabet.2011.11.004]

51 **Hogan AE**, Gaoatswe G, Lynch L, Corrigan MA, Woods C, O'Connell J, O'Shea D. Glucagon-like peptide 1 analogue therapy directly modulates innate immune-mediated inflammation in individuals with type 2 diabetes mellitus. *Diabetologia* 2014; **57**: 781-784 [DOI: 10.1007/s00125-013-3145-0]

52 **Seufert J**, Gallwitz B. The extra-pancreatic effects of GLP-1 receptor agonists: a focus on the cardiovascular, gastrointestinal and central nervous systems. *Diabetes Obes Metab* 2014; **16**: 673-688 [PMID: 24373150 DOI: 10.1111/dom.12251]

53 **Eng J**, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem* 1992; **267**: 7402-7405 [PMID: 1313797]

54 **Göke R**, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, Göke B. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. *J Biol Chem* 1993; **268**: 19650-19655 [PMID: 8396143]

55 **Kolterman OG**, Kim DD, Shen L, Ruggles JA, Nielsen LL, Fineman MS, Baron AD. Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus. *Am J Health Syst Pharm* 2005; **62**: 173-181 [PMID: 15700891]

56 **Klonoff DC**, Buse JB, Nielsen LL, Guan X, Bowlus CL, Holcombe JH, Wintle ME, Maggs DG. Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years. *Curr Med Res Opin* 2008; **24**: 275-286 [PMID: 18053320]

57 **Kim D**, MacConell L, Zhuang D, Kothare PA, Trautmann M, Fineman M, Taylor K. Effects of once-weekly dosing of a long-acting release formulation of exenatide on glucose control and body weight in subjects with type 2 diabetes. *Diabetes Care* 2007; **30**: 1487-1493 [PMID: 17353504 DOI: 10.2337/dc06-2375]

58 **Buse JB**, Rosenstock J, Sesti G, Schmidt WE, Montanya E, Brett JH, Zychma M, Blonde L. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). *Lancet* 2009; **374**: 39-47 [PMID: 19515413]

59 **Fadini GP**, Simioni N, Frison V, Dal Pos M, Bettio M, Rocchini P, Avogaro A. Independent glucose and weight-reducing effects of Liraglutide in a real-world population of type 2 diabetic outpatients. *Acta Diabetol* 2013; **50**: 943-949 [PMID: 23754673 DOI: 10.1007/s00592-013-0489-3]

60 **Derosa G**, Franzetti IG, Querci F, Carbone A, Ciccarelli L, Piccinni MN, Fogari E, Maffioli P. Exenatide plus metformin

compared with metformin alone on β-cell function in patients with Type 2 diabetes. *Diabet Med* 2012; **29**: 1515-1523 [PMID: 22540883 DOI: 10.1111/j.14645491.2012.03699.x]

61 **Bunck MC**, Diamant M, Cornér A, Eliasson B, Malloy JL, Shaginian RM, Deng W, Kendall DM, Taskinen MR, Smith U, Yki-Järvinen H, Heine RJ. One-year treatment with exenatide improves beta-cell function, compared with insulin glargine, in metformin-treated type 2 diabetic patients: a randomized, controlled trial. *Diabetes Care* 2009; **32**: 762-768 [PMID: 19196887 DOI: 10.2337/dc08-1797]

62 **Gallwitz B**, Guzman J, Dotta F, Guerci B, Simó R, Basson BR, Festa A, Kiljański J, Sapin H, Trautmann M, Schernthaner G. Exenatide twice daily versus glimepiride for prevention of glycaemic deterioration in patients with type 2 diabetes with metformin failure (EUREXA): an open-label, randomised controlled trial. *Lancet* 2012; **379**: 2270-2278 [PMID: 22683137 DOI: 10.1016/S0140-6736(12)60479-6]

63 **Buse JB**, Bergenstal RM, Glass LC, Heilmann CR, Lewis MS, Kwan AY, Hoogwerf BJ, Rosenstock J. Use of twice-daily exenatide in Basal insulin-treated patients with type 2 diabetes: a randomized, controlled trial. *Ann Intern Med* 2011; **154**: 103-112 [PMID: 21138825 DOI: 10.7326/0003-4819-154-2-201101180-00300]

64 **Grimm M**, Han J, Weaver C, Griffin P, Schulteis CT, Dong H, Malloy J. Efficacy, safety, and tolerability of exenatide once weekly in patients with type 2 diabetes mellitus: an integrated analysis of the DURATION trials. *Postgrad Med* 2013; **125**: 47-57 [PMID: 23748506 DOI: 10.3810/pgm.2013.05.2660]

65 **Astrup A**, Carraro R, Finer N, Harper A, Kunesova M, Lean ME, Niskanen L, Rasmussen MF, Rissanen A, Rössner S, Savolainen MJ, Van Gaal L. Safety, tolerability and sustained weight loss over 2 years with the once-daily human GLP-1 analog, liraglutide. *Int J Obes* (Lond) 2012; **36**: 843-854 [PMID: 21844879 DOI: 10.1038/ijo.2011.158]

66 **Armstrong MJ**, Barton D, Gaunt P, Hull D, Guo K, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide efficacy and action in non-alcoholic steatohepatitis (LEAN): study protocol for a phase II multicentre, double-blinded, randomised, controlled trial. *BMJ Open* 2013; **3**: e003995 [PMID: 24189085 DOI: 10.1136/bmjopen-2013-003995]

67 **Seino Y**, Min KW, Niemoeller E, Takami A; EFC10887 GETGOAL- L Asia Study Investigators. Randomized, double-blind, placebo-controlled trial of the once-daily GLP-1 receptor agonist lixisenatide in Asian patients with type 2 diabetes insufficiently controlled on basal insulin with or without a sulfonylurea (GetGoal-L-Asia). *Diabetes Obes Metab* 2012; **14**: 910-917 [DOI: 10.1111/j.1463-1326.2012.01618.x]

68 **Fonseca VA**, Alvarado-Ruiz R, Raccah D, Boka G, Miossec P, Gerich JE; EFC6018 GetGoal-Mono Study Investigators Efficacy and safety of the once-daily GLP-1 receptor agonist lixisenatide in monotherapy: a randomized, double-blind, placebo-controlled trial in patients with type 2 diabetes (GetGoal-Mono). *Diabetes Care* 2012; **35**: 1225-1231 [DOI: 10.2337/dc11-1935]

69 **Kapitza C**, Forst T, Coester HV, Poitiers F, Ruus P, Hincelin-Méry A. Pharmacodynamic characteristics of lixisenatide once daily versus liraglutide once daily in patients with type 2 diabetes insufficiently controlled on metformin. *Diabetes Obes Metab* 2013; **15**: 642-649 [PMID: 23368510 DOI: 10.1111/dom.12076]

70 **Brown DX**, Butler EL, Evans M. Lixisenatide as add-on therapy to basal insulin. *Drug Des Devel Ther* 2013; **8**: 25-38 [PMID: 24363554 DOI: 10.2147/DDDT.S45108]

71 **Nauck M**, Horton E, Andjelkovic M, Ampudia-Blasco FJ, Parusel CT, Boldrin M, Balena R. Taspoglutide, a once-weekly glucagon-like peptide 1 analogue, vs insulin glargine titrated to target in patients with Type 2 diabetes: an open-label randomized trial. *Diabet Med* 2013; **30**: 109-113 [PMID: 22937895]

72 **Rosenstock J**, Balas B, Charbonnel B, Bolli GB, Boldrin M, Ratner R, Balena R. The fate of taspoglutide, a weekly GLP-1 receptor agonist, versus twice-daily exenatide for type 2 diabetes: the T-emerge 2 trial. *Diabetes Care* 2013; **36**: 498-504 [PMID: 23139373 DOI: 10.2337/dc12-0709]

73 **Dennis MS**, Zhang M, Meng YG, Kadkhodayan M, Kirchhofer D, Combs D, Damico LA. Albumin binding as a general strategy for improving the pharmacokinetics of proteins. *J Biol Chem* 2002; **277**: 35035-35043 [PMID: 12119302]

74 **Baggio LL**, Huang Q, Brown TJ, Drucker DJ. A recombinant human glucagon-like peptide (GLP)-1-albumin protein (albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. *Diabetes* 2004; **53**: 2492-2500 [PMID: 15331566]

75 **Tomkin GH**. Albiglutide, an albumin-based fusion of glucagon-like peptide 1 for the potential treatment of type 2 diabetes. *Curr Opin Mol Ther* 2009; **11**: 579-588 [PMID: 19806507]

76 **Buse JB**, Garber A, Rosenstock J, Schmidt WE, Brett JH, Videbæk N, Holst J, Nauck M. Liraglutide treatment is associated with a low frequency and magnitude of antibody formation with no apparent impact on glycemic response or increased frequency of adverse events: results from the Liraglutide Effect and Action in Diabetes (LEAD) trials. *J Clin Endocrinol Metab* 2011; **96**: 1695-1702 [PMID: 21450987]

77 **Madsbad S**, Kielgast U, Asmar M, Deacon CF, Torekov SS, Holst JJ. An overview of once-weekly glucagon-like peptide-1 receptor agonists--available efficacy and safety data and perspectives for the future. *Diabetes Obes Metab* 2011; **13**: 394-407 [PMID: 21208359 DOI: 10.1111/j.1463-1326.2011.01357.x]

78 **Nyström T**, Gutniak MK, Zhang Q, Zhang F, Holst JJ, Ahrén B, Sjöholm A. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab* 2004; **287**: E1209-E1215 [PMID: 15353407]

79 **Cleveland JC**, Meldrum DR, Cain BS, Banerjee A, Harken AH. Oral sulfonylurea hypoglycemic agents prevent ischemic preconditioning in human myocardium. Two paradoxes revisited. *Circulation* 1997; **96**: 29-32 [PMID: 9236412]

80 **Tomai F**, Danesi A, Ghini AS, Crea F, Perino M, Gaspardone A, Ruggeri G, Chiariello L, Gioffrè PA. Effects of K(ATP) channel blockade by glibenclamide on the warm-up phenomenon. *Eur Heart J* 1999; **20**: 196-202 [PMID: 10082152]

81 **Ovünç K**. Effects of glibenclamide, a K(ATP) channel blocker, on warm-up phenomenon in type II diabetic patients with chronic stable angina pectoris. *Clin Cardiol* 2000; **23**: 535-539 [PMID: 10894443]

82 **Luptak I**, Yan J, Cui L, Jain M, Liao R, Tian R. Long-term effects of increased glucose entry on mouse hearts during normal aging and ischemic stress. *Circulation* 2007; **116**: 901-909 [PMID: 17679614]

83 **Bao W**, Aravindhan K, Alsaid H, Chendrimada T, Szapacs M, Citerone DR, Harpel MR, Willette RN, Lepore JJ, Jucker BM. Albiglutide, a long lasting glucagon-like peptide-1 analog, protects the rat heart against ischemia/reperfusion injury: evidence for improving cardiac metabolic efficiency. *PLoS One* 2011; **6**: e23570 [PMID: 21887274 DOI: 10.1371/journal.pone.0023570]

84 **Rahmi RM**, Uchida AH, Rezende PC, Lima EG, Garzillo CL, Favarato D, Strunz CM, Takiuti M, Girardi P, Hueb W, Kalil Filho R, Ramires JA. Effect of hypoglycemic agents on ischemic preconditioning in patients with type 2 diabetes and symptomatic coronary artery disease. *Diabetes Care* 2013; **36**: 1654-1659 [PMID: 23250803]

85 **Anderson SL**, Trujillo JM. Association of pancreatitis with glucagon-like peptide-1 agonist use. *Ann Pharmacother* 2010; **44**: 904-909 [PMID: 20371755 DOI: 10.1345/aph.1M676]

86 **Wenten M**, Gaebler JA, Hussein M, Pelletier EM, Smith DB, Girase P, Noel RA, Braun DK, Bloomgren GL. Relative risk of acute pancreatitis in initiators of exenatide twice daily

compared with other anti-diabetic medication: a follow-up study. *Diabet Med* 2012; **29**: 1412-1418 [PMID: 22416857 DOI: 10.1111/j.1464-5491.2012.03652.x]
87 **Gale EA**. GLP-1-based therapies and the exocrine pancreas: more light, or just more heat? *Diabetes* 2012; **61**: 986-988 [PMID: 22517653]
88 **Gier B**, Matveyenko AV, Kirakossian D, Dawson D, Dry SM, Butler PC. Chronic GLP-1 receptor activation by exendin-4 induces expansion of pancreatic duct glands in rats and accelerates formation of dysplastic lesions and chronic pancreatitis in the Kras(G12D) mouse model. *Diabetes* 2012; **61**: 1250-1262 [PMID: 22266668 DOI: 10.2337/db11-1109]
89 **Gier B**, Butler PC, Lai CK, Kirakossian D, DeNicola MM, Yeh MW. Glucagon like peptide-1 receptor expression in the human thyroid gland. *J Clin Endocrinol Metab* 2012; **97**: 121-131 [PMID: 22031513]
90 **Karagiannis T**, Paschos P, Paletas K, Matthews DR, Tsapas A. Dipeptidyl peptidase-4 inhibitors for treatment of type 2 diabetes mellitus in the clinical setting: systematic review and meta-analysis. *BMJ* 2012; **344**: e1369 [PMID: 22411919 DOI: 10.1136/bmj.e1369]
91 **Kim YG**, Hahn S, Oh TJ, Kwak SH, Park KS, Cho YM. Differences in the glucose-lowering efficacy of dipeptidyl peptidase-4 inhibitors between Asians and non-Asians: a systematic review and meta-analysis. *Diabetologia* 2013; **56**: 696-708 [PMID: 23344728]
92 **Gitt AK**, Bramlage P, Binz C, Krekler M, Deeg E, Tschöpe D. Prognostic implications of DPP-4 inhibitor vs. sulfonylurea use on top of metformin in a real world setting - results of the 1 year follow-up of the prospective DiaRegis registry. *Int J Clin Pract* 2013; **67**: 1005-1014 [PMID: 23981060 DOI: 10.1111/ijcp.12179]
93 **Kosaraju J**, Murthy V, Khatwal RB, Dubala A, Chinni S, Muthureddy Nataraj SK, Basavan D. Vildagliptin: an anti-diabetes agent ameliorates cognitive deficits and pathology observed in streptozotocin-induced Alzheimer's disease. *J Pharm Pharmacol* 2013; **65**: 1773-1784 [PMID: 24117480 DOI: 10.1111/jphp.12148]
94 **Omar BA**, Vikman J, Winzell MS, Voss U, Ekblad E, Foley JE, Ahrén B. Enhanced beta cell function and anti-inflammatory effect after chronic treatment with the dipeptidyl peptidase-4 inhibitor vildagliptin in an advanced-aged diet-induced obesity mouse model. *Diabetologia* 2013; **56**: 1752-1760 [PMID: 23636640 DOI: 10.1007/s00125-013-2927-8]
95 **Duttaroy A**, Voelker F, Merriam K, Zhang X, Ren X, Subramanian K, Hughes TE, Burkey BF. The DPP-4 inhibitor vildagliptin increases pancreatic beta cell mass in neonatal rats. *Eur J Pharmacol* 2011; **650**: 703-707 [PMID: 21070766 DOI: 10.1016/j.ejphar]
96 **Takeda Y**, Fujita Y, Honjo J, Yanagimachi T, Sakagami H, Takiyama Y, Makino Y, Abiko A, Kieffer TJ, Haneda M. Reduction of both beta cell death and alpha cell proliferation by dipeptidyl peptidase-4 inhibition in a streptozotocin-induced model of diabetes in mice. *Diabetologia* 2012; **55**: 404-412 [PMID: 22072158 DOI: 10.1007/s00125-011-2365-4]
97 **Aston-Mourney K**, Subramanian SL, Zraika S, Samarasekera T, Meier DT, Goldstein LC, Hull RL. One year of sitagliptin treatment protects against islet amyloid-associated β-cell loss and does not induce pancreatitis or pancreatic neoplasia in mice. *Am J Physiol Endocrinol Metab* 2013; **305**: E475-E484 [PMID: 23736544 DOI: 10.1152/ajpendo]
98 **Nabeno M**, Akahoshi F, Kishida H, Miyaguchi I, Tanaka Y, Ishii S, Kadowaki T. A comparative study of the binding modes of recently launched dipeptidyl peptidase IV inhibitors in the active site. *Biochem Biophys Res Commun* 2013; **434**: 191-196 [PMID: 23501107]
99 **Scheen AJ**, Charpentier G, Ostgren CJ, Hellqvist A, Gause-Nilsson I. Efficacy and safety of saxagliptin in combination with metformin compared with sitagliptin in combination with metformin in adult patients with type 2 diabetes mellitus. *Diabetes Metab Res Rev* 2010; **26**: 540-549 [PMID: 20824678 DOI: 10.1002/dmrr.1114]
100 **Capuano A**, Sportiello L, Maiorino MI, Rossi F, Giugliano D, Esposito K. Dipeptidyl peptidase-4 inhibitors in type 2 diabetes therapy--focus on alogliptin. *Drug Des Devel Ther* 2013; **7**: 989-1001 [PMID: 24068868 DOI: 10.2147/DDDT.S37647]
101 **Aschner P**, Chan J, Owens DR, Picard S, Wang E, Dain MP, Pilorget V, Echtay A, Fonseca V. Insulin glargine versus sitagliptin in insulin-naive patients with type 2 diabetes mellitus uncontrolled on metformin (EASIE): a multicentre, randomised open-label trial. *Lancet* 2012; **379**: 2262-2269 [PMID: 22683131 DOI: 10.1016/S0140-6736(12)60439-5]
102 **Barnett AH**, Huisman H, Jones R, von Eynatten M, Patel S, Woerle HJ. Linagliptin for patients aged 70 years or older with type 2 diabetes inadequately controlled with common antidiabetes treatments: a randomised, double-blind, placebo-controlled trial. *Lancet* 2013; **382**: 1413-1423 [PMID: 23948125 DOI: 10.1016/S0140-6736(13)61500-7]
103 **Strain WD**, Lukashevich V, Kothny W, Hoellinger MJ, Paldánius PM. Individualised treatment targets for elderly patients with type 2 diabetes using vildagliptin add-on or lone therapy (INTERVAL): a 24 week, randomised, double-blind, placebo-controlled study. *Lancet* 2013; **382**: 409-416 [PMID: 23706759 DOI: 10.1016/S0140-6736(13)60995-2]
104 **Sinclair A**, Morley J. Frailty and diabetes. *Lancet* 2013; **382**: 1386-1387 [PMID: 23948126 DOI: 10.1016/S0140-6736(13)61676-1]
105 **Morley JE**, Sinclair A. Individualising treatment for older people with diabetes. *Lancet* 2013; **382**: 378-380 [PMID: 23706758 DOI: 10.1016/S0140-6736(13)61055-7]
106 **Sinclair A**, Morley JE, Rodriguez-Mañas L, Paolisso G, Bayer T, Zeyfang A, Bourdel-Marchasson I, Vischer U, Woo J, Chapman I, Dunning T, Meneilly G, Rodriguez-Saldana J, Gutierrez Robledo LM, Cukierman-Yaffe T, Gadsby R, Schernthaner G, Lorig K. Diabetes mellitus in older people: position statement on behalf of the International Association of Gerontology and Geriatrics (IAGG), the European Diabetes Working Party for Older People (EDWPOP), and the International Task Force of Experts in Diabetes. *J Am Med Dir Assoc* 2012; **13**: 497-502 [PMID: 22748719 DOI: 10.1016/j.jamda.2012.04.012]
107 **Lukashevich V**, Prato SD, Araga M, Kothny W. Efficacy and safety of vildagliptin in patients with type 2 diabetes mellitus inadequately controlled with dual combination of metformin and sulphonylurea. *Diabetes Obes Metab* 2014; **16**: 403-409 [PMID: 24199686]
108 **Rosenstock J**, Gross JL, Aguilar-Salinas C, Hissa M, Berglind N, Ravichandran S, Fleming D. Long-term 4-year safety of saxagliptin in drug-naive and metformin-treated patients with Type 2 diabetes. *Diabet Med* 2013; **30**: 1472-1476 [PMID: 23802840 DOI: 10.1111/dme.12267]
109 **Hiatt WR**, Kaul S, Smith RJ. The cardiovascular safety of diabetes drugs--insights from the rosiglitazone experience. *N Engl J Med* 2013; **369**: 1285-1287 [PMID: 23992603 DOI: 10.1056/NEJMp1309610]
110 **Scirica BM**, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Frederich R, Wiviott SD, Hoffman EB, Cavender MA, Udell JA, Desai NR, Mosenzon O, McGuire DK, Ray KK, Leiter LA, Raz I. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013; **369**: 1317-1326 [PMID: 23992601 DOI: 10.1056/NEJMoa1307684]
111 **Graefe-Mody U**, Retlich S, Friedrich C. Clinical pharmacokinetics and pharmacodynamics of linagliptin. *Clin Pharmacokinet* 2012; **51**: 411-427 [PMID: 22568694 DOI: 10.2165/11630900-000000000-00000]
112 **Friedrich C**, Emser A, Woerle HJ, Graefe-Mody U. Renal impairment has no clinically relevant effect on the long-term exposure of linagliptin in patients with type 2 diabetes. *Am J Ther* 2013; **20**: 618-621 [PMID: 23411609 DOI: 10.1097/

MJT.0b013e31826232dc]
113 **Gallwitz B**, Rosenstock J, Rauch T, Bhattacharya S, Patel S, von Eynatten M, Dugi KA, Woerle HJ. 2-year efficacy and safety of linagliptin compared with glimepiride in patients with type 2 diabetes inadequately controlled on metformin: a randomised, double-blind, non-inferiority trial. *Lancet* 2012; **380**: 475-483 [PMID: 22748821 DOI: 10.1016/S01406736(12)60691-6]
114 **Del Prato S**, Taskinen MR, Owens DR, von Eynatten M, Emser A, Gong Y, Chiavetta S, Patel S, Woerle HJ. Efficacy and safety of linagliptin in subjects with type 2 diabetes mellitus and poor glycemic control: pooled analysis of data from three placebo-controlled phase III trials. *J Diabetes Complications* 2013; **27**: 274-279 [PMID: 23403068 DOI: 10.1016/j.jdiacomp.2012.11.008]
115 **Ndefo UA**, Okoli O, Erowele G. Alogliptin: A new dipeptidyl peptidase-4 inhibitor for the management of type 2 diabetes mellitus. *Am J Health Syst Pharm* 2014; **71**: 103-109 [PMID: 24375601 DOI: 10.2146/ajhp130131]
116 **White WB**, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med* 2013; **369**: 1327-1335 [PMID: 23992602 DOI: 10.1056/NEJMoa1305889]
117 **Goda M**, Kadowaki T. Teneligliptin for the treatment of type 2 diabetes. *Drugs Today* (Barc) 2013; **49**: 615-629 [PMID: 24191255 DOI: 10.1358/dot.2013.49.10.2035882]
118 **Younce CW**, Burmeister MA, Ayala JE. Exendin-4 attenuates high glucose-induced cardiomyocyte apoptosis via inhibition of endoplasmic reticulum stress and activation of SERCA2a. *Am J Physiol Cell Physiol* 2013; **304**: C508-C518 [PMID: 23302777 DOI: 10.1152/ajpcell]
119 **Matsubara J**, Sugiyama S, Akiyama E, Iwashita S, Kurokawa H, Ohba K, Maeda H, Fujisue K, Yamamoto E, Kaikita K, Hokimoto S, Jinnouchi H, Ogawa H. Dipeptidyl peptidase-4 inhibitor, sitagliptin, improves endothelial dysfunction in association with its anti-inflammatory effects in patients with coronary artery disease and uncontrolled diabetes. *Circ J* 2013; **77**: 1337-1344 [PMID: 23386232]
120 **Butler AE**, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes* 2013; **62**: 2595-2604 [PMID: 23524641 DOI: 10.2337/db12-1686]
121 **Crickx E**, Marroun I, Veyrie C, Le Beller C, Schoindre Y, Bouilloud F, Blétry O, Kahn JE. DPP4 inhibitor-induced polyarthritis: a report of three cases. *Rheumatol Int* 2014; **34**: 291-292 [PMID: 23462883 DOI: 10.1007/s00296-013-2710-7]
122 **Ambrosio ML**, Monami M, Sati L, Marchionni N, Di Bari M, Mannucci E. GLP-1 receptor agonist-induced polyarthritis: a case report. *Acta Diabetol* 2013; Epub ahead of print [PMID: 24158775 DOI: 10.2337/db12-1686.Epub2013Mar]

P- Reviewer: Apikoglu-Rabus S, Conteduca V, Kumar KVS
S- Editor: Wen LL **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.651

World J Diabetes 2014 October 15; 5(5): 651-658
ISSN 1948-9358 (online)

REVIEW

Diabetes treatment in patients with renal disease: Is the landscape clear enough?

Ioannis Ioannidis

Ioannis Ioannidis, 2nd Department of Internal Medicine, Konstantopoulio Hospital, Nea Ionia, 14233 Athens, Greece
Author contributions: Ioannidis I solely contributed to this paper.
Correspondence to: Ioannis Ioannidis, MD, PhD, Director of Diabetes Outpatient Clinic, 2nd Department of Internal Medicine, Konstantopoulio Hospital, Nea Ionia, Agias Olgas 3-5, 14233 Athens, Greece. kefion@otenet.gr
Telephone: +30-213-2057216 Fax: +30-210-2773845
Received: November 30, 2013 Revised: July 9, 2014
Accepted: July 18, 2014
Published online: October 15, 2014

Abstract

Diabetes is the most important risk factors for chronic kidney disease (CKD). The risk of CKD attributable to diabetes continues to rise worldwide. Diabetic patients with CKD need complicated treatment for their metabolic disorders as well as for related comorbidities. They have to treat, often intensively, hypertension, dyslipidaemia, bone disease, anaemia, and frequently established cardiovascular disease. The treatment of hypoglycaemia in diabetic persons with CKD must tie their individual goals of glycaemia (usually less tight glycaemic control) and knowledge on the pharmacokinetics and pharmacodynamics of drugs available to a person with kidney disease. The problem is complicated from the fact that in many efficacy studies patients with CKD are excluded so data of safety and efficacy for these patients are missing. This results in fear of use by lack of evidence. Metformin is globally accepted as the first choice in practically all therapeutic algorithms for diabetic subjects. The advantages of metformin are low risk of hypoglycaemia, modest weight loss, effectiveness and low cost. Data of UKPDS indicate that treatment based on metformin results in less total as well cardiovascular mortality. Metformin remains the drug of choice for patients with diabetes and CKD provided that their estimate Glomerular Filtration Rate (eGFR) remains above 30 mL/min per square meter. For diabetic patients with eGFR between 30-60 mL/min per square meter more frequent monitoring of renal function and dose reduction of metformin is needed. The use of sulfonylureas, glinides and insulin carry a higher risk of hypoglycemia in these patients and must be very careful. Lower doses and slower titration of the dose is needed. Is better to avoid sulfonylureas with active hepatic metabolites, which are renally excreted. Very useful drugs for this group of patients emerge dipeptidyl peptidase 4 inhibitors. These drugs do not cause hypoglycemia and most of them (linagliptin is an exception) require dose reduction in various stages of renal disease.

Key words: Chronic kidney disease; Diabetes; Antidiabetic drugs; Metformin; Dipeptidyl peptidase 4 inhibitors; Therapeutic algorithm

Core tip: Chronic kidney disease (CKD) is very often among diabetic persons. In every day clinical practice doctors worldwide have to deal with these patients and help them to achieve their metabolic goals. Despite this, many studies of antidiabetic drugs have excluded people with CKD. So, we lack solid evidence on the effectiveness and safety of these drugs. In this review I propose therapeutic algorithms for diabetic patients in different stages of CKD and clarify some questions about the use of popular antidiabetic drugs as metformin and sulfonylureas.

Ioannidis I. Diabetes treatment in patients with renal disease: Is the landscape clear enough? *World J Diabetes* 2014; 5(5): 651-658 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/651.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.651

INTRODUCTION

Chronic kidney disease (CKD) affects million of people

worldwide. CKD is becoming a major problem for public health as it leads to increased morbidity and mortality. Patients with end stage chronic kidney disease often need kidney transplantation[1].

The prevalence of chronic kidney disease to the estimated 11% of the United States population. Patients with chronic kidney disease have an increased risk of cardiovascular disease and progression of renal disease in end-stage renal failure. End stage renal failure leads to dialysis or transplantation[2,3].

Diabetes is the most important risk factors for CKD. The risk of CKD attributable to diabetes continues to rise worldwide.

The National Kidney Foundation and the American Heart Association have recently issued guidelines for the management of cardiovascular risk in people with kidney disease by stating emphatically that these individuals are at very high cardiovascular risk.

For diabetic patients with chronic kidney disease, the risk of cardiovascular disease is even higher classifying these individuals in the highest risk group for cardiovascular disease. Diabetic subjects with microalbuminuria have increased risk (2x) of cardiovascular disease than those with normoalbuminuria. Proteinuria and decreased Glomerular Filtration Rate (GFR) contribute synergistically to increase cardiovascular risk. Most diabetic patients with CKD stage 3 will suffer a serious cardiovascular event, possibly fatal before their chronic kidney disease progress to end stage kidney failure.

Diabetic patients need complicated treatment for their metabolic problems as well as for related comorbidities. They have to treat, often intensively, hypertension, dyslipidaemia, bone disease, anaemia, and frequently established cardiovascular disease (CVD). Thus, the problem for the appropriate selection of antidiabetic treatment for patients with diabetes and CKD is usual in every day clinical practice[4,5].

DIABETES TREATMENT: DIFFERENT GOALS AND DIFFERENT DRUGS

Recent guidelines for the treatment of diabetes (ADA, EASD 2012) propose personalization of glycaemic goals. For the majority of diabetic patients the appropriate goal is a haemoglobin A1c (HbA1c) < 7% but for patients with severe comorbidities a goal between 7% and 8% is acceptable. Diabetic subjects with CKD usually belong to this group.

The glycated HbA1c is the most popular and well-accepted biological marker for the assessment of long-term glycaemic control. This also applies to patients with diabetes and renal disease. However, the method has significant limitations in these patients. The measurement is influenced by both renal function and complications of chronic kidney disease such as haemolysis, iron deficiency and metabolic acidosis.

In most cases diabetic subjects with chronic kidney disease must rely more on self-monitoring of blood glucose with usual glucose meters. Patients with diabetes and CKD have usually already established CVD. These patients are also in greater risk of hypoglycaemia. We know from physiology that normal renal function conveys a 30% of neoglycogenesis, which is necessary to avoid hypoglycaemia especially in prolonged fasting periods[6].

Many diabetics with uraemia have also nutritional problems and some times cachexia. The use of insulin as well as of sulfonylureas or glinides (short acting secretagogues) leads to increased rate of hypoglycaemia in this group of patients[7,8].

On the other hand, many drugs have renal metabolism and their metabolites are usually active prolonging their time of action. The use of antidiabetic drugs, especially the new classes, is conflicted. The major problem is that in many efficacy studies patients with CKD are excluded so data of safety and efficacy for these patients are missing. This results in fear of use by lack of evidence[9].

Nevertheless, pharmacokinetics and pharmacodynamics data for many new drugs help us to understand the potential risks and benefits for these subjects. Even if these basic data are reassuring the clinical point remains critical: We cannot use new drugs based only on these evidence! We need results form efficacy studies and then approval from FDA and EMEA[10].

Finally, the use of antidiabetic drugs is more complicated in these patients because many people with kidney disease are often elderly, and have long lasting disease and significant co-morbidities. These people take many drugs and they have high risk of drug interactions.

ESTIMATION OF RENAL FUNCTION IN DIABETIC PATIENTS

For all diabetic subjects we have to estimate their renal function. 1st step: Serum creatinine/annually (or every 3-4 mo in selected patients); 2nd step: Based on serum creatinine we estimate GFR (eGFR). eGFR is usually based on patient characteristics (as age, sex and race) as well as serum creatinine levels. The most popular method of assessment of renal

$$\text{MDRD: GFR} = 175 \times \text{SerumCr}^{-1.154} \times \text{age}^{-0.203}$$

[× 1.212 (if patient is black) × 0.742 (if female)]

function with the greater precision is the Modification of Diet in Renal Disease (MDRD) equation. This equation is based on data of MDRD Study. This equation (MDRD) is especially accurate in GFR < 60 mL/min.

For higher GFR another equation can also be used: Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) method based on data of CKD-EPI.

$$\text{CKD-EPI: GFR} = 141 \times \min(\text{Scr}/\kappa, 1)^{\alpha} \times \max(\text{Scr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018 \text{ (if female)} \times 1.159 \text{ (if black)}$$

Where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

Usually we use friendly calculators to estimate GFR.

Many programs are also free available for smartphones.

The classical formula of Cockcroft-Gault is not used anymore because it overestimates GFR. (Body weight in the formula must be lean weight and not total weight).

METFORMIN

Metformin is globally accepted as the first choice in practically all therapeutic algorithms for diabetic subjects. The advantages of metformin are low risk of hypoglycaemia, modest weight loss, effectiveness and low cost. Data of UKPDS indicate that treatment based on metformin results in less total as well cardiovascular mortality.

Many diabetologists as well as practitioners are fear to use metformin in patients with renal problems even if they have only albuminuria. There is a lot of confusion about the real restriction of its use in patients with CKD[11].

Metformin is slowly absorbed when administered orally. The bioavailability of the drug is low (50%-60%).

Metformin achieves a maximum plasma concentration one to three hours after ingestion, if taken in the form of immediate release or in 4-8 h with the extended release form. Metformin is not connected with albumin or any other protein in plasma. This results in a high volume of distribution up to 1000 even after the first dose[12].

In patients with moderate and severe CKD Cmax is increased 173% and 390%, respectively, compared with patients with normal renal function.

In normal pH metformin remains as hydrophilic cation. Less than 0.01% of the drug is unionized in blood. Lipid solubility of metformin is low. So, metformin can not diffuses through cell membranes. Phenformin, another member of antidiabetic drug class biguanides, which is no longer in the market, is more lipophilic than metformin due to different side chain. Metformin is not metabolized in the liver. Metformin is actively excreted by the urinary tube and found unchanged in the urine. After 24 h, if renal function is normal, metformin is not detected in the blood after administration of a single dose. The half-life of metformin is plasma is about 6 h[13].

The absorption of metformin in the intestine is mediated by a transporter known as plasma membrane monoamine transporter. Several metformin transporters are implicated in its intestinal absorption as well as in its hepatic uptake and renal excretion. These transporters are either Organic Cation Transporters (OCTs) or multidrug and toxin extrusion proteins (MATEs).

The kidneys also actively excrete metformin. Metformin enters renal cells of the renal tubule from circulation. This procedure takes place on the basolateral membrane of the cells and is mediated by OCT2.

Then, metformin is excreted into the lumen. This excretion is facilitated by MATE (1 and 2-K). These extrusion proteins are located in the apical membrane of renal proximal tubule cells.

Metformin is also reabsorbed in renal tubules and this action is mediated by OCT1, which is located also in proximal and distal tubules.

The molecular mechanisms underlying metformin action appear to be complex. Metformin entries into the hepatic cell and facilitate phosphorylation and activation of AMP-activated protein kinase (AMPK). Activation of this key-kinase (energy status sensor) lead to many effects related to metabolism of glucose and lipids. Metformin inhibits hepatic neoglycogenesis also in a direct manner. Metformin inhibits complex I of the mitochondrial respiratory chain. This inhibition, leads to an incretion of AMP:ATP ratio, which activate AMPK. This inhibition leads also to increased anaerobic metabolism of glucose in cytoplasm and the production of lactic acid. Thus, metformin is related with increased risk of lactic acidosis when renal elimination of lactic acid is decreased (renal disease, reduced GFR) or hepatic function is severely damaged (lactic acid is used in hepatocytes to produce glucose-neoglycogenesis-). The risk of lactic acidosis is also increased in patients with tissue hypoxia (shock, severe heart failure, sepsis, surgery related hypotension, *etc.*)[14].

Risk of lactic acidosis was greater with phenformin because it's a more potent inhibitor of mitochondrial respiration. Phenformin has hepatic metabolism with an inactive metabolite. The enzyme CYP2D6 metabolizes phenformin into an inactive metabolite. A small ratio of patients (about 2.8%) has a polymorphism of the enzyme that makes them poor metabolizers. In these patients the risk of lactic acid is even greater (due to higher levels of phenformin).

Nevertheless, analysis of data from may trials (347 comparative trials and cohort studies) from Cochrane Database systematic review in 2010, showed no cases of lactic acidosis in 70490 patient-years of metformin.

Statistical analysis of these data suggested that the upper limit for the incidence of lactic acidosis per 100000 patient-years was 4.3 cases (lower than 5.4 cases in the non-metformin group).

In this analysis also, levels of lactic acid seems to be no different in the two groups.

In most studies however lactic acidosis was not a pre specified end point and there were no data about lactic acid levels.

In the Table 1 we summarize the current recommendations about the use of metformin in CKD.

All diabetic subjects at risk of acute renal failure must discontinue at least temporally metformin. Clinical situations related to increase of acute renal failure includes hepatic insufficiency and use of radiocontrast agents and antimicrobial drugs. Fluid substitution as well as support of cardiac output is useful in certain clinical conditions. Monitoring of urine output and serum creatinine lack sensitivity and specificity in acute renal failure, they remain the most used parameters in clinical practice.

At last, when we change the dose of drugs affecting blood pressure and potentially renal perfusion we have to monitor renal function closely and to reduce the dose of metformin (use of diuretics or increase of their dose,

Table 1 Use of metformin in chronic kidney disease

eGFR (mL/min per 1.73 m^2)	Use of metformin
> 60 (CKD 1 and 2)	No contraindication Check of renal function annually
45-60 (CKD 3a)	Use of metformin-reduce dose (no more than 1.5-2 g daily) Frequent check of renal function (every 3-6 mo)
30-45 (CKD 3b)	Reduce dose (no more than 1-1.5 g daily) No new cases Frequent check of renal function (every 3-6 mo)
< 30 (CKD 4 and 5)	Stop metformin

CKD: Chronic kidney disease; eGFR: Estimate Glomerular Filtration Rate.

start of use of ACEIs and ARBs, unstable heart failure with frequent hospitalizations, *etc.*).

PIOGLITAZONE

Pioglitazone has only and exclusively hepatic metabolism. It does not cause hypoglycemia and it can be given theoretically without dose adjustment at all stages of CKD. Pioglitazone is related with fluid retention, anemia and osteoporosis. These side effects complicate the existing problems with anemia and bone disease in subjects with diabetes and CKD[15,16].

The use of pioglitazone is generally limited in these patients and in decreased dose (usually 15 mg once daily).

SULFONYLUREAS

Sulfonylureas are old drugs widely used worldwide. These drugs ease the secretion of insulin and are related with increased risk of hypoglycemia, which is a major issue for CKD patients.

Glibenclamide

Glibenclamide (glyburide) is metabolized in the liver and excreted by the kidneys equally and intestine. Some metabolites are active and can accumulate in CKD despite the fact that biliary removal partially counteracts the limited renal excretion.

Hypoglycemia may be serious and lasting more than 24 h in CKD.

The use of glibenclamide in subjects with moderate CKD (eGFR 60-90 mL/min) should be limited (reduced dose, frequent monitoring due to increased risk of hypoglycemia). The drug and is contraindicated in stage $\geqslant$ 3 CKD (eGFR < 60 mL/min)[17].

Glimepiride

Glimepiride is metabolized by the liver to two major metabolites each of which has hypoglycemic activity. In renal disease these metabolites summed. Although the half-life is 5-7 h, the drug can cause severe hypoglycemia that lasts more than 24 h. Its use is safe in GFR > 60 mL/min and with a reduced dose of up to 30 mL/min. In CKD stage 4 or 5 the use of glimepiride is dangerous[18].

Gliclazide

Gliclazide is metabolized by the liver to inactive metabolites that are eliminated in the urine. Thus, gliclazide causes less hypoglycemia than other sulfonylureas. In CKD sage 1, 2, 3 (eGFR > 30 mL/min) gliclazide can be used. There are no data in patients with severe CKD but according to its metabolism the use (in reduced dose) of gliclazide is also permitted in these subjects[19].

Glipizide

Glipizide also does not need dose adjustment in severe and moderate renal disease and can be used safely. (The only caution remains the risk of hypoglycemia).

GLINIDES

Glinides, repaglinide and nateglinide, are short acting secretagogues. The short duration of their action means reduced risk of hypoglycemia compared to sulfonylureas. This is an advantage for diabetic subjects with CKD because they belong in the high risk for hypoglycemia group as already mentioned.

Repaglinide is absorbed from the gastrointestinal tract and metabolized in the liver by oxidation and conjugation with glucuronic acid. The major metabolites of repaglinide are M1, M2 and M4. These metabolites are excreted *via* the bile into the feces and have no hypoglycemic activity[20].

Repaglinide can be used even in CKD stages 4 and 5 without dose reduction.

Nateglinide is also rapidly absorbed from the gastrointestinal tract and metabolized in liver to 9 main metabolites (M1-M9). These metabolites have much weaker hypoglycemic activity than the parent compound. The only metabolite that retains high activity is the metabolite M7. The concentration of this metabolite however is low (< 7%), resulting in a hypoglycemic effect, which is attributed mainly to intact nateglinide. The excretion of the drug in urine is unchanged form at 16% and by 84% in the form of metabolites.

In CKD stage 5 we avoid nateglinide, and in stage 4 we adjust the dose (60 mg × 3)[21].

GLIPTINES (DIPEPTIDYL PEPTIDASE 4 INHIBITORS)

Dipeptidyl peptidase 4 (DPP-4) inhibitors (gliptins) constitute a new class of antidiabetic drugs with a very favorable profile: safety, efficacy, and low risk of hypoglycemia and weight neutrality[22].

Gliptins are inhibitors of the enzyme DPP-4. This enzyme degrades and inactivates many active peptides. Among them are incretin hormones. These hormones, namely glucagon like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide stimulates glucose

Table 2 Dose adjustment of dipeptidyl peptidase 4 inhibitors in chronic kidney disease

	CKD			
	CKD 1, 2 and 3a (Cl_{cr} > 50 mL/min)	CKD 3b (Cl_{cr} 30-50 mL/min)	CKD stage 4 (Cl_{cr} 15-30 mL/min)	CKD stage 5 (ESRD)
Sitagliptin (Januvia)	√ (100 mg × 1)	1/2 dose (50 mg × 1)	1/4 dose (25 mg × 1)	1/4 dose (25 mg × 1)
Vildagliptin (Galvus)	√ (50 mg × 2)	50 mg × 1		50 mg (no experience)
Saxagliptin (Onglyza)	√ (5 mg × 1)	1/2 dose (2.5 mg × 1)	1/2 dose (2.5 mg × 1)	1/2 dose (2.5 mg × 1)
Linagliptin (Trajenta)	√ (5 mg × 1)	√ (5 mg × 1)	√ (5 mg × 1)	P (5 mg × 1)
Alogliptin (Nesina)	√ (25 mg × 1)	1/2 dose (12.5 mg × 1)	1/4 dose (6.25 mg × 1)	1/4 dose (6.25 mg × 1)

CKD: Chronic kidney disease; ESRD: End stage renal disease.

dependent insulin secretion by b cells in pancreatic islets. At the same time they suppress glucagon production by a cells in the same islets. Their role in glucose homeostasis seems to be important. These hormones are secreted in low levels when we are fasting but their secretion is rapidly increased after meal consumption. Their action results also in reduced glucagon secretion, which in turns reduces hepatic glucose production.

Vildagliptin, Sitagliptin, Saxagliptin, Linagliptin and Alogliptin belong to this class and are already available in the market. Their place in algorithms for patients with diabetes and CKD is important. We can use them all in CKD but with dose adjustment for the majority of the members of this class. (Only linagliptin does not need dose adjustment in any stage of CKD)[23].

In Table 2 we summarize the dose adjustments for all gliptins in diabetic subjects with CKD.

Sitagliptin

Sitagliptin does not undergo extensive metabolism. In the liver sitagliptin partially metabolized by oxidation in a limited rate by the enzyme CYP3A4. Nevertheless, most of the drug is excreted in the intact form in the urine (more than 80%). Sitagliptin is filtered in renal glomerulus but also is actively excreted by active tubular secretion[24].

Six metabolites are detected in amounts of < 1% to 7%. These metabolites M1 to M6 are products of hepatic metabolism.

Chemically the changes in these metabolites are: M1: N-sulfation, M4: N-carbamyl glucuronidation, M6: hydroxylation followed by either glucuronidation (M3), and oxidative desaturation followed by cyclization (M2 and M5). These metabolites are practically inactive.

In renal disease the elimination of the drug is reduced resulting in 2- or 4-fold increase of the concentration of the drug (for CI_{cr} 30-50 mL/min and < 30 mL/min respectively). The dose adjustment is based on these properties.

In Phase I studies of sitagliptin dosing up to 600 mg daily doe not results to dose-related side effects, at least in the short term (up to 28 d). These data indicates that if we don't adjust the dose in CKD practically it might be safe at least for a short period[25,26].

Vildagliptin

Vildagliptin is absorbed quickly (85.4% of the drug). The maximum plasma level is detected at 1.1-h post dose.

Plasma radioactivity (after the administration of radioactive labeled drug) due to vildagliptin is 25.7% and to its major metabolite M20.7 is 55%. The half- life of vildagliptin is 2.8 h. Eighty-five percent of the drug is excreted in the urine (22.6% as vildagliptin the rest as inactive metabolites) and the remaining 15% in feces (4.54% as vildagliptin). In humans, the main pathway of metabolism of the drug is carboxylation, which results in the form of the active metabolite M20.7. DPP-4 contributes to formation of this metabolite. Other minor metabolites are: M15.3, which results from hydrolysis of amide bonds, M20.2 from glucuronidation of the pyrrolidine ring and M20.9, M21.6 from oxidation of the pyrrolidine ring. All these metabolites are inactive[27].

Hydrolysis takes place in multiple tissues or organs. Exposure to vildagliptin in subjects with type 2 diabetes and renal disease of various stages cannot be accurately predicted because the kidneys play a small role in the removal of the drug while participating in metabolism *via* hydrolysis[28].

In diabetic subjects with chronic kidney disease stage 1 or 2 (eGFR > 50 mL/min per 1.73 m^2), dose adjustment of vildagliptin is not required.

In patients with chronic kidney disease stage ⩾ 3, both vildagliptin and its active metabolite M20.7 are less excreted *via* the kidneys. In these patients a dose adjustment is required. (When eGFR is < 50 mg/mL per 1.73 m^2 the dose is 50 mg × 1).

Saxagliptin

Saxagliptin is primarily hepatic metabolized by the cytochrome P450 3A4/5 (CYP3A4/5). The major metabolite of this drug is also active as also a DPP-4 inhibitor, and retains half of the potency of parent drug.

All the drugs, which are also metabolized in this cytochrome CYP3A4/5, may alter the pharmacokinetics of the drug and its active metabolite. Twenty-four percent of the drug is excreted in the urine as saxagliptin and 36% as its active metabolite. There is also some active renal excretion of the drug. A significant part (more than 20%) can be found in the feces as a sum of excreted in bile drug and unabsorbed drug[29].

In diabetic patients with chronic kidney disease stages 1 and 2 increased concentration of saxagliptin and its active metabolite remains clinically irrelevant and no dose adjustment is needed.

In diabetic subjects with chronic kidney disease stages ⩾ 3 half dose is recommended (2.5 mg × 1 daily) to

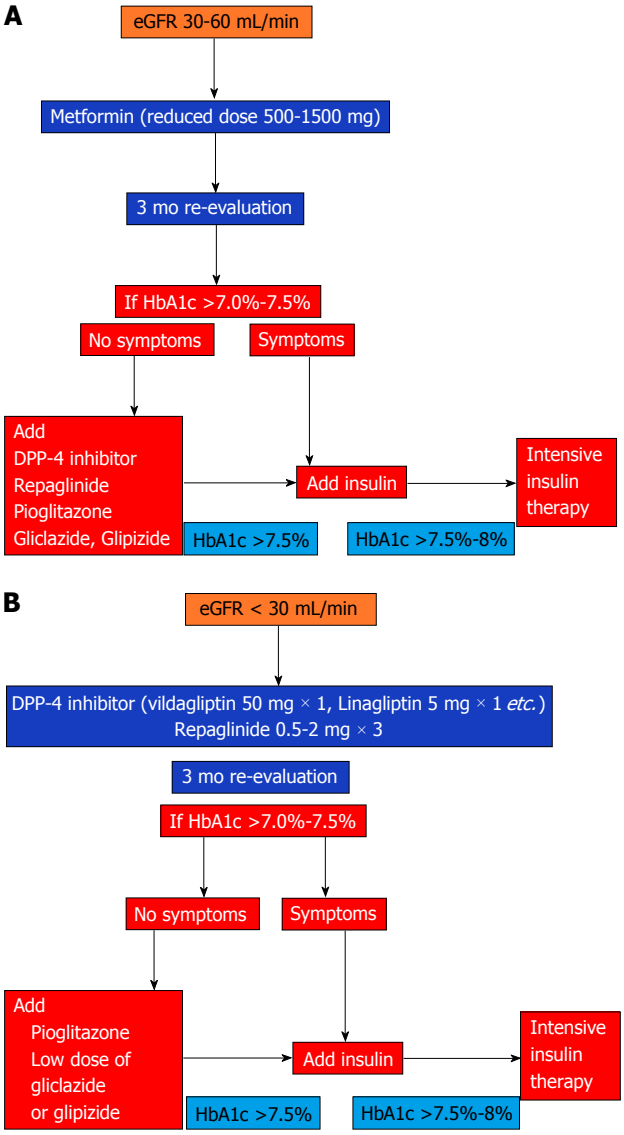

Figure 1 Therapeutic algorithm (A and B). eGFR: Estimate Glomerular Filtration Rate; HbA1c: Haemoglobin A1c; DPP-4: Dipeptidyl peptidase 4.

achieve the same plasma concentrations compared to subjects with normal renal function. The same dose is recommended in patients with end-stage renal disease (requiring hemodialysis).

Alogliptin

This DPP-4 inhibitor is not practically metabolized and is excreted unchanged in the urine. (More than 70% of the parent drug). One minor metabolite named M1 is active but its concentration remains quite low (< 1%)[30]. Alogliptin is excreted by glomerulus filtration as well as by active tubular secretion.

In patients with CKD stage 1 and 2 no dose adjustment is needed (25 mg × 1 daily). In patients with CKD stage 3 (Cr_{Cl} ⩾ 30 to < 60 mL/min), the recommended dose is 12.5 mg once daily and in patients with CKD stage ⩾ 4 the recommended dose is 6.25 mg once daily. The same dose is required in patients with end-stage renal disease requiring dialysis.

Linagliptin

Linagliptin is primarily nonrenally excreted: 80% of the drug is eliminated *via* the bile and gut and only 5% is eliminated *via* the kidney[31]. The drug is not practically metabolized and is excreted unchanged. There is no need of dose adjustment in any stage of CKD (5 mg × 1 for all diabetic subjects).

GLP-1 RA (RECEPTORS AGONISTS)

These drugs are injectable and are potent without risk of hypoglycemia. They have to be used with caution in patients with CKD because their gastrointestinal side effects can induce deterioration of renal disease. (Dehydration due to vomiting or diarrhea).

Exenatide

Exenatide is excreted only by the kidneys and undergoes fragmentation in the renal tubule. It does not metabolized by DPP-4 nor the neutral endopeptidase (NEP). There is no hepatic metabolism of exenatide[32].

In CKD stage 3 dose reduction is needed (5 μg × 2 and close monitoring). In CKD stage 4 and 5 (clearance < 30 mL/min) is not allowed.

Liraglutide

Liraglutide is cleaved *in vivo* by the enzyme DPP-4 that elicits two amino acids at the N terminus of the peptide. NEP also metabolizes liraglutide into several metabolites[33].

Of the administered drug (radioactive labeled) only 26.3% appears in the urine and feces, while breathing excretes 15%. Twenty point one percent of radioactivity is excreted in the urine mainly as water and only 6.3% in substances other than water.

Liraglutide is degraded entirely in the body and is not excreted in urine and feces. These characteristics indicate that we can use in all stages of CKD. Nevertheless we have not yet clinical studies in patients with eGFR < 60 mL/min[34] (there is ongoing studies with preliminary, not yet published, positive results of safety and effectiveness in patients with CKD stage ⩾ 3).

INSULIN

The kidneys carry out one third of exogenous insulin degradation. It is filtered at the glomerulus and is absorbed by the proximal tubule. Sixty percent of the renal clearance is due to glomerular filtration and 40% in the secretion by uptake from peritubular vessels. Reduction in renal filtration is partially counterbalanced by secretion[35]. The dose of exogenous insulin is reduced 25% when eGFR is 10-50 mL/min and 50% when eGFR is < 10 mL/min[36].

CONCLUSION

The landscape is not clear enough in diabetes treatment in CKD. The risk of hypoglycaemia, which is higher in

subjects with both diabetes and CKD, leads to selection of appropriate drugs with low risk of hypoglycaemia such as metformin (reduced dose) and DPP-4 inhibitors. When insulin treatment is appropriate, dose adjustment is usually required especially in CKD stages 4 and 5. Finally, many people with diabetes have a less strict target of glycaemia.

ACKNOWLEDGMENTS

Based on all these data I propose the algorithms as shown in Figure 1.

REFERENCES

1 **de Boer IH**, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA* 2011; **305**: 2532-2539 [PMID: 21693741 DOI: 10.1001/jama.2011.861]

2 **Collins AJ**, Foley RN, Chavers B, Gilbertson D, Herzog C, Johansen K, Kasiske B, Kutner N, Liu J, St Peter W, Guo H, Gustafson S, Heubner B, Lamb K, Li S, Li S, Peng Y, Qiu Y, Roberts T, Skeans M, Snyder J, Solid C, Thompson B, Wang C, Weinhandl E, Zaun D, Arko C, Chen SC, Daniels F, Ebben J, Frazier E, Hanzlik C, Johnson R, Sheets D, Wang X, Forrest B, Constantini E, Everson S, Eggers P, Agodoa L. 'United States Renal Data System 2011 Annual Data Report: Atlas of chronic kidney disease & end-stage renal disease in the United States. *Am J Kidney Dis* 2012; **59**: A7, e1-420 [PMID: 22177944]

3 **National Kidney Foundation**. KDOQI Clinical Practice Guideline for Diabetes and CKD: 2012 Update. *Am J Kidney Dis* 2012; **60**: 850-886 [PMID: 23067652 DOI: 10.1053/j.ajkd.2012.07.005]

4 **Kerri L**, Cavanaugh. Diabetes Management Issues for Patients With Chronic Kidney Disease. *Clin Diabetes* 2007; **25**: 90-97 [DOI: 10.2337/diaclin.25.3.90]

5 **Lubowsky ND**, Siegel R, Pittas AG. Management of glycemia in patients with diabetes mellitus and CKD. *Am J Kidney Dis* 2007; **50**: 865-879 [PMID: 17954300 DOI: 10.1053/j.ajkd.2007.08.012]

6 **Gerich JE**, Meyer C, Woerle HJ, Stumvoll M. Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care* 2001; **24**: 382-391 [PMID: 11213896 DOI: 10.2337/diacare.24.2.382]

7 **Schernthaner G**, Ritz E, Schernthaner GH. Strict glycaemic control in diabetic patients with CKD or ESRD: beneficial or deadly? *Nephrol Dial Transplant* 2010; **25**: 2044-2047 [PMID: 20388630 DOI: 10.1093/ndt/gfq199]

8 **Snyder RW**, Berns JS. Use of insulin and oral hypoglycemic medications in patients with diabetes mellitus and advanced kidney disease. *Semin Dial* 2004; **17**: 365-370 [PMID: 15461745 DOI: 10.1111/j.0894-0959.2004.17346.x]

9 **Haneda M**, Morikawa A. Which hypoglycaemic agents to use in type 2 diabetic subjects with CKD and how? *Nephrol Dial Transplant* 2009; **24**: 338-341 [PMID: 19015168 DOI: 10.1093/ndt/gfn616]

10 **Zanchi A**, Lehmann R, Philippe J. Antidiabetic drugs and kidney disease--recommendations of the Swiss Society for Endocrinology and Diabetology. *Swiss Med Wkly* 2012; **142**: w13629 [PMID: 22987488]

11 **Gong L**, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenet Genomics* 2012; **22**: 820-827 [PMID: 22722338]

12 **Nye HJ**, Herrington WG. Metformin: the safest hypoglycaemic agent in chronic kidney disease? *Nephron Clin Pract* 2011; **118**: c380-c383 [PMID: 21325870 DOI: 10.1159/000323739]

13 **Lipska KJ**, Bailey CJ, Inzucchi SE. Use of metformin in the setting of mild-to-moderate renal insufficiency. *Diabetes Care* 2011; **34**: 1431-1437 [PMID: 21617112 DOI: 10.2337/dc10-2361]

14 **Cusi K**, Consoli A, DeFronzo RA. Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1996; **81**: 4059-4067 [PMID: 8923861]

15 **Budde K**, Neumayer HH, Fritsche L, Sulowicz W, Stompôr T, Eckland D. The pharmacokinetics of pioglitazone in patients with impaired renal function. *Br J Clin Pharmacol* 2003; **55**: 368-374 [PMID: 12680885 DOI: 10.1046/j.1365-2125.2003.01785.x]

16 **Abe M**, Kikuchi F, Okada K, Matsumoto K. Plasma concentration of pioglitazone in patients with type 2 diabetes on hemodialysis. *Ther Apher Dial* 2009; **13**: 238-239 [PMID: 19527473 DOI: 10.1111/j.1744-9987.2009.00694.x]

17 **Jönsson A**, Rydberg T, Sterner G, Melander A. Pharmacokinetics of glibenclamide and its metabolites in diabetic patients with impaired renal function. *Eur J Clin Pharmacol* 1998; **53**: 429-435 [PMID: 9551701 DOI: 10.1007/s002280050403]

18 **Rosenkranz B**, Profozic V, Metelko Z, Mrzljak V, Lange C, Malerczyk V. Pharmacokinetics and safety of glimepiride at clinically effective doses in diabetic patients with renal impairment. *Diabetologia* 1996; **39**: 1617-1624 [PMID: 8960852 DOI: 10.1007/s001250050624]

19 **Sarkar A**, Tiwari A, Bhasin PS, Mitra M. Pharmacological and Pharmaceutical Profile of Gliclazide: A ReviewJournal of Applied Pharmaceutical Science 01 (09). 2011: 11-19

20 **Marbury TC**, Ruckle JL, Hatorp V, Andersen MP, Nielsen KK, Huang WC, Strange P. Pharmacokinetics of repaglinide in subjects with renal impairment. *Clin Pharmacol Ther* 2000; **67**: 7-15 [PMID: 10668848 DOI: 10.1067/mcp.2000.103973]

21 **Weaver ML**, Orwig BA, Rodriguez LC, Graham ED, Chin JA, Shapiro MJ, McLeod JF, Mangold JB. Pharmacokinetics and metabolism of nateglinide in humans. *Drug Metab Dispos* 2001; **29**: 415-421 [PMID: 11259325]

22 **Idris I**, Donnelly R. Dipeptidyl peptidase-IV inhibitors: a major new class of oral antidiabetic drug. *Diabetes Obes Metab* 2007; **9**: 153-165 [PMID: 17300591 DOI: 10.1111/j.1463-1326.2007.00705.x]

23 **Scheen AJ**. Pharmacokinetics of dipeptidylpeptidase-4 inhibitors. *Diabetes Obes Metab* 2010; **12**: 648-658 [PMID: 20590741 DOI: 10.1111/j.1463-1326.2010.01212.x]

24 **Chan JC**, Scott R, Arjona Ferreira JC, Sheng D, Gonzalez E, Davies MJ, Stein PP, Kaufman KD, Amatruda JM, Williams-Herman D. Safety and efficacy of sitagliptin in patients with type 2 diabetes and chronic renal insufficiency. *Diabetes Obes Metab* 2008; **10**: 545-555 [PMID: 18518892 DOI: 10.1111/j.1463-1326.2008.00914.x]

25 **Vincent SH**, Reed JR, Bergman AJ, Elmore CS, Zhu B, Xu S, Ebel D, Larson P, Zeng W, Chen L, Dilzer S, Lasseter K, Gottesdiener K, Wagner JA, Herman GA. Metabolism and excretion of the dipeptidyl peptidase 4 inhibitor [14C]sitagliptin in humans. *Drug Metab Dispos* 2007; **35**: 533-538 [PMID: 17220239 DOI: 10.1124/dmd.106.013136]

26 **Bergman AJ**, Cote J, Yi B, Marbury T, Swan SK, Smith W, Gottesdiener K, Wagner J, Herman GA. Effect of renal insufficiency on the pharmacokinetics of sitagliptin, a dipeptidyl peptidase-4 inhibitor. *Diabetes Care* 2007; **30**: 1862-1864 [PMID: 17468348 DOI: 10.2337/dc06-2545]

27 **Kothny W**, Shao Q, Groop PH, Lukashevich V. One-year safety, tolerability and efficacy of vildagliptin in patients with type 2 diabetes and moderate or severe renal impairment. *Diabetes Obes Metab* 2012; **14**: 1032-1039 [PMID: 22690943 DOI: 10.1111/j.1463-1326.2012.01634.x]

28 **Lukashevich V**, Schweizer A, Shao Q, Groop PH, Kothny W. Safety and efficacy of vildagliptin versus placebo in patients with type 2 diabetes and moderate or severe renal impairment: a prospective 24-week randomized placebo-controlled trial. *Diabetes Obes Metab* 2011; **13**: 947-954 [PMID: 21733061

DOI: 10.1111/j.1463-1326.2011.01467.x]
29 **Nowicki M**, Rychlik I, Haller H, Warren M, Suchower L, Gause-Nilsson I, Schützer KM. Long-term treatment with the dipeptidyl peptidase-4 inhibitor saxagliptin in patients with type 2 diabetes mellitus and renal impairment: a randomised controlled 52-week efficacy and safety study. *Int J Clin Pract* 2011; **65**: 1230-1239 [PMID: 21977965 DOI: 10.1111/j.1742-1241.2011.02812.x]
30 **Christopher R**, Covington P, Davenport M, Fleck P, Mekki QA, Wann ER, Karim A. Pharmacokinetics, pharmacodynamics, and tolerability of single increasing doses of the dipeptidyl peptidase-4 inhibitor alogliptin in healthy male subjects. *Clin Ther* 2008; **30**: 513-527 [PMID: 18405789 DOI: 10.1016/j.clinthera.2008.03.005]
31 **Graefe-Mody U**, Friedrich C, Port A, Ring A, Retlich S, Heise T, Halabi A, Woerle HJ. Effect of renal impairment on the pharmacokinetics of the dipeptidyl peptidase-4 inhibitor linagliptin(*). *Diabetes Obes Metab* 2011; **13**: 939-946 [PMID: 21672124 DOI: 10.1111/j.1463-1326.2011.01458.x]
32 **Linnebjerg H**, Kothare PA, Park S, Mace K, Reddy S, Mitchell M, Lins R. Effect of renal impairment on the pharmacokinetics of exenatide. *Br J Clin Pharmacol* 2007; **64**: 317-327 [PMID: 17425627 DOI: 10.1111/j.1365-2125.2007.02890.x]
33 **Malm-Erjefält M**, Bjørnsdottir I, Vanggaard J, Helleberg H, Larsen U, Oosterhuis B, van Lier JJ, Zdravkovic M, Olsen AK. Metabolism and excretion of the once-daily human glucagon-like peptide-1 analog liraglutide in healthy male subjects and its in vitro degradation by dipeptidyl peptidase IV and neutral endopeptidase. *Drug Metab Dispos* 2010; **38**: 1944-1953 [PMID: 20709939 DOI: 10.1124/dmd.110.034066]
34 **Jacobsen LV**, Hindsberger C, Robson R, Zdravkovic M. Effect of renal impairment on the pharmacokinetics of the GLP-1 analogue liraglutide. *Br J Clin Pharmacol* 2009; **68**: 898-905 [PMID: 20002084 DOI: 10.1111/j.1365-2125.2009.03536.x]
35 **Rabkin R**, Ryan MP, Duckworth WC. The renal metabolism of insulin. *Diabetologia* 1984; **27**: 351-357 [PMID: 6389240 DOI: 10.1007/BF00304849]
36 **Reilly JB**, Berns JS. Selection and dosing of medications for management of diabetes in patients with advanced kidney disease. *Semin Dial* 2010; **23**: 163-168 [PMID: 20210915 DOI: 10.1111/j.1525-139X.2010.00703.x]

P- Reviewer: Mitra A, Tamemoto H, Zhang Q
S- Editor: Wen LL **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.659

World J Diabetes 2014 October 15; 5(5): 659-665
ISSN 1948-9358 (online)

REVIEW

Acute effects of physical exercise in type 2 diabetes: A review

Ricardo Yukio Asano, Marcelo Magalhães Sales, Rodrigo Alberto Vieira Browne, José Fernando Vila Nova Moraes, Hélio José Coelho Júnior, Milton Rocha Moraes, Herbert Gustavo Simões

Ricardo Yukio Asano, Universidade Mogi das Cruzes, Center of Health Sciences, Mogi das Cruzes 08770-490, Brazil
Marcelo Magalhães Sales, Milton Rocha Moraes, Hebert Gustavo Simões, Universidade Católica de Brasília, School of Physical Education, Brasília 72030-170, Brazil
Marcelo Magalhães Sales, UDF-Centro Universitário, School Health, Brasilia 70390-045, Brazil
Rodrigo Alberto Vieira Browne, Universidade Federal do Rio Grande do Norte, Center of Health Sciences, Natal 59078-970, Brazil
José Fernando Vila Nova Moraes, Universidade Federal do Vale do São Francisco, School of Physical Education, Petrolina 56304205, Brazil
Hélio José Coelho Júnior, Universidade Estadual de Campinas, School of Physical Education, Campinas 130883-851, Brazil
Author contributions: All authors contributed in all phases of the study: search articles, literature review, writing and reviewing of the manuscript.
Supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)
Correspondence to: Ricardo Yukio Asano, PhD, Universidade Mogi das Cruzes, Center of Health Sciences, 200 Dr. Cândido Xavier de Almeida Souza Avenue, Mogi das Cruzes 08770-490, Brazil. ricardokiu@ig.com.br
Telephone: +55-11-970115500 Fax: +55-11-40331129
Received: June 10, 2014 Revised: July 9, 2014
Accepted: July 25, 2014
Published online: October 15, 2014

Abstract

The literature has shown the efficiency of exercise in the control of type 2 diabetes (T2D), being suggested as one of the best kinds of non-pharmacological treatments for its population. Thus, the scientific production related to this phenomenon has growing exponentially. However, despite its advances, still there is a lack of studies that have carried out a review on the acute effects of physical exercise on metabolic and hemodynamic markers and possible control mechanisms of these indicators in individuals with T2D, not to mention that in a related way, these themes have been very little studied today. Therefore, the aim of this study was to organize and analyze the current scientific production about the acute effects of physical exercise on metabolic and hemodynamic markers and possible control mechanisms of these indicators in T2D individuals. For such, a research with the following keywords was performed: -exercise; diabetes and post-exercise hypotension; diabetes and excess post-exercise oxygen consumption; diabetes and acute effects in PUBMED, SCIELO and HIGHWIRE databases. From the analyzed studies, it is possible to conclude that, a single exercise session can promote an increase in the bioavailability of nitric oxide and elicit decreases in postexercise blood pressure. Furthermore, the metabolic stress from physical exercise can increase the oxidation of carbohydrate during the exercise and keep it, in high levels, the post exercise consumption of O_2, this phenomenon increases the rate of fat oxidation during recovery periods after exercise, improves glucose tolerance and insulin sensitivity and reduces glycemia between 2-72 h, which seems to be dependent on the exercise intensity and duration of the effort.

Key words: Metabolic diseases; Hypertension; Nitric oxide; Blood glucose; Oxygen consumption

Core tip: Physical exercise is one of the best kinds of non-pharmacological treatments to prevent and control type 2 diabetes (T2D), being recommended by important medical associations, such as American College of Sports Medicine and the American Diabetes Association. In the literature, studies about the effects of a single exercise session on the population, its changes in blood pressure, glycemia, carbohydrate oxidation, fat oxidation, increase in nitric oxide and others are increasing exponentially. In this review, we report the most recent and important findings in the literature about the ef-

fects of acute exercise in T2D.

Asano RY, Sales MM, Browne RAV, Moraes JFVN, Coelho Júnior HJ, Moraes MR, Simões HG. Acute effects of physical exercise in type 2 diabetes: A review. *World J Diabetes* 2014; 5(5): 659-665 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/659.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.659

INTRODUCTION

Physical exercise, along with a proper diet are central factors in the prevention and control of diabetes mellitus (DM), since their effects include appropriate values of blood pressure, glycemia and lipidemia[1]. Several studies have shown the efficiency of exercise programs in the control of DM, being suggested as one of the best types of non-pharmacological treatments to the population in question[2-5]. Aerobic, resistance or combined exercise programs can help in the control of glycemia of diabetes mellitus type 2 (T2D), mainly by the increase of the need of glucose consumption by skeletal muscle in activity and the hypoglycaemic effect after exercise has been performed[1,6-9].

Currently, the guidelines to physical exercise prescription by the American College of Sports Medicine and American Diabetes Association to T2D provide general information, such as exercise daily, accumulate 150 min of exercise in a moderate intensity or 75 min of high intensity exercise per week; resistance exercises should be included at least 2-3 times per week[1]. On the other hand, despite the advances made in discovering the effects of exercise in the treatment and control of T2D and associated diseases, still there is a lack of studies that have carried out a review on the acute effects of physical exercise on metabolic and hemodynamic markers and possible control mechanisms of these indicators in individuals with type 2 diabetes, not to mention that in a related way, these themes have been very little studied today. Mainly concerning the magnitude of different intensities and durations of exercise on glucose uptake, oxidation of macronutrients and blood pressure response after performing only one session of exercise (acute exercise) and biomolecular mechanisms involved in this phenomenon[1]. Hence, the aim of this study was to synthetize the current knowledge pertaining the acute effects of physical exercises in T2D; analyze the implications of exercise and determinate trends to future researches about this topic.

The method used in the present study was a review of the literature. As inclusion criteria and search of scientific articles, the following keywords were used: diabetes and exercise; diabetes and postexercise hypotension; diabetes and excess postexercise oxygen consumption; diabetes and acute effect of physical exercise, in the databases PubMed, Scielo and HIGHWIRE. The studies that have not addressed the acute effects of physical exercise on type 2 diabetes and did not show relevant results on the subject were excluded from the analysis.

ACUTE EFFECTS OF PHYSICAL EXERCISE ON GLYCEMIA AND INSULINEMIA

The control of glycemia is dependent of the activities of the neuroendocrine system. In resting conditions, the glucose uptake by the cells is mainly insulin dependent, where the glucose transporter 4 (GLUT-4) is translocated to the cell membrane, facilitating glucose entrance in the cell cytoplasm[10]. During exercise, an increase in the uptake and utilization of glucose occurs, and it seems to be dependent on the intensity and duration of the effort. The more intense the effort is, more carbohydrate will be metabolized[11,12]. Therewith, exercise promotes a reduction in glycemia, which is initially controlled by glucagon, epinephrine and norepinephrine. Afterwards, with the assistance of growth hormone and glucagon, production and release of glucose by the liver in the bloodstream is increased, thus, regulating again the glycemia[13].

This acute effect of exercise is benefic in euglycemic and T2D individuals. Exercise increases the concentration of GLUT-4 in the cell membrane, which leads to the increase in glucose uptake, even with low insulin levels[14]. On the other hand, the mechanisms surrounding this phenomenon are still inconclusive. Higher expression of key-proteins related to the insulin pathway, such as insulin receptor substrate 1 and phosphatidylinositide 3-kinases, and insulin independent mechanisms, such as the increase in the activity of AMP-activated protein kinase, the activation of the calcium-calmodulim pathway, and the kallikreins-kinins components can be involved in this process[10,15-20].

Furthermore, both exercise models, aerobic and resistance, promote improvements in glucose tolerance, insulin sensitivity and reduction in glycemia between 2-72 h, which seems to be dependent on the intensity and duration of the effort[1,21,22].

Although, there is some knowledge about the benefits of acute exercise in T2D, more studies are still made necessary to elucidate some questions, such as the effects of intense exercise in general population, since the most studies and exercise prescription to this population are of moderate intensity[1].

CARBOHYDRATE AND FAT OXIDATION DURING AND POST EXERCISE IN T2D

Insulin resistance, along with elevated oxidative stress, impairs energy metabolism at rest, as well as during and after exercising in T2D. At rest, the lowest availability of glucose and muscular glycogen in T2D increases the predominance of fat oxidation when compared to euglycemic individuals[1].

Although glucose uptake by insulin dependent pathways are impaired in T2D, exercise increases carbohydrate

oxidation, and this capacity seems to be preserved in T2D, since the glucose uptake during the effort occurs mainly by insulin independent pathways[23]. Nevertheless, T2D demonstrates lower capacity to utilize carbohydrate during exercise when compared to euglycemic individuals[24].

Other peculiarities occur during exercise in T2D, such as the decrease in rate of fatty acids oxidation when compared to euglycemic[25]. However, the effects of different lactate threshold intensities, during and after aerobic exercises, have been little studied and are yet inconclusive.

Ghanassia *et al*[25] observed that the predominance of carbohydrate oxidation in T2D during exercise seems to be independent of the intensity of effort. Nevertheless, the use of carbohydrate as substrate seems to be dependent of intensity, since it is available in the muscle (glycogen) and in the blood (glucose)[1].

Lima *et al*[26] observed an increase in fat oxidation after a cycle ergometer session, when compared to resting values in T2D. Furthermore, high exercise intensities extend this increase, and fat oxidation after exercise was higher in T2D in comparison to euglycemic.

The increase in carbohydrate oxidation during exercise, as well as fat oxidation during the post exercise recovery period can contribute to augment insulin sensitivity, and collaborate to reduce body fat percentage. It is noteworthy that the accumulation of intramuscular fat has a direct relation on insulin resistance, and consequently the appearance of T2D[27,28].

POST-EXERCISE HYPOTENSION IN T2D

Individuals with T2D present other impairments, such as endothelial dysfunction[3,29], increased sympathetic tonus and other cardiovascular diseases, including hypertension[30], which lead to the increase in morbidity and mortality.

One session of aerobic or resistance exercise can promote postexercise hypotension (PEH). The exercise-induced mechanical stress on the wall of the arteries, can increase the release of vasodilating substances by the endothelium (*e.g.*, nitric oxide, bradykinin), augments baroreflex sensitivity, and decreased sympathetic nervous activity in the solitary tract nucleus caused by the release of substance P by skeletal muscles[31-34]. This adaptation can bring benefits to health, because it helps to keep low levels of blood pressure, avoiding and controlling blood pressure increase at rest. However, the magnitude of this effect seems to be diminished in T2D individuals, since this population presents endothelial dysfunction, which collaborates to a decrease in the capacity of nitric oxide (NO) release when compared with euglycemic individuals[35-37]. Increased sympathetic tonus and other cardiovascular diseases are also observed in T2D[30].

Studies have demonstrated that the occurrence of PEH in T2D can be intense depending on the effort. Lima *et al*[4] demonstrated that T2D individuals seem to be more responsive to high intensity exercise sessions, since exercise above lactate threshold (LT) (110% of the LT) resulted in a significant decrease in systolic blood pressure (SBP) values up to 90 min after the session, whereas exercise performed below lactate threshold (90% of the LT) only reduced SBP during 45 min post exercise.

Simões *et al*[38] comparing two resistance training exercise intensities (23% and 43% of 1RM), observed that only the higher session (43%) promoted PEH. Similar results were found by Motta *et al*[29], when studying the effects of a 20 min high intensity cycle ergometer (90% of lactate threshold) in individuals with and without T2D. Both studies only observed significant blood pressure decreases in non T2D individuals.

Although the physiological mechanism responsible by this process still remains inconclusive, it is known that high intensity exercise promotes increases the activity of the kallikrein-kinin system, and consequently, augments the synthesis and release of nitric oxide[29]. However, more studies are still made necessary to elucidate this question.

EXCESS POSTEXERCISE OXYGEN CONSUMPTION IN T2D

Exercise increases oxygen consumption after exercising and during rest, this phenomenon is known as excess postexercise oxygen consumption (EPOC), which has a fast component (2-3 min), and a slow component which can persist for more than 30 min. The duration and magnitude of EPOC depends on the intensity and duration of the effort[39-42].

The need to resynthesize creatine phosphate, restore intramuscular oxygen, body temperature and muscular glycogen, increased activity of the sodium-potassium pump, clearance of lactate, high levels of epinephrine and norepinephrine are factors that can lead to EPOC[40,41].

However, T2D individuals present metabolic impairments, such as lower capacity to utilize carbohydrate, due to lower enzymatic regulation and intracellular signalling and gene transcription[43]. Thus, these modifications can change the pattern of metabolic and respiratory alterations elicited during and after exercise[4]. Therefore, it decreased the benefits of EPOC when compared to euglycemic individuals.

Studies about EPOC in T2D are scarce. Therefore, determining which intensity and duration could be more beneficial to promote this event in T2D is important to increase post-exercise fat oxidation, once the accumulation of intramuscular fat has been associated to the development of T2D[43].

NITRIC OXIDE AND EXERCISE IN TYPE 2 DIABETES

NO is a gaseous, inorganic and colorless free radical, which has seven electrons of nitrogen and eight of oxygen, having an unpaired electron[44]. NO is synthetized from oxidation one of the two guanidine nitrogens of

L-arginine, which is converted to L-citrulline[45].

NO produced by endothelial cells has an essential function in the process of relaxing of blood vessels. In physiological conditions, vascular relaxing occurs when the membrane receptors of endothelial cells are activated by soluble stimulus, which include: acetylcholine, bradykinin, adenosine diphosphate, substance P, serotonin and others, or when there is an increase of friction exerted by circulating cells in the endothelial layer (shear stress), generating the activation of endothelial NO synthases (eNOS) present in these cells, causing an increase of synthesis and release of NO[46].

NO produced by eNOS in endothelial cells spreads out to smooth muscle cells and vascular lumen. In the smooth muscle, NO interacts with the iron from heme group of enzyme guanylate cyclase (GC), causing an alteration in the structure of this enzyme, becoming activated (GCa). GCa catalyzes the departure of two phosphate groups from the molecule guanosine triphosphate, similar to the adenosine triphosphate (ATP), forming the cyclic guanosine monophosphate (cGMP). An increase in the levels of cGMP occurs when NO activates GC inside the cells[47], resulting in: (1) maintenance of vascular tonus; (2) blood pressure regulation; (3) prevention of platelet aggregation (by increase of cGMP and decrease in Ca^{2+}); (4) inhibition in adhesion of monocytes and neutrophils in the vascular endothelium; (5) anti-proliferative effect; and (6) anti-oxidant effect decreasing the production of peroxynitrite anion (ONOO-)[36]. Recent studies have shown that having a physically active lifestyle can contribute to maintain the functional capacity of the vascular endothelium, measured by the preservation of ability to produce NO[48-50].

The acute effects of exercise in the bioavailability of NO in physical performance and health, mainly in endothelial function, have been previously studied. Studies have demonstrated that exercise promotes an increase in NO levels after a single session. This acute effect of exercise in NO can induce positive adaptations in the cardiovascular, hepatic, esqueleto muscle systems and others[35,51].

This effect can influence health parameters, such as the control of blood pressure (BP). Faria *et al*[52] induced spontaneously hypertensive rats to one session of exercise (squat), using vests as load. They observed a decrease in BP, lower vascular reactivity, and endothelium-dependent vasodilatation mediated by the NO after exercising.

Augeri *et al*[53] examined the influence of the T786C gene of eNOS in post-exercise hypotension (PEH) and NO after a low (40% VO_2max) and moderate intensity exercise (60% VO_2max) in the cycle ergometer in prehypertensive individuals. The individuals, who carried the TT genotype, demonstrated less PEH than heterozygous individuals 9 h after exercising.

On the other hand, Long *et al*[54] determined the preventive effects of exercise in the coronary blood flow and macrovascular atherosclerosis in aerobic trained Yucatan pigs, which passed by a high cholesterol and fat concentrated diet. The short aerobic training kept the endothelium independent relaxation (adenosine) and increased the coronary endothelium-dependent relaxation through the action of bradykinin, that is a mediator of NO production, and decreased the developing of atheromatous plaques in the aerobic trained pigs.

In the venous system, Chies *et al*[55] evaluated the effects of angiotensin Ⅱ in the portal vein and vena cava of trained rats. The exposition of trained animals to consecutive sessions of acute aerobic exercise in a treadmill improved the portal vein response in the presence of angiotensin Ⅱ. This upgrading seems to be specific in portal vein, once the researches didn't observe this phenomenon in vena cava. The authors concluded that these adaptations are influenced by NO, endothelin and prostanoids.

Regarding vascular damage, Cubbon *et al*[56] studied the association of NO induced by exercise in the proliferation and mobilization of circulating progenitor cells (CPC), which are potential mediators of cell repair. The mobilization of CPC is critically dependent of NO, and south Asians are associated with low CPC levels. The mobilization of CPC was measured during a moderate-intensity exercise session. Mediators of vasodilatation and CPC were lower in the Asian group than in Europeans. During the exercise, the CPC also was lower in Asians. A decrease in the release of NO can contribute to inappropriate balance between vascular damage and muscular repair in the population.

The acute effects of exercise in NO have also been studied in other tissues. In the skeletal muscle, Lee-Young *et al*[57] observed that in mice without eNOS, ATP is reduced (40%), in sedentary conditions exercise tolerance is markedly impaired during a 30 min session. The researchers observed that a partial reduction of eNOS expression is enough to induce physiological changes in ATP and NO production, and consequently, reducing the tolerance to the effort.

Besides exercise, diet also seems to influence the availability of NO. Bailey *et al*[58] administrated oral L-arginine in nine healthy individuals and performed "step" exercise in two intensities (moderate and high) one hour after ingestion. Plasma nitrite was significantly higher in the group that consumed L-arginine, resulting in a decrease in SBP. Submaximal VO_2max was 7% lower in the moderate intensity exercise, while in the high intensity exercise the slow component was reduced and the time to exhaustion delayed with L-arginine supplementation. As a conclusion, the authors stated that diet with L-arginine showed similar results with nitrite, increasing the bioavailability of NO, and reducing the cost of O_2 in the moderate exercise and time to exhaustion in the maximal exercise.

One exercise session seems to increase the bioavailability of NO, collaborating with the regulation of vascular tonus, balance between damage and muscle repair and preventing diseases such as atherosclerosis and hypertension[59]. Studies related to the bioavailability of NO in different exercise intensities are inexistent. The production

Table 1 Summary of human studies about acute effects of physical exercise in type 2 diabetes

Ref.	Sample	Exercise intervention	Results
Lima *et al*[4]	T2D = 11	20 min of cycle ergometer at 90% and 110% LT, and control session	Higher intensity exercise (110% LT) was more effective than lower intensity (90% LT)
Sriwijitkamol *et al*[5]	Obese T2D = 12 Obese CG = 8 Lean CG = 8	40 min of cycle ergometer at 50% and 70% VO_{2max}	Obese and T2D had attenuated exercise-stimulated AMPK activity and AS160 phosphorylation. T2D had reduced basal *PGC-1* gene expression but normal exercise-induced increases in PGC-1 expression
Borghouts *et al*[12]	T2D = 8 CG = 8	1 h of cycle ergometer at 40% VO_{2peak} and control session	Muscle glycogen oxidation was lower in T2D than in CG. Plasma glucose contributed more to energy expenditure in T2D than CG
Braun *et al*[24]	Insulin-resistant = 6 Insulin-sensitive = 6	50 min of treadmill walking at 45% VO_{2max}	Carbohydrate oxidation and estimated muscle glycogen use were significantly lower in the insulin-resistance group
Ghanassia *et al*[25]	T2D = 30 CG = 38	Increasing exercise intensity in cycle ergometer	Lipid oxidation was lower in T2D. Maximal lipid oxidation point and the crossover point were lower in T2D
Lima *et al*[26]	T2D = 9 CG = 11	20 min of cycle ergometer at 90% LT, increasing exercise intensity and control session	T2D have a better fat oxidation after high-intensity exercise than moderate exercise. T2D had less fat oxidation than CG after moderate exercise
Motta *et al*[29]	T2D = 10 CG = 10	20 min of cycle ergometer at 90% LT and control session	CG presented PEH, but not in the T2D. Plasma kallikrein activity increased postexercise in the CG, but not in the T2D
Simões *et al*[38]	T2D = 10 CG = 10	Resistance exercise circuit at 43% and 23% 1RM (approximately 25 min), and control session	43% 1RM promoted PEH, whereas the 23% did not
Asano *et al*[60]	T2D = 11	20 min of cycle ergometer at 80% and 120% LT, and control session	Exercise above LT (120% LT) increase nitric oxide and decrease SBP post-exercise, but about 80% LT not

T2D: Type 2 diabetics; LT: Lactate threshold; CG: Control group; VO_{2max}: Maximal oxygen uptake; VO_{2peak}: Peak oxygen uptake; PEH: Post-exercise hypotension; 1RM: 1-repetition maximum; AMPK: AMP-activated protein kinase; PGC-1: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

of knowledge about this important topic is essential to define better exercise strategies to increase the bioavailability of NO in individuals with T2D after one exercise session.

A summary of acute effects of physical exercise in T2D, along with the reference, number of volunteers and the kind of intervention, can be observed in Table 1.

CONCLUSION

A single session of exercise can promote beneficial effects regarding blood pressure control, glycemia, carbohydrate oxidation during exercise and fat oxidation after exercise. Evidence has shown that exercise, especially at intense domains, can increase the bioavailability of nitric oxide, promoting a decrease in blood pressure after exercising. Furthermore, metabolic stress from exercising is able to increase the oxidation of carbohydrates during exercise, keeping an elevated O_2 consumption after exercising. This, in consequence, increases fat oxidation during at rest and improves glucose tolerance, insulin sensibility and can reduce glucose levels between 2 to 72 h depending of intensity and duration of the effort.

These acute effects of physical exercise are important to T2D, because they help to improve conditions such as high blood pressure, hyperglycaemia and lipidemia.

ACKNOWLEDGMENTS

The authors are grateful for the students' scholarships at undergraduate (CNPq), masters (CAPES) and PhD (CAPES and CNPq), as well as for the research productivity scholarships (CNPq).

REFERENCES

1 **Colberg SR**, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. *Diabetes Care* 2010; **33**: 2692-2696 [PMID: 21115771 DOI: 10.2337/dc10-1548]

2 **Bassuk SS**, Manson JE. Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease. *J Appl Physiol* (1985) 2005; **99**: 1193-1204 [PMID: 16103522 DOI: 10.1152/japplphysiol.00160.2005]

3 **Maiorana A**, O'Driscoll G, Goodman C, Taylor R, Green D. Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes. *Diabetes Res Clin Pract* 2002; **56**: 115-123 [PMID: 11891019 DOI: 10.1016/S0168-8227(01)00368-0]

4 **Lima LC**, Assis GV, Hiyane W, Almeida WS, Arsa G, Baldissera V, Campbell CS, Simões HG. Hypotensive effects of exercise performed around anaerobic threshold in type 2 diabetic patients. *Diabetes Res Clin Pract* 2008; **81**: 216-222 [PMID: 18571267 DOI: 10.1016/j.diabres.2008.04.019]

5 **Sriwijitkamol A**, Coletta DK, Wajcberg E, Balbontin GB, Reyna SM, Barrientes J, Eagan PA, Jenkinson CP, Cersosimo E, DeFronzo RA, Sakamoto K, Musi N. Effect of acute exercise on AMPK signaling in skeletal muscle of subjects with type 2 diabetes: a time-course and dose-response study. *Diabetes* 2007; **56**: 836-848 [PMID: 17327455 DOI: 10.2337/db06-1119]

6 **Gentil P**, Oliveira E, Fontana K, Molina G, Oliveira RJD, Bottaro M. The acute effects of varied resistance training methods on blood lactate and loading char acteristics in recreationally trained men. *Rev Bras Med Esporte* 2006; **12**: 303-307 [DOI: 10.1590/S1517-86922006000600001]

7 **Hiyane WC**, Simões HG, Campbell CSG. Critical velocity as a noninvasive method to estimate the lactate minimum velocity on cycling. *Rev Bras Med Es-porte* 2006; **12**: 381-385 [DOI: 10.1590/S1517-86922006000600016]

8 **Oliveira JCD**, Baldissera V, Simões HG, Aguiar APD, Azevedo PHSMD, Poi-an PAFDO, Perez SEDA. Identification

of lactate and glucose threshold in resis-tance exercises. *Rev Bras Med Esporte* 2006; **12**: 333-338 [DOI: 10.1590/S1517-86922006000600007]
9 **Briscoe VJ**, Tate DB, Davis SN. Type 1 diabetes: exercise and hypoglycemia. *Appl Physiol Nutr Metab* 2007; **32**: 576-582 [PMID: 17510699 DOI: 10.1139/H07-025]
10 **Suh SH**, Paik IY, Jacobs K. Regulation of blood glucose homeostasis during prolonged exercise. *Mol Cells* 2007; **23**: 272-279 [PMID: 17646701]
11 **Burke LM**, Hawley JA. Carbohydrate and exercise. *Curr Opin Clin Nutr Metab Care* 1999; **2**: 515-520 [PMID: 10678682 DOI: 10.1097/00075197-199911000-00015]
12 **Borghouts LB**, Wagenmakers AJ, Goyens PL, Keizer HA. Substrate utilization in non-obese Type II diabetic patients at rest and during exercise. *Clin Sci* (Lond) 2002; **103**: 559-566 [PMID: 12444908]
13 **Powers SK**, Howley ET. Exercise physiology: theory and application to fit-ness and performance. 6th ed. São Paulo: Manole, 2000
14 **Krook A**, Wallberg-Henriksson H, Zierath JR. Sending the signal: molecular mechanisms regulating glucose uptake. *Med Sci Sports Exerc* 2004; **36**: 1212-1217 [PMID: 15235328 DOI: 10.1249/01.MSS.0000132387.25853.3B]
15 **Taguchi T**, Kishikawa H, Motoshima H, Sakai K, Nishiyama T, Yoshizato K, Shirakami A, Toyonaga T, Shirontani T, Araki E, Shichiri M. Involvement of bradykinin in acute exercise-induced increase of glucose uptake and GLUT-4 translocation in skeletal muscle: studies in normal and diabetic humans and rats. *Metabolism* 2000; **49**: 920-930 [PMID: 10910005 DOI: 10.1053/meta.2000.6755]
16 **Koopman R**, van Loon LJ. Aging, exercise, and muscle protein metabolism. *J Appl Physiol* (1985) 2009; **106**: 2040-2048 [PMID: 19131471]
17 **Kishi K**, Muromoto N, Nakaya Y, Miyata I, Hagi A, Hayashi H, Ebina Y. Bradykinin directly triggers GLUT4 translocation via an insulin-independent pathway. *Diabetes* 1998; **47**: 550-558 [PMID: 9568686 DOI: 10.2337/diabetes.47.4.550]
18 **Schweitzer GG**, Castorena CM, Hamada T, Funai K, Arias EB, Cartee GD. The B2 receptor of bradykinin is not essential for the post-exercise increase in glucose uptake by insulin-stimulated mouse skeletal muscle. *Physiol Res* 2011; **60**: 511-519 [PMID: 21401298]
19 **Jessen N**, Goodyear LJ. Contraction signaling to glucose transport in skeletal muscle. *J Appl Physiol* (1985) 2005; **99**: 330-337 [PMID: 16036906 DOI: 10.1152/japplphysiol.00175.2005]
20 **Montanari D**, Yin H, Dobrzynski E, Agata J, Yoshida H, Chao J, Chao L. Kallikrein gene delivery improves serum glucose and lipid profiles and cardiac function in streptozotocin-induced diabetic rats. *Diabetes* 2005; **54**: 1573-1580 [PMID: 15855348 DOI: 10.2337/diabetes.54.5.1573]
21 **Ceysens G**, Rouiller D, Boulvain M. Exercise for diabetic pregnant women. *Cochrane Database Syst Rev* 2006; (**3**): CD004225 [PMID: 16856038 DOI: 10.1002/14651858.CD004225]
22 **Boulé NG**, Kenny GP, Haddad E, Wells GA, Sigal RJ. Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. *Diabetologia* 2003; **46**: 1071-1081 [PMID: 12856082]
23 **Sigal RJ**, Kenny GP, Wasserman DH, Castaneda-Sceppa C, White RD. Physical activity/exercise and type 2 diabetes: a consensus statement from the American Diabetes Association. *Diabetes Care* 2006; **29**: 1433-1438 [PMID: 16732040 DOI: 10.2337/diacare.27.10.2518]
24 **Braun B**, Sharoff C, Chipkin SR, Beaudoin F. Effects of insulin resistance on substrate utilization during exercise in overweight women. *J Appl Physiol* (1985) 2004; **97**: 991-997 [PMID: 15133003]
25 **Ghanassia E**, Brun JF, Fedou C, Raynaud E, Mercier J. Substrate oxidation during exercise: type 2 diabetes is associated with a decrease in lipid oxidation and an earlier shift towards carbohydrate utilization. *Diabetes Metab* 2006; **32**: 604-610 [PMID: 17296514 DOI: 10.1152/japplphysiol.00231.2004]
26 **Lima L**, Cunha G, Motta D, Almeida W, Asano R, Sales M, Melo G, Campbell C, Simões H. Effect of exercise intensity on the oxidation of carbohydrates and fats during post-exercise recovery in type 2 diabetics. *R Bras Ci e Mov* 2011; **19**: 33-41
27 **Curi R**, Lagranha CJ, Hirabara SM, Folador A, Jr OT, Fernandes LC, Pellegrinotti IL, Pithon-Curi TC, Procópio J. A limiting factor for fatty acid oxidation during aerobic exercise: the Krebs cycle. *R Bras Ci e Mov* 2003; **11**: 87-94
28 **Atkinson FS**, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. *Diabetes Care* 2008; **31**: 2281-2283 [PMID: 18835944 DOI: 10.2337/dc08-1239]
29 **Motta DF**, Lima LC, Arsa G, Russo PS, Sales MM, Moreira SR, Morais PK, Almeida WS, Araujo RC, Moraes MR, Pesquero JL, Simões HG, Campbell CS. Effect of type 2 diabetes on plasma kallikrein activity after physical exercise and its relationship to post-exercise hypotension. *Diabetes Metab* 2010; **36**: 363-368 [PMID: 20579916 DOI: 10.1016/j.diabet.2010.03.008]
30 **Arauz-Pacheco C**, Parrott MA, Raskin P. Treatment of hypertension in adults with diabetes. *Diabetes Care* 2003; **26** Suppl 1: S80-S82 [PMID: 12502624]
31 **Moraes MR**, Bacurau RF, Ramalho JD, Reis FC, Casarini DE, Chagas JR, Oliveira V, Higa EM, Abdalla DS, Pesquero JL, Pesquero JB, Araujo RC. Increase in kinins on post-exercise hypotension in normotensive and hypertensive volunteers. *Biol Chem* 2007; **388**: 533-540 [PMID: 17516849]
32 **Moraes MR**, Bacurau RF, Simões HG, Campbell CS, Pudo MA, Wasinski F, Pesquero JB, Würtele M, Araujo RC. Effect of 12 weeks of resistance exercise on post-exercise hypotension in stage 1 hypertensive individuals. *J Hum Hypertens* 2012; **26**: 533-539 [PMID: 21734721]
33 **Forjaz CL**, Cardoso CG, Rezk CC, Santaella DF, Tinucci T. Postexercise hypotension and hemodynamics: the role of exercise intensity. *J Sports Med Phys Fitness* 2004; **44**: 54-62 [PMID: 15181391]
34 **Chen CY**, Bonham AC. Postexercise hypotension: central mechanisms. *Exerc Sport Sci Rev* 2010; **38**: 122-127 [PMID: 20577060 DOI: 10.1097/JES.0b013e3181e372b5]
35 **Asano RY**, Sales MM, Coelho JM, Moraes JFVN, Pereira LA, Campbell CSG, Simões HG. Exercise, nitric oxide, and endothelial dysfunction: a brief review. *J Exerc Physiol Online* 2012; **15**: 76-86
36 **Stabler T**, Kenjale A, Ham K, Jelesoff N, Allen J. Potential mechanisms for reduced delivery of nitric oxide to peripheral tissues in diabetes mellitus. *Ann N Y Acad Sci* 2010; **1203**: 101-106 [PMID: 20716290 DOI: 10.1111/j.1749-6632.2010.05599.x]
37 **Simões HG**, Asano RY, Sales MM, Browne RA, Arsa G, Motta-Santos D, Puga GM, Lima LC, Campbell CS, Franco OL. Type 2 diabetes elicits lower nitric oxide, bradykinin concentration and kallikrein activity together with higher DesArg(9)-BK and reduced post-exercise hypotension compared to non-diabetic condition. *PLoS One* 2013; **8**: e80348 [PMID: 24265812 DOI: 10.1371/journal.pone.0080348]
38 **Simões GC**, Moreira SR, Kushnick MR, Simões HG, Campbell CS. Postresistance exercise blood pressure reduction is influenced by exercise intensity in type-2 diabetic and non-diabetic individuals. *J Strength Cond Res* 2010; **24**: 1277-1284 [PMID: 20386125 DOI: 10.1519/JSC.0b013e3181d67488]
39 **Børsheim E**, Bahr R. Effect of exercise intensity, duration and mode on post-exercise oxygen consumption. *Sports Med* 2003; **33**: 1037-1060 [PMID: 14599232]
40 **Bahr R**, Sejersted OM. Effect of feeding and fasting on excess postexercise oxygen consumption. *J Appl Physiol* (1985) 1991; **71**: 2088-2093 [PMID: 1778897]
41 **Thornton MK**, Potteiger JA. Effects of resistance exercise bouts of different intensities but equal work on EPOC. *Med Sci Sports Exerc* 2002; **34**: 715-722 [PMID: 11932584]

42 **Børsheim E**, Bahr R, Høstmark AT, Knardahl S. Effect of beta-adrenoceptor blockade on postexercise oxygen consumption and triglyceride/fatty acid cycling. *Metabolism* 1998; **47**: 439-448 [PMID: 9550543 DOI: 10.1016/0026-0495(94)90197-X]

43 **Bruce CR**, Hawley JA. Improvements in insulin resistance with aerobic exercise training: a lipocentric approach. *Med Sci Sports Exerc* 2004; **36**: 1196-1201 [PMID: 15235325]

44 **Beckman JS**, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 1996; **271**: C1424-C1437 [PMID: 8944624]

45 **Marletta MA**. Nitric oxide synthase: aspects concerning structure and catalysis. *Cell* 1994; **78**: 927-930 [PMID: 7522970 DOI: 10.1016/0092-8674(94)90268-2]

46 **Busconi L**, Michel T. Endothelial nitric oxide synthase membrane targeting. Evidence against involvement of a specific myristate receptor. *J Biol Chem* 1994; **269**: 25016-25020 [PMID: 7523377]

47 **Moncada S**, Higgs EA. The discovery of nitric oxide and its role in vascular biology. *Br J Pharmacol* 2006; **147** Suppl 1: S193-S201 [PMID: 16402104 DOI: 10.1038/sj.bjp.0706458]

48 **Souza HC**, Penteado DM, Martin-Pinge MC, Barbosa Neto O, Teixeira Vde P, Blanco JH, Silva VJ. Nitric oxide synthesis blockade increases hypertrophy and cardiac fibrosis in rats submitted to aerobic training. *Arq Bras Cardiol* 2007; **89**: 88-93, 99-104 [PMID: 17874014 DOI: 10.1590/S0066-782X2007001400005]

49 **Dominguez JM**, Prisby RD, Muller-Delp JM, Allen MR, Delp MD. Increased nitric oxide-mediated vasodilation of bone resistance arteries is associated with increased trabecular bone volume after endurance training in rats. *Bone* 2010; **46**: 813-819 [PMID: 19892040 DOI: 10.1016/j.bone.2009.10.029]

50 **Colleran PN**, Li Z, Yang HT, Laughlin MH, Terjung RL. Vasoresponsiveness of collateral vessels in the rat hindlimb: influence of training. *J Physiol* 2010; **588**: 1293-1307 [PMID: 20194126 DOI: 10.1113/jphysiol.2009.18624]

51 **Souza Junior TP**, Asano RY, Prestes J, Sales MPM, Coelho JMO, Simões HG. Óxido Nítrico e exercício: uma revisão. *Rev Educ Fís UEM* 2012; **23**: 469-481 [DOI: 10.4025/reveducfis.v23i3.11738]

52 **Faria Tde O**, Targueta GP, Angeli JK, Almeida EA, Stefanon I, Vassallo DV, Lizardo JH. Acute resistance exercise reduces blood pressure and vascular reactivity, and increases endothelium-dependent relaxation in spontaneously hypertensive rats. *Eur J Appl Physiol* 2010; **110**: 359-366 [PMID: 20499250 DOI: 10.1007/s00421-010-1508-5]

53 **Augeri AL**, Tsongalis GJ, Van Heest JL, Maresh CM, Thompson PD, Pescatello LS. The endothelial nitric oxide synthase -786 T& gt; C polymorphism and the exercise-induced blood pressure and nitric oxide responses among men with elevated blood pressure. *Atherosclerosis* 2009; **204**: e28-e34 [PMID: 19155013 DOI: 10.1016/j.atherosclerosis]

54 **Long X**, Bratz IN, Alloosh M, Edwards JM, Sturek M. Short-term exercise training prevents micro- and macrovascular disease following coronary stenting. *J Appl Physiol* (1985) 2010; **108**: 1766-1774 [PMID: 20299615 DOI: 10.1152/japplphysiol.01014.2009]

55 **Chies AB**, de Souza Rossignoli P, Daniel EF. Exercise increases the angiotensin II effects in isolated portal vein of trained rats. *Peptides* 2010; **31**: 883-888 [PMID: 20172009 DOI: 10.1016/j.peptides.2010.02.011]

56 **Cubbon RM**, Murgatroyd SR, Ferguson C, Bowen TS, Rakobowchuk M, Baliga V, Cannon D, Rajwani A, Abbas A, Kahn M, Birch KM, Porter KE, Wheatcroft SB, Rossiter HB, Kearney MT. Human exercise-induced circulating progenitor cell mobilization is nitric oxide-dependent and is blunted in South Asian men. *Arterioscler Thromb Vasc Biol* 2010; **30**: 878-884 [PMID: 20110574 DOI: 10.1161/ATVBAHA.109.201012]

57 **Lee-Young RS**, Ayala JE, Hunley CF, James FD, Bracy DP, Kang L, Wasserman DH. Endothelial nitric oxide synthase is central to skeletal muscle metabolic regulation and enzymatic signaling during exercise in vivo. *Am J Physiol Regul Integr Comp Physiol* 2010; **298**: R1399-R1408 [PMID: 20200137 DOI: 10.1152/ajpregu.00004.2010]

58 **Bailey SJ**, Winyard PG, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Jones AM. Acute L-arginine supplementation reduces the O2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. *J Appl Physiol* (1985) 2010; **109**: 1394-1403 [PMID: 20724562 DOI: 10.1152/japplphysiol.00503.2010]

59 **Allen JD**, Stabler T, Kenjale A, Ham KL, Robbins JL, Duscha BD, Dobrosielski DA, Annex BH. Plasma nitrite flux predicts exercise performance in peripheral arterial disease after 3months of exercise training. *Free Radic Biol Med* 2010; **49**: 1138-1144 [PMID: 20620208 DOI: 10.1016/j.freeradbiomed.2010.06.033]

60 **Asano RY**, Browne RAV, Sotero RC, Sales MM, Moraes JFVN, Campbell CSG, Simões HG. Cycling above rather than below lactate threshold is more effective for nitric oxide release and post-exercise blood pressure reduction in individuals with type-2 diabetes. *Motriz Rev Educ Fis* 2013; **19**: 633-640 [DOI: 10.1590/S1980-65742013000300015]

P- Reviewer: Pamidi N, Ray S **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.666

World J Diabetes 2014 October 15; 5(5): 666-677
ISSN 1948-9358 (online)

REVIEW

Molecular mechanisms of protein induced hyperinsulinaemic hypoglycaemia

Suresh Chandran, Fabian Yap, Khalid Hussain

Suresh Chandran, Department of Neonatology, KK Women's and Children's Hospital, Singapore 229899, Singapore
Suresh Chandran, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117598, Singapore
Fabian Yap, Department of Paediatric Endocrinology, KK Women's and Children's Hospital, Singapore 229899, Singapore
Suresh Chandran, Fabian Yap, Duke-NUS Graduate Medical School, Singapore 169857, Singapore
Suresh Chandran, Fabian Yap, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 308232, Singapore
Khalid Hussain, Departments of Paediatric Endocrinology, Great Ormond Street Hospital for Children NHS Foundation Trust, London WC1N 3JH, United Kingdom
Khalid Hussain, Developmental Endocrinology Research Group, Molecular Genetics Unit, Institute of Child Health, University College London, London WC1N 1EH, United Kingdom
Author contributions: Chandran S and Yap F wrote the review under the supervision of Hussain K; Hussain K contributed to abstract, introduction written and final editing of the manuscript.
Correspondence to: Dr. Khalid Hussain, Reader/Honorary Consultant Paediatric Endocrinologist, Developmental Endocrinology Research Group, Molecular Genetics Unit, Institute of Child Health, University College London, 30 Guildford Street, London WC1N 1EH, United Kingdom. khalid.hussain@ucl.ac.uk
Telephone: +44-20-79052128 Fax: +44-20-74046191
Received: January 21, 2014 Revised: April 29, 2014
Accepted: May 28, 2014
Published online: October 15, 2014

Abstract

The interplay between glucose metabolism and that of the two other primary nutrient classes, amino acids and fatty acids is critical for regulated insulin secretion. Mitochondrial metabolism of glucose, amino acid and fatty acids generates metabolic coupling factors (such as ATP, NADPH, glutamate, long chain acyl-CoA and diacylglycerol) which trigger insulin secretion. The observation of protein induced hypoglycaemia in patients with mutations in *GLUD1* gene, encoding the enzyme glutamate dehydrogenase (GDH) and *HADH* gene, encoding for the enzyme short-chain 3-hydroxyacyl-CoA dehydrogenase has provided new mechanistic insights into the regulation of insulin secretion by amino acid and fatty acid metabolism. Metabolic signals arising from amino acid and fatty acid metabolism converge on the enzyme GDH which integrates both signals from both pathways and controls insulin secretion. Hence GDH seems to play a pivotal role in regulating both amino acid and fatty acid metabolism.

Key words: Hyperinsulinaemic hypoglycaemia; K_{ATP} channel; Glutamate dehydrogenase; Hyperinsulinism/Hyperammonaemia syndrome; Short-chain-3-hydroxyacyl-CoA dehydrogenase; Glutamine

Core tip: The interplay between glucose, amino acid and fatty acid metabolism is critical for regulated insulin secretion. Mitochondrial metabolism of glucose, amino acid and fatty acids generates metabolic coupling factors (such as ATP, NADPH, glutamate, long chain acyl-CoA and diacylglycerol) which trigger insulin secretion. The observation of protein induced hypoglycaemia in patients with mutations in *GLUD1* [encoding for the enzyme glutamate dehydrogenase (GDH)] and *HADH* genes, has provided novel mechanistic insights into the regulation of insulin secretion by amino acid and fatty acid metabolism. Metabolic signals arising from amino acid and fatty acid metabolism converge on the enzyme GDH which integrates both signals from both pathways and controls insulin secretion. Hence GDH seems to play a pivotal role in regulating both amino acid and fatty acid metabolism.

Chandran S, Yap F, Hussain K. Molecular mechanisms of protein induced hyperinsulinaemic hypoglycaemia. *World J Diabetes* 2014; 5(5): 666-677 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/666.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.666

INTRODUCTION

Glucose, amino acids and fatty acids are the substrates available for metabolic homeostasis and play important roles in insulin secretion. Pancreatic β-cells synthesise and secrete insulin in response to signals generated from glucose, amino acid and fatty acid metabolism but glucose is the prime stimulus for insulin secretion. Regulated insulin release requires tight coupling in the β-cell between glucose metabolism and insulin secretory response. As β-cells are continually exposed to a complex milieu of nutrients and other circulating factors (like incretins), it is important to understand the interplay between glucose metabolism and that of the two other primary nutrient classes, the amino acids and fatty acids. Specific amino acids are now known to acutely and chronically regulate insulin secretion from pancreatic β-cells *in vivo* and *in vitro* and lipid metabolism in the β-cell is critical for the regulation of insulin secretion[1].

The metabolism of glucose, amino acids and fatty acids results in the generation of metabolic coupling factors involved in regulating insulin exocytosis. These metabolic coupling factors generated from the metabolism of glucose, amino acids and fatty acids in the β-cell include ATP, NADPH, glutamate, long chain acyl-CoA and diacylglycerol[2]. Each of these coupling factors plays a key role in regulating insulin secretion. The exocytotic process is closely controlled by signals generated from nutrient metabolism as well as by neurotransmitters and circulating hormones.

Under normal physiological conditions the metabolism of glucose, amino acids and fatty acids is intricately controlled and will result in the regulated secretion of insulin. The secretion of insulin is precisely regulated to keep fasting blood glucose concentrations between 3.5-5.9 mmol/L. In some pathological states the signals generated from glucose, amino acid and fatty acid metabolism cause insulin hyper-secretion or dysregulation of insulin secretion. In these states insulin secretion becomes inappropriate for the level of blood glucose causing hyperinsulinaemic hypoglycaemia (HH).

HH is a major cause of persistent hypoglycaemia in the childhood period[3]. In the newborn and infancy periods HH can be either congenital or secondary to certain risk factors (such as intrauterine growth retardation). Congenital forms of HH are due to defects in key genes (*ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *HNF4/HNF1A*, *SLC23A* and *UCP2*) involved in regulating insulin secretion[4]. Loss of function mutations in the genes *ABCC8* and *KCNJ11* (which encode for the SUR 1 and KIR6.2, components of the β-cell potassium (KATP) channel subunits respectively) lead to the most severe forms of HH, which is usually medically unresponsive[5]. Clinically HH presents with fasting hypoglycaemia but in some patients the HH is typically triggered by the ingestion of protein (amino acids). Protein induced HH is observed in patients with gain of function mutations of *GLUD1*[6], loss-of-function mutations of *ABCC8/KCJN11*[7] and loss of function mutations in the *HADH*[8].

This state of the art review article will firstly discuss the molecular mechanisms of glucose, amino acid and fatty acid regulated insulin secretion and then focus on the current understanding of the molecular mechanisms involved in protein induced HH.

GLUCOSE MEDIATED INSULIN SECRETION BY THE PANCREATIC β-CELL

Glucose mediated secretion of insulin is initiated by the uptake of glucose by the β-cells *via* the glucose transporter. Glucose is then phosphorylated to glucose-6-phosphate by islet-specific glucokinase. Further metabolism of glucose increases the cellular ATP: ADP ratio, which closes ATP-dependent KATP channels in the β-cell membrane, causing membrane depolarization and influx of calcium. Intracellular free calcium then promotes margination of secretory granules, which fuse with the cell membrane before releasing their contents into the extracellular space by exocytosis (Figure 1)[9]. The functional integrity of both SUR 1 and KIR 6.2 proteins is necessary for KATP channel function and the genes encoding for these two proteins are localized very closely to each other on the short arm of chromosome 11 (11p14-15.1).

Although KATP channels have an essential role in linking the metabolism of glucose to the secretion of insulin, there is now evidence that there may well be other mechanisms of insulin secretion, the so-called KATP channel independent pathways of insulin secretion[10]. This pathway leads to augmented insulin release in the presence of raised cytosolic calcium (Ca^{2+}) concentrations. Increases in the intracellular Ca^{2+} concentration in the pancreatic β-cell cause modest increases in insulin secretion, which can be dramatically increased by modulators of protein kinases and phosphatases. This suggests that steps distal to the elevation of cytosolic Ca^{2+} are of greater quantitative importance in controlling insulin secretion. It has also been shown that glucose can cause pronounced insulin secretion in Ca^{2+} depleted islets in the presence of activators of protein kinases A and C[11].

Given the key role of pancreatic β-cell KATP channels in regulating insulin secretion it is no surprise that genetic defects in the genes regulating the function of these channels lead to severe forms of HH. Recessive inactivating mutations in KATP channel subunits are the most common cause of HH[5,12]. So far, over 150 mutations have been identified in the *ABCC8* and 25 in *KCNJ11*[13]. These include missense, frame shift, nonsense, insertions/deletions, splice site and regulatory mutations, either present in homozygous or compound heterozygous state. In the Ashkenazi Jewish population, two common (F1388del and c.3992-9G4A) mutations account for 90% of all cases of congenital HH[4].

The molecular basis of recessive inactivating *ABCC8* and *KCNJ11* mutations involves multiple defects in KATP channel biogenesis and turnover, in channel trafficking

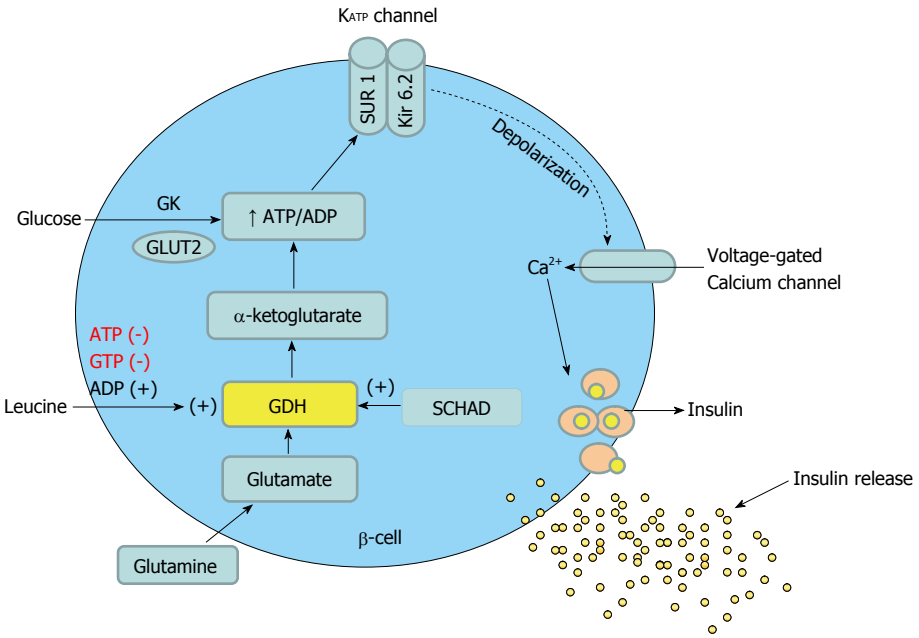

Figure 1 Glucose and protein mediated insulin secretion in the beta cell of pancreas. GDH: Glutamate dehydrogenase; SCHAD: Short-chain 3-hydroxyacyl-CoA dehydrogenase; GK: Glucokinase; SUR: Sulfonylurea receptor; Kir 6.2: Potassium channel inwardly rectifying; GLUT2: Glucose transporter 2.

from the endoplasmic reticulum and Golgi apparatus to the plasma membrane and alterations of channels in response to both nucleotide regulation and open state frequency.

AMINO ACID MEDIATED INSULIN SECRETION

The observations that plasma levels of insulin increase consistently and significantly when healthy subjects ingest protein meals[14] or when intravenous mixtures of amino acids are administered[15], provide fundamental scientific evidence of the relationship between protein metabolism, amino acids and insulin secretion.

Protein metabolism begins when dietary proteins are broken down to amino acids by intestinal enzymes[16]. Large differences in capacity of individual amino acids to stimulate insulin release are noted in both animal and human studies[15,17]. For example, when 30 g each of 10 amino acids in a mixture was administered individually, arginine proved the most effective and histidine the least in stimulating insulin release[15]. Although leucine itself can stimulate insulin secretion, the phenomenon of protein meal or amino acid stimulated insulin secretion does not solely or largely depend on the presence of leucine[14,15].

Amino acids, alone[18] or in combination[19], act synergistically with glucose to potentiate the release of insulin. Synergism was also observed between amino acid pairs, where the synergistic effect was significantly greater with arginine-leucine than with arginine-phenylalanine and their combined effects greater than when amino acids were administered alone[20]. Indeed, the oral ingestion of amino acid mixtures in combination with carbohydrates produce stronger insulinotropic effects compared with carbohydrate-only preparations[21], a phenomenon mediated by the incretin hormones gastric inhibitory polypeptide and glucagon-like peptide-1 (GLP-1)[22]. Amino acids shown to have the highest insulinotropic effect include leucine, valine, lysine, and isoleucine[23]. Metabolism of amino acids can occur either by transamination or by oxidative deamination.

Transamination is an early step in the degradation of most amino acids and involves a chemical reaction between two molecules, an amino acid (with an amine NH_2 group) and a keto acid (with a keto = O group), catalysed by a family of enzymes known as aminotransferases. Different aminotransferases are each specific for an amino acid or a group of chemically similar ones such as branch chain amino acids (BCAA). The keto acid that accepts the amino group is always alpha-ketoglutarate (α-KG), a metabolically important biological compound and key intermediate in the citric acid cycle. For example, alanine transaminase catalyses the transfer of an amino group from alanine to α-KG giving rise to pyruvate and glutamate.

On the other hand, oxidative deamination involves conversion of an amino acid into the corresponding keto acid by removing the amine group as ammonia, which goes into the urea cycle. As glutamate is the end product of many transamination reactions, oxidative deamination occurs primarily on glutamate, generating α-KG[16,24]. The main enzyme involved in oxidative deamination is glutamate dehydrogenase (GDH).

Glutamine and alanine are the most abundant amino acids in the blood and extracellular fluids. Whereas glutamine and alanine require the presence of glucose for insulin secretion, leucine is able to stimulate insulin secretion independently through the allosteric activation of GDH[25-28], generating α-KG. The further metabolism of α-KG is then involved in insulin production in two ways.

First, by entering the TCA cycle, the ATP:ADP ratio is raised causing closure of the K_{ATP} channel and depolarisation of the β-cell. The voltage dependant calcium channel opens leading to an increase in cellular calcium concentration, triggering the release of insulin from storage granules (Figure 1)[29]. Second, α-KG inhibits isocitrate dehydrogenase resulting in increased cytosolic citrate needed for the synthesis of short and long chain acyl-CoA, which are coupling factors closely involved in insulin secretion[30].

LEUCINE

Leucine is one of the most potent insulin secretagogues among the BCAA that facilitates glucose-induced insulin release from pancreatic β-cells[31]. It does so *via* several mechanisms. First, in pancreatic β-cells, leucine and its non-metabolizable analogue 2-aminobicyclo (2.2.1) heptane-2-carboxylic acid, stimulate the secretion of insulin by acting indirectly as a positive allosteric activator of GDH to enhance glutaminolysis. Activated GDH facilitates the oxidation of glutamate to α-KG, which raises the ATP:ADP ratio resulting in closure of K_{ATP} channel, cellular depolarization, influx of calcium and exocytosis of insulin from the storage granules (Figure 1)[32]. Second, the transaminated product of leucine, α-ketoisocaproate (KIC) can cause insulin secretion through direct inhibition of the K_{ATP} channel[33]. Glucose completely blocks the effects of leucine but not of KIC on stimulation of insulin secretion by β-cells[34]. Third, leucine plays an important role in the regulation of the mammalian target of Rapamycin (mTOR) pathway, which was recently recognized as a critical regulator of metabolic response to nutrients and growth factors[35]. Recent data strongly suggest that leucine down-regulates the surface expression of α2 adrenergic receptors in pancreatic islets through activation of mTOR, leading to insulin secretion[36].

GLUTAMINE

As the most abundant amino acid found in the blood, glutamine has both nutritive and non-nutritive effects[37]. Glutamine is physiologically important for maintaining cellular function in tissues of the intestine, kidney, brain and liver[38]. It is an important precursor substrate for the synthesis of peptides, proteins and nucleotides[39], in particular ATP which is central in the β-cell signalling pathway. In SUR 1 knockout (KO) β-cells models, isolated pancreatic islets respond briskly to a physiological mixture of 20 amino acids even though these islets cannot be stimulated by glucose or by leucine. Glutamine played an important role in mediating amino acid stimulation of insulin release as 60% of the insulin response was attributable to glutamine even though it comprised 16% of the amino acid load[7].

Although glutamine itself functions as a key precursor for nucleic acids and nucleotides, in many physiological circumstances it acts to provide glutamate, which promotes a wider array of metabolic functions compared to glutamine. By oxidative deamination of glutamate, GDH liberates free ammonia and the α-KG is then oxidized in the tricarboxylic acid cycle (TCA) cycle, raising ATP levels that close K_{ATP} channels and depolarize the cell membrane to release insulin. Ammonia is added to glutamate by glutamine synthetase to form glutamine, the major inert-organ carrier for ammonia.

Glutamine can be cleaved by glutaminase to yield glutamate and NH3. The mitochondrial carbamoyl phosphate synthetase (CPS 1) then can catalyze the conversion of ammonia to carbamoyl phosphate. The CPS 1 enzyme is allosterically activated by N-Acetyl glutamate (NAG) produced from glutamate by NAG synthase and may thus be indirectly regulated by glutamate concentration. Carbamoyl phosphate thus formed combines with ornithine in the urea cycle. Thus glutamate also aids in ammonia detoxification and promotion of urea synthesis in the liver (Figure 2)[40,41]. However, the exact mechanism of glutamine linked hyperinsulinemia remains less well understood. Glutamine can also potentiate insulin secretion by stimulating enteroendocrine L-cells to synthesise and secrete the incretin GLP-1. This effect is attributable to a triggering pathway that elevates intracellular Ca^{2+} and an amplifying pathway mediated by elevated cAMP[42].

ALANINE

The mechanism of action of alanine as an insulin secretagogue is still unclear. Upon entry into the β-cell cytosol, alanine is deaminated and takes part in the TCA cycle through pyruvate and acetyl-CoA. This results in increase of the cellular content of ATP, closure of the K_{ATP} channel, depolarization of the plasma membrane, activation of voltage-gated calcium channel, increase in calcium influx and insulin exocytosis (Figure 3)[43]. Insulinotropic property of alanine has been reported by Dunne *et al*[44] and McClenaghan *et al*[45] to be the result of co-transport with Na^+, leading to β-cell membrane depolarization and increase in cellular calcium. Current evidence suggests that the mode of action of alanine as an insulin secretagogue involves a combination of increased ATP generation, co-transport with Na^+ and signal transduction[26,46].

ARGININE

The mechanism of insulin release by arginine involves the mCAT2A amino acid transporter which electrogenically transports arginine into the β-cell, leading to increased intracellular calcium[47]. Accumulation of intracellular arginine leads to membrane depolarization, a further rise in intracellular calcium through opening of voltage-gated calcium channels, and insulin secretion[48]. Arginine can also influence insulin secretion by its conversion to glutamate, which allows the generation of metabolic coupling factors[49], however the detailed metabolism of arginine in the β-cell remains to be investigated.

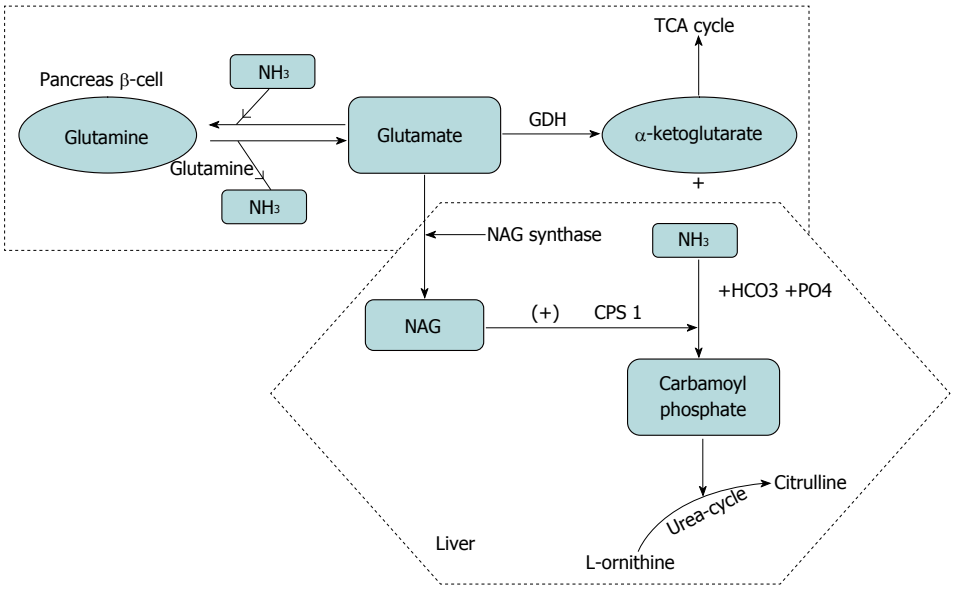

Figure 2 Glutamate metabolism. Oxidation of glutamate by glutamate dehydrogenase liberates free ammonia (NH_3) and alpha ketoglutarate, which enters tricarboxylic acid cycle cycle and generates ATP. In the liver glutamate also generates N-acetylglutamate (NAG), which in turn allosterically activates carbomyl phosphate synthetase (CPS) to regulate ammonia detoxification into urea. Glutamine provides a substrate for ammonia buffering, by adding ammonia to glutamate to form glutamine. TCA: Tricarboxylic acid cycle; GDH: Glutamate dehydrogenase.

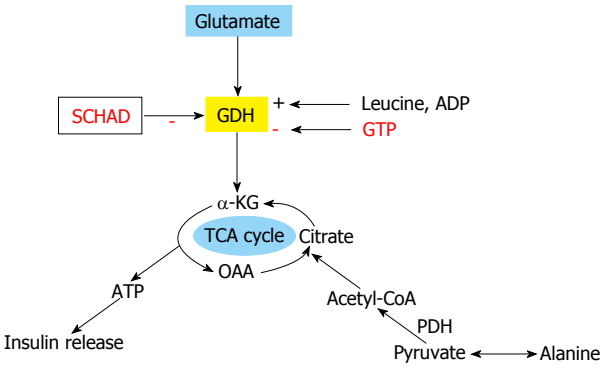

Figure 3 Glutamate and alanine as insulin secretagogues. Protein induced hyperinsulinaemic hypoglycaemia due to loss of function mutation in HADH gene (SCHAD). Alanine is deaminated to pyruvate and pyruvate dehydrogenase (PDH) converts it to acetyl CoA, which can enter TCA cycle to generate ATP for closing KATP channel. TCA: Tricarboxylic acid cycle; α-KG: Alpha ketoglutarate; GDH: Glutamate dehydrogenase; OAA: Oxaloacetic acid.

FATTY ACID β-OXIDATION PATHWAY

During the fasting state fatty acids (FA) are the most important substrates for ketogenesis to provide the brain with an "alternative fuel" source. Triglycerides are broken down to FA and glycerol in the process of lipolysis. β-oxidation of FA occurs in the peroxisomes and mitochondria. Short and medium chain FA can diffuse directly into the mitochondria and are then activated by acyl-CoA synthetase to acyl-CoA in the mitochondrial matrix, whereas long and very long chains FA are activated by acyl-CoA synthetase on the mitochondrial outer membrane. The "carnitine shuttle" allows acyl-CoA to penetrate the outer and inner mitochondrial membranes, catalysed by carnitine palmitoyltransferase- Ⅰ and Ⅱ (CPT- Ⅰ and Ⅱ) respectively, facilitated by the inner membrane exchange transporter, carnitine-acylcarnitine translocase[50].

In the mitochondrial matrix, acetyl-coA is generated by β-oxidation of acyl-CoA *via* a 4-step process involving dehydrogenation, hydration, oxidation and thiolysis (Figure 4)[50]. Acetyl-CoA finally enters the Krebs cycle. The short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD), an intramitochondrial homodimer enzyme is essential for catalysing the penultimate reaction of 3-hydroxyacyl CoA to 3-ketoacyl-CoA. Possible molecular mechanisms involved in the pathogenesis of HH due to deficiency of SCHAD have been reported recently[8,51].

PROTEIN INDUCED INSULIN SECRETION-HISTORICAL PERSPECTIVE

The history of protein induced hypoglycaemia dates back to 1956 when Cochrane described three children with severe hypoglycaemia while on low carbohydrate and high protein diet. Even though amino acid-stimulated insulin secretion by pancreatic β-cells was known for long[52], the molecular mechanisms involved in the dysregulated islet cell function leading to HH due to genetic mutations remain poorly understood. In 1970 researchers reported that amino acids could induce insulin secretion only in the presence of glucose except in case of leucine where the insulin-stimulatory effect is abolished in presence of glucose[53]. Animal studies have suggested that amino acid oxidation and signalling effects are two vital steps in which the amino acid amplifies insulin release from the stored vesicles following β-cell depolarisation and influx of calcium. By early 80's leucine's property to induce insulin secretion by allosterically stimulating GDH was identified[29].

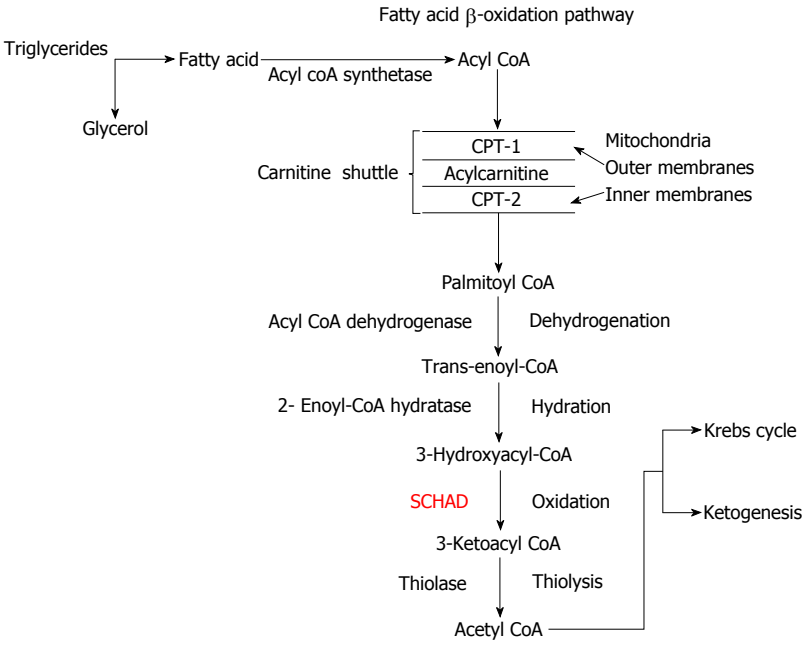

Figure 4 β-oxidation of fatty acids. Acyl-CoA is converted to acetyl-CoA through dehydrogenation, hydration, oxidation and thiolysis. Acetyl-CoA can enter the Krebs cycle or can lead to ketogenesis. CPT-1: Carnitine palmitoyltransferase-1; SCHAD: 3-hydroxyacyl CoA dehydrogenase.

MOLECULAR MECHANISMS OF AMINO ACID INDUCED HYPERINSULINAEMIC HYPOGLYCAEMIA

Amino acids are known to enhance insulin secretion from primary islet β-cell lines under appropriate conditions. Leucine can stimulate insulin release on its own by allosterically activating GDH. In the β-cell mitochondria, GDH can stimulate insulin secretion by oxidative deamination of glutamate by raising α-KG, NADH/NAD and NADPH/NADP ratios. Protein sensitive hyperinsulinaemic hypoglycaemia occurs in three forms; gain-of-function mutations of *GLUD1*[6], loss of function mutations of *ABCC8/KCNJ11*[7] and loss of function mutations in *HADH*[8].

HYPERINSULINISM/ HYPERAMMONAEMIA SYNDROME

Hyperinsulinism/hyperammonaemia syndrome (HI/HA) syndrome is the second most common cause of congenital hyperinsulinism-(CHI), characterized by both fasting and protein sensitive hypoglycaemia together with persistently elevated plasma ammonia levels[6]. HI/HA is likely the disorder described by Cochrane *et al*[52] in 1955, with leucine sensitive hypoglycaemia in a child and her father. Zammarchi *et al*[54] first reported a case of hyperammonaemia with leucine sensitive hypoglycaemia. Activating mutations in the *GLUD1* gene were reported to be the cause of HI/HA syndrome by Stanley *et al*[6,32] in 1998. Children usually present with recurrent symptomatic hypoglycaemic episodes (leucine sensitive) and persistent hyperammonaemia.

Molecular basis of HI/HA

The enzyme, GDH has a complex allosteric regulatory mechanism and is highly expressed in the pancreas, liver, kidney and brain. GDH catalyses the reversible oxidative deamination of glutamate to α-KG and ammonia, using NAD or NADP as co-factors. GDH is allosterically inhibited by GTP and activated by ADP and leucine[55].

In patients with HI/HA syndrome there is impairment of allosteric inhibition of GDH by GTP leading to gain-of GDH function. This causes increased leucine induced glutamate oxidation to α-KG, which explains the leucine sensitivity following a protein meal and postprandial hypoglycaemia. These patients on fasting develop hypoglycaemia following release of alanine and glutamine from skeletal muscle, which can stimulate insulin release mediated through GDH[56]. The mechanism of hyperammonaemia in HI/HA syndrome is still unclear. In liver, increased GDH activity may lead to hyperammonaemia through 2 possible mechanisms: elevated activity of GDH causing increased levels of ammonia from glutamate and excessive depletion of glutamate pool, reducing the availability of N-acetyl glutamate (NAG) *via* NAG synthase reaction. NAG is an allosteric activator of CPS 1 and deficiency of this can impair urea synthesis[32,57]. An alternative hypothesis for hyperammonaemia in HI/HA syndrome is that the excessive ammonia is due to abnormal muscle catabolism[58]. More recently the source of the hyperammonaemia in the HI/HA syndrome is thought to be the kidney[59].

Sirtuins and insulin secretion

Sirtuins are a family of NAD^+ dependant enzymes having a critical role in metabolic adaptation to stress. Sirtuin4 (SIRT4), an intramitochondrial enzyme highly expressed

in pancreatic β-cells, also regulates GDH. SIRT4 repress the activity of GDH by ADP-ribosylation in pancreatic β-cell mitochondria, down regulating insulin secretion mediated through amino acids. In normal glucose states, SIRT4 blunts amino acid-induced insulin secretion by repressing the activity of GDH[60,61]. In contrary GDH is released from the SIRT4-mediated inhibition *via* an undefined mechanism during fasting, thereby enhancing amino acid-induced insulin secretion[61]. In SIRT4 knockout mice, GDH activity is enhanced in β-cells, leading to the enhancement of glucose and amino acid-stimulated insulin secretion[61]. So loss of function mutation of *SIRT4* can present with a phenotype similar to gain of function mutation of GLUD1. However no humans have yet been described with protein induced hyperinsulinism due to *SIRT4* mutations[62].

Clinical presentation of HI/HA

The infants with HI/HA syndrome are usually born at term and not macrosomic. The major clinical feature is recurrent episodes of symptomatic HH after first few months of life. These may occur with fasting or can be provoked by protein feeding. Hypoglycaemia in HI/HA syndrome is not as severe as seen in HH due to KATP channel mutations. Hyperammonaemia, a characteristic biochemical marker of HI/HA syndrome, is typically mild to moderate (up to 3-5 times the upper limit of normal) and is not associated with lethargy, irritability, or coma. The plasma amino acid profile remains normal in HI/HA syndrome in contrast to abnormal profile observed in the other causes of hyperammonaemia[62,63].

Protein diet or blood glucose levels do not affect the plasma ammonia levels in patients with HI/HA syndrome[54,64]. Kapoor *et al*[62] reported some patients who have mutations in GLUD1 with HH but with normal serum ammonia levels and the authors proposed that this could be due to mosaicism for the mutation in the liver, where the mutation is absent or seen in < 50% in hepatocytes. Hyperammonaemia is resistant to detoxification compounds (sodium benzoate and N-carbamylglutamate) or protein-restricted diet[65].

Kapoor *et al*[62] have published the clinical characteristics of patients with HI/HA due to GLUD mutations. Of the twenty patients most of them were appropriate for the gestational age and presented at a mean age of 23.4 wk. Nineteen of them had hyperammonaemia. Thirteen of the 17-screened probands had 7 different heterozygous mutations and three novel mutations were identified (N410D, D451V, P436L). More than 90% cases responded to diazoxide. Seizure was the most common (94%) symptom, 43% of them developed generalized epilepsy with a higher preponderance in cases with mutations in exons 6 and 7 of *GLUD1* gene[62,66].

Earlier in 2004, Stanley *et al*[32,57] has reported that over activity of GDH in the brain decreases the levels of glutamate and glutamine, protecting the central nervous system from the neurotoxicity of its accumulation.

GDH transgenic mice harbouring the human *GDH-HI H454Y* mutation develop a hypoglycaemia phenotype[67] and insulin secretion studies in these mice are associated with increased oxidative deamination of glutamate *via* GDH, this confirming the key role of GDH in amino acid stimulated insulin secretion.

Using a β-cell-specific GDH KO mouse model [βGlud1 (-/-)] islets isolated from these mice showed diminished of insulin release when stimulated by glutamine combined with 2-aminobicyclo (2.2.1) heptane-2-carboxylic acid or l-leucine[68]. Further studies in these mice showed that permissive levels of glutamate were required for the full development of glucose-stimulated insulin secretion and that GDH plays an indispensable role in this process.

Management of HI/HA

Treatment of HI/HA is aimed at correction of fasting and protein induced hypoglycaemia. Diazoxide remains the main stay of treatment and affected patients are well controlled with a dose of 5-15 mg/kg per day[69]. Being a KATP channel agonist, diazoxide prevents β-cell membrane depolarization and inhibits insulin secretion by keeping KATP channels open. Diazoxide is usually combined with hydrochlorothiazide in neonates to counteract its fluid retention side effects. Hypertrichosis seen in infants on diazoxide usually resolves on discontinuation[70]. Recent reports of large symptomatic pericardial effusion in infants on diazoxide, warrants meticulous cardiovascular monitoring while on treatment[71].

Green tea flavonoids and HI/HA

Naturally occurring compounds from green tea, discovered by the Chinese Emperor Shen-Nung in 2737 B.C. has been used as a remedy to treat a number of ailments, including diabetes mellitus[72]. Green tea is a significant source of a type of flavonoid called catechin, which includes epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate (ECG) and epicatechin, of which EGCG and ECG have a strong inhibitory effect on GDH function[72,73].

Animal studies have shown that ECG binds to the same site as the allosteric regulator ADP and hijacks the ADP activation site. In pancreatic islet cells of transgenic mice expressing a human HI/HA form of GDH, a hyper-response to glutamine caused by dysregulated GDH is blocked by the addition of EGCG[73]. Above all EGCG has the property to inhibit GTP-insensitive GDH mutations, opening the window of therapeutic potential to treat GDH hyperinsulinism. EGCG also has been shown to block glutamine stimulated calcium influx and insulin secretion in GDH transgenic mice islets[74].

Several novel GDH inhibitors are identified and are under trial[75]. Current evidence support the pathological basis of hyperammonaemia to be due to gain in GDH activity and excessive oxidation of glutamate, reducing the level needed for the synthesis of NAG and thereby slowing the clearance of ammonia (Figure 2). In this context N-carbamylglutamate (Carglumic acid), a carbamoyl

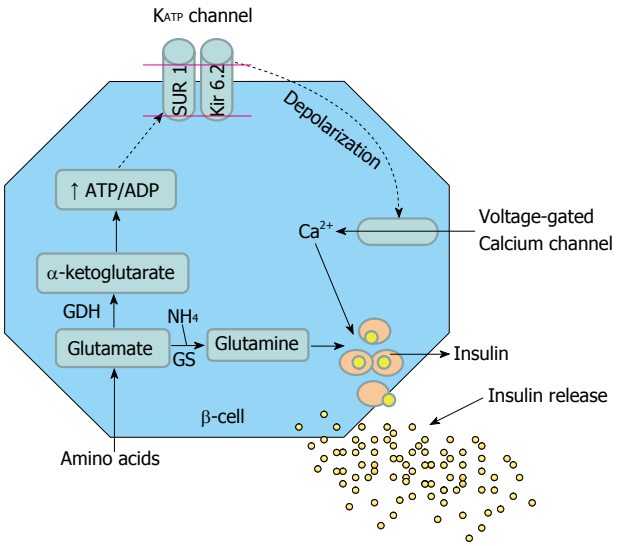

Figure 5 Protein Induced Hypoglycaemia due to defects in KATP channel genes. GDH: Glutamate dehydrogenase; GK: Glucokinase.

phosphate synthetase activator has a potential role in the treatment of hyperammonaemia in HI/HA syndrome[69,76,77]. De novo mutations in GLUD1 have been reported in 70% of GDH-HI cases with the remainder inherited in an autosomal dominant pattern[69].

PROTEIN INDUCED HYPOGLYCAEMIA DUE TO DEFECTS IN KATP CHANNEL GENES

Mutations in the *ABCC8/KCNJ11* genes are the most common cause of CHI[5]. The observation that patients with KATP channel null mutations can develop HH following high protein meal in the absence of leucine sensitivity[78], demonstrates that amino acids can induce HH *via* GDH and KATP channel independent pathways. Patient with GLUD1 mutations show leucine sensitive hypoglycaemia whereas those with *ABCC8/KCNJ11* mutations are not leucine sensitive. Thus, protein-induced HH is not necessarily synonymous with leucine-sensitive HH. The GDH and KATP channel independent mechanism of protein induced HH can be explained through the direct induction of insulin release by glutamine, formed by the ATP-dependent condensation of glutamate with ammonia, catalysed by glutamine synthetase (Figure 5).

Role of glutamine in insulin secretion in patients with KATP channel defects

Glutamine plays a pivotal role in glucose and amino acid stimulated insulin secretion as a signalling molecule, which is followed by β-cell depolarization and influx of calcium and insulin release. Prerequisites for glutamine to function in β-cell include elevated ATP levels and increased cytosolic calcium[7]. Role of glutamine in stimulation of insulin release has been shown in patients with mutations of SUR 1[78]. Animal studies have shown that β-cells of SUR $1^{-/-}$ mice are markedly sensitive to glutamine stimulation[7,67]. Li *et al*[7,67] has shown that β-cells lacking SUR 1 protein were hyper-responsive to glutamine and amino acid mixture but were refractory to glucose stimulation. This amino acid response was reduced by 60% when glutamine was omitted from the amino acid mixture[7]. Two possible mechanisms are considered but still remain unsettled: Metabolism of amino acids is enhanced while glucose is impaired in SUR 1 lacking β-cell which could be the result of persistent elevation of cytosolic calcium and secondly glutamine may be triggering insulin release by a hypothetical novel mechanisms like activation of protein kinase pathways[11,78,79].

PROTEIN INDUCED HYPERINSULINAEMIC HYPOGLYCAEMIA DUE TO LOSS OF FUNCTION MUTATION IN HADH GENE

Mutations causing genetic defects have been described in many of the enzymes involved in mitochondrial fatty acid oxidation. Recently, mutations in the penultimate enzyme in the fatty acid oxidation chain have been described that result in quite different symptoms from those normally seen. Patients with the mutations in *HADH* present with protein (leucine)-induced HH, suggesting a link between fatty acid oxidation, amino acid metabolism and insulin secretion[80].

Short-chain-3-hydroxyacyl-CoA dehydrogenase catalyses the penultimate reaction of the β-oxidation cycle for medium and short chain 3-hydroxy fatty-acyl-CoA's. SCHAD deficiency impairs short chain fatty acid oxidation. First insights into the molecular mechanism involved in SCHAD deficiency came with the observation of Clayton *et al*[8] (2001) that fatty acid beta oxidation defect is associated with HH, supporting the concept of lipid signalling pathway in the control of insulin secretion[81].

Clinical aspects of patients with HADH mutations

Affected children with SCHAD deficiency on fasting as well as following a protein meal, either present with mild late onset hypoglycaemia or severe neonatal hypoglycaemia with raised levels of fatty acid metabolites including plasma hydroxybutyrylcarnitine and urinary 3-hydroxyglutaric acid[82,83]. Most often they present with hypoglycaemic seizures. Kapoor *et al*[84] in 2009 reported for the first time that human mutations of *HADH* gene cause severe dietary protein sensitivity leading to HH and they may have normal acylcarnitine and urinary organic acid profiles. These cases had novel *HADH* gene mutations. The enzymes GDH and SCHAD have a direct protein-protein interaction, which is lost in patients with *HADH* mutations causing leucine induced HH. Leucine sensitivity is evident in patients with *HADH* gene mutations (Figure 3). There is no associated loss of inhibitory effect of GTP on GDH, as seen with *GLUD1* mutations[85].

The interaction between SCHAD and GLUD1

SCHAD has a vital role in insulin secretion, suggested by the high degree of expression of *HADH* gene in β-cells

of pancreas[86]. Hardy *et al*[87], using RNA interference, identified *HADH* gene as one of the 4 essential genes required for normal insulin secretion. FOXA2, a transcription factor encoded by the gene *FOXA2*, is essential for β-cell differentiation and its function has been shown to regulate *HADH* expression[88]. Additionally, severe hyperinsulinism after leucine tolerance testing was reported in all patients having *HADH* gene mutations[85]. Further reports on the loss of protein-protein interaction in human cases between SCHAD and GDH were published[51,83,89]. Heslegrave *et al*[85] made a similar observation of the loss of interaction between SCHAD and GDH in lymphoblasts.

Sund *et al*[90] showed severe HH in FOXA2 β-cell KO mice. Islets from these mice were shown to have reduced expression of both SCHAD and Kir6.2 and had severe HH. Li *et al*[51] showed that HADH KO mice developed a hyperinsulinaemic response following leucine loading and an exacerbation of the same on addition of glutamine and alanine. When glutamine and leucine were removed from the amino acid mixture, KO mice islets failed to induce HH, suggesting the role of GDH activation for abnormal insulin secretion.

Recent studies on HADH KO mice showed an increased sensitivity to amino acid stimulated insulin secretion indicating activation of the glutaminolysis pathway *via* GDH to increase ATP production and thereby insulin. Binding of SCHAD to GDH was also shown in immunoprecipitation experiments. These research works indicate that hyperinsulinism in SCHAD-deficient states is caused by loss of "moonlighting function" (a protein having additional functions in other pathways) of SCHAD protein, which otherwise provides a direct inhibitory regulation of GDH in β-cells[51,91]. So in pancreatic β-cells, mutations resulting in the absence of SCHAD protein leads to abnormal activation of GDH, causing hyperinsulinism.

The activation of GDH in *HADH* gene mutant patients or mouse KO models is limited to pancreatic β-cells and hence deficiency of SCHAD enzyme does not lead to hyperammonemia unlike in HI/HA syndrome[51,92]. Further evidence for protein-protein interaction between enzymes came from Zhang *et al*[93]. They showed the co-precipitation of GDH with SCHAD when anti-SCHAD antibody was used as bait in wild type mouse liver mitochondria, confirming the previous observation that GDH activation in SCHAD deficiency is due to loss of protein-protein interaction.

Diazoxide remains the treatment of choice in HH due to *HADH* gene mutations. This also confirms the intactness of K_{ATP} channel in patients with SCHAD deficiency[8,82,84,92].

CONCLUSION

The interplay between glucose metabolism and that of the two other primary nutrient classes, amino acids and fatty acids is critical for regulated insulin secretion. Protein induced HH is observed in patients with mutations in *GLUD1*, *HADH* and *ABCC8/KCNJ11*. GDH and SCHAD play important roles in integrating amino acid and fatty acid signals for insulin secretion.

REFERENCES

1 **Newsholme P**, Brennan L, Rubi B, Maechler P. New insights into amino acid metabolism, beta-cell function and diabetes. *Clin Sci* (Lond) 2005; **108**: 185-194 [PMID: 15544573]

2 **Maechler P**, Carobbio S, Rubi B. In beta-cells, mitochondria integrate and generate metabolic signals controlling insulin secretion. *Int J Biochem Cell Biol* 2006; **38**: 696-709 [PMID: 16443386 DOI: 10.1016/j.biocel.2005.12.006]

3 **Aynsley-Green A**, Hussain K, Hall J, Saudubray JM, Nihoul-Fékété C, De Lonlay-Debeney P, Brunelle F, Otonkoski T, Thornton P, Lindley KJ. Practical management of hyperinsulinism in infancy. *Arch Dis Child Fetal Neonatal Ed* 2000; **82**: F98-F107 [PMID: 10685981 DOI: 10.1136/fn.82.2.F98]

4 **James C**, Kapoor RR, Ismail D, Hussain K. The genetic basis of congenital hyperinsulinism. *J Med Genet* 2009; **46**: 289-299 [PMID: 19254908 DOI: 10.1136/jmg.2008.064337]

5 **Thomas PM**, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gagel RF, Bryan J. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 1995; **268**: 426-429 [PMID: 7716548 DOI: 10.1126/science.7716548]

6 **Stanley CA**, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, Perlman K, Rich BH, Zammarchi E, Poncz M. Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med* 1998; **338**: 1352-1357 [PMID: 9571255 DOI: 10.1056/NEJM199805073381904]

7 **Li C**, Buettger C, Kwagh J, Matter A, Daikhin Y, Nissim IB, Collins HW, Yudkoff M, Stanley CA, Matschinsky FM. A signaling role of glutamine in insulin secretion. *J Biol Chem* 2004; **279**: 13393-13401 [PMID: 14736887 DOI: 10.1074/jbc.M311502200]

8 **Clayton PT**, Eaton S, Aynsley-Green A, Edginton M, Hussain K, Krywawych S, Datta V, Malingre HE, Berger R, van den Berg IE. Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of beta-oxidation in insulin secretion. *J Clin Invest* 2001; **108**: 457-465 [PMID: 11489939 DOI: 10.1172/JCI11294]

9 **Inagaki N**, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J. Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 1995; **270**: 1166-1170 [PMID: 7502040]

10 **Henquin JC**. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 2000; **49**: 1751-1760 [PMID: 11078440 DOI: 10.2337/diabetes]

11 **Ammälä C**, Eliasson L, Bokvist K, Berggren PO, Honkanen RE, Sjöholm A, Rorsman P. Activation of protein kinases and inhibition of protein phosphatases play a central role in the regulation of exocytosis in mouse pancreatic beta cells. *Proc Natl Acad Sci USA* 1994; **91**: 4343-4347 [PMID: 8183910]

12 **Thomas P**, Ye Y, Lightner E. Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet* 1996; **5**: 1809-1812 [PMID: 8923010 DOI: 10.1093/hmg/5.11.1809]

13 **Flanagan SE**, Clauin S, Bellanné-Chantelot C, de Lonlay P, Harries LW, Gloyn AL, Ellard S. Update of mutations in the genes encoding the pancreatic beta-cell K(ATP) channel subunits Kir6.2 (KCNJ11) and sulfonylurea receptor 1 (ABCC8) in diabetes mellitus and hyperinsulinism. *Hum Mutat* 2009; **30**: 170-180 [PMID: 18767144 DOI: 10.1002/humu.20838]

14 **Floyd JC**, Fajans SS, Conn JW, Knopf RF, Rull J. Insulin secretion in response to protein ingestion. *J Clin Invest* 1966; **45**: 1479-1486 [PMID: 5919349]

15 **Floyd JC**, Fajans SS, Conn JW, Knopf RF, Rull J. Stimulation of insulin secretion by amino acids. *J Clin Invest* 1966; **45**: 1487-1502 [PMID: 5919350 DOI: 10.1172/JCI105456]

16 **Bateman KS**, Chernaia MM, Tarasova NI, James MN. Crystal structure of human pepsinogen A. *Adv Exp Med Biol* 1998; **436**: 259-263 [PMID: 9561228]

17 **Rocha DM**, Faloona GR, Unger RH. Glucagon-stimulating activity of 20 amino acids in dogs. *J Clin Invest* 1972; **51**: 2346-2351 [PMID: 4639019]

18 **Floyd JC**, Fajans SS, Pek S, Thiffault CA, Knopf RF, Conn JW. Synergistic effect of essential amino acids and glucose upon insulin secretion in man. *Diabetes* 1970; **19**: 109-115 [PMID: 4905636]

19 **van Loon LJ**, Kruijshoop M, Menheere PP, Wagenmakers AJ, Saris WH, Keizer HA. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. *Diabetes Care* 2003; **26**: 625-630 [PMID: 12610012 DOI: 10.2337/diacare.26.3.625]

20 **Floyd JC**, Fajans SS, Pek S, Thiffault CA, Knopf RF, Conn JW. Synergistic effect of certain amino acid pairs upon insulin secretion in man. *Diabetes* 1970; **19**: 102-108 [PMID: 5414363]

21 **van Loon LJ**, Saris WH, Verhagen H, Wagenmakers AJ. Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. *Am J Clin Nutr* 2000; **72**: 96-105 [PMID: 10871567]

22 **Asmar M**, Holst JJ. Glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide: new advances. *Curr Opin Endocrinol Diabetes Obes* 2010; **17**: 57-62 [PMID: 19881341 DOI: 10.1097/MED.0b013e3283339051]

23 **Nilsson M**, Stenberg M, Frid AH, Holst JJ, Björck IM. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins. *Am J Clin Nutr* 2004; **80**: 1246-1253 [PMID: 15531672]

24 **Smith MB**, March J. Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th ed. Wiley, 2001

25 **Malaisse WJ**, Sener A, Carpinelli AR, Anjaneyulu K, Lebrun P, Herchuelz A, Christophe J. The stimulus-secretion coupling of glucose-induced insulin release. XLVI. Physiological role of L-glutamine as a fuel for pancreatic islets. *Mol Cell Endocrinol* 1980; **20**: 171-189 [PMID: 7002657 DOI: 10.1016/0303-7207(80)90080-5]

26 **Sener A**, Malaisse WJ. The stimulus-secretion coupling of amino acid-induced insulin release. Insulinotropic action of L-alanine. *Biochim Biophys Acta* 2002; **1573**: 100-104 [PMID: 12383948 DOI: 10.1016/S0304-4165(02)00337-9]

27 **Malaisse WJ**, Malaisse-Lagae F, Sener A. The stimulus-secretion coupling of amino acid-induced insulin release metabolic interaction of L-asparagine and L-leucine in pancreatic islets. *Biochim Biophys Acta* 1984; **797**: 194-202 [PMID: 6365174]

28 **Sener A**, Malaisse WJ. L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. *Nature* 1980; **288**: 187-189 [PMID: 7001252]

29 **Arya VB**, Senniappan S, Guemes M, Hussain K. Neonatal hypoglycemia. *Indian J Pediatr* 2014; **81**: 58-65 [PMID: 23904063 DOI: 10.4103/2230-8210.107822]

30 **Deeney JT**, Gromada J, Høy M, Olsen HL, Rhodes CJ, Prentki M, Berggren PO, Corkey BE. Acute stimulation with long chain acyl-CoA enhances exocytosis in insulin-secreting cells (HIT T-15 and NMRI beta-cells). *J Biol Chem* 2000; **275**: 9363-9368 [PMID: 10734079 DOI: 10.1074/jbc.275.13.9363]

31 **Hutton JC**, Sener A, Malaisse WJ. Interaction of branched chain amino acids and keto acids upon pancreatic islet metabolism and insulin secretion. *J Biol Chem* 1980; **255**: 7340-7346 [PMID: 6993486]

32 **Stanley CA**. Hyperinsulinism/hyperammonemia syndrome: insights into the regulatory role of glutamate dehydrogenase in ammonia metabolism. *Mol Genet Metab* 2004; **81** Suppl 1: S45-S51 [PMID: 15050973 DOI: 10.1016/j.ymgme.2003.10.013]

33 **Bränström R**, Efendić S, Berggren PO, Larsson O. Direct inhibition of the pancreatic beta-cell ATP-regulated potassium channel by alpha-ketoisocaproate. *J Biol Chem* 1998; **273**: 14113-14118 [PMID: 9603910]

34 **Gao Z**, Young RA, Li G, Najafi H, Buettger C, Sukumvanich SS, Wong RK, Wolf BA, Matschinsky FM. Distinguishing features of leucine and alpha-ketoisocaproate sensing in pancreatic beta-cells. *Endocrinology* 2003; **144**: 1949-1957 [PMID: 12697702 DOI: 10.1210/en.2002-0072]

35 **Avruch J**, Long X, Ortiz-Vega S, Rapley J, Papageorgiou A, Dai N. Amino acid regulation of TOR complex 1. *Am J Physiol Endocrinol Metab* 2009; **296**: E592-E602 [PMID: 18765678 DOI: 10.1152/ajpendo.90645.2008]

36 **Yang J**, Dolinger M, Ritaccio G, Mazurkiewicz J, Conti D, Zhu X, Huang Y. Leucine stimulates insulin secretion via down-regulation of surface expression of adrenergic α2A receptor through the mTOR (mammalian target of rapamycin) pathway: implication in new-onset diabetes in renal transplantation. *J Biol Chem* 2012; **287**: 24795-24806 [PMID: 22645144 DOI: 10.1074/jbc.M112.344259]

37 **Williamson DH**, Brosnan JT. Concentrations of metabolites in animal tissues. In: Bergmeyer HU (Editor), Methods in Enzymatic Analysis. Academic Press, New York and London, 1974

38 **Newsholme P**, Lima MM, Procopio J, Pithon-Curi TC, Doi SQ, Bazotte RB, Curi R. Glutamine and glutamate as vital metabolites. *Braz J Med Biol Res* 2003; **36**: 153-163 [PMID: 12563517 DOI: 10.1590/S0100-879X2003000200002]

39 **Young VR**, Ajami AM. Glutamine: the emperor or his clothes? *J Nutr* 2001; **131**: 2449S-2459S; discussion 2486S-2487S [PMID: 11533293]

40 **Cho SW**, Ahn JY, Lee J, Choi SY. Identification of a peptide of the guanosine triphosphate binding site within brain glutamate dehydrogenase isoproteins using 8-azidoguanosine triphosphate. *Biochemistry* 1996; **35**: 13907-13913 [PMID: 8909287 DOI: 10.1021/bi9618575]

41 **Wrzeszczynski KO**, Colman RF. Activation of bovine liver glutamate dehydrogenase by covalent reaction of adenosine 5′-O-[S-(4-bromo-2,3-dioxobutyl)thiophosphate] with arginine-459 at an ADP regulatory site. *Biochemistry* 1994; **33**: 11544-11553 [PMID: 7918368]

42 **Tolhurst G**, Zheng Y, Parker HE, Habib AM, Reimann F, Gribble FM. Glutamine triggers and potentiates glucagon-like peptide-1 secretion by raising cytosolic Ca2+ and cAMP. *Endocrinology* 2011; **152**: 405-413 [PMID: 21209017 DOI: 10.1210/en.2010-0956]

43 **Brennan L**, Shine A, Hewage C, Malthouse JP, Brindle KM, McClenaghan N, Flatt PR, Newsholme P. A nuclear magnetic resonance-based demonstration of substantial oxidative L-alanine metabolism and L-alanine-enhanced glucose metabolism in a clonal pancreatic beta-cell line: metabolism of L-alanine is important to the regulation of insulin secretion. *Diabetes* 2002; **51**: 1714-1721 [PMID: 12031957 DOI: 10.2337/diabetes.51.6.1714]

44 **Dunne MJ**, Yule DI, Gallacher DV, Petersen OH. Effects of alanine on insulin-secreting cells: patch-clamp and single cell intracellular Ca2+ measurements. *Biochim Biophys Acta* 1990; **1055**: 157-164 [PMID: 2242384 DOI: 10.1016/01674889(90)]

45 **McClenaghan NH**, Barnett CR, Flatt PR. Na+ cotransport by metabolizable and nonmetabolizable amino acids stimulates a glucose-regulated insulin-secretory response. *Biochem Biophys Res Commun* 1998; **249**: 299-303 [PMID: 9712690

46 **Dura E**, Jijakli H, Zhang HX, Oguzhan B, Sener A, Malaisse WJ. Insulinotropic action of amino acids at their physiological concentrations: I. Experiments in incubated islets. *Int J Mol Med* 2002; **9**: 527-531 [PMID: 11956661 DOI: 10.3892/ijmm.9.5.527]

47 **Sener A**, Best LC, Yates AP, Kadiata MM, Olivares E, Louchami K, Jijakli H, Ladrière L, Malaisse WJ. Stimulus-

secretion coupling of arginine-induced insulin release: comparison between the cationic amino acid and its methyl ester. *Endocrine* 2000; **13**: 329-340 [PMID: 11216645 DOI: 10.1385/ENDO:13:3:329]
48 **Malaisse WJ**, Plasman PO, Blachier F, Herchuelz A, Sener A. Stimulus-secretion coupling of arginine-induced insulin release: significance of changes in extracellular and intracellular pH. *Cell Biochem Funct* 1991; **9**: 1-7 [PMID: 1712259]
49 **Broca C**, Brennan L, Petit P, Newsholme P, Maechler P. Mitochondria-derived glutamate at the interplay between branched-chain amino acid and glucose-induced insulin secretion. *FEBS Lett* 2003; **545**: 167-172 [PMID: 12804769]
50 **Wood PA**. Defects in mitochondrial beta-oxidation of fatty acids. *Curr Opin Lipidol* 1999; **10**: 107-112 [PMID: 10327278]
51 **Li C**, Chen P, Palladino A, Narayan S, Russell LK, Sayed S, Xiong G, Chen J, Stokes D, Butt YM, Jones PM, Collins HW, Cohen NA, Cohen AS, Nissim I, Smith TJ, Strauss AW, Matschinsky FM, Bennett MJ, Stanley CA. Mechanism of hyperinsulinism in short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency involves activation of glutamate dehydrogenase. *J Biol Chem* 2010; **285**: 31806-31818 [PMID: 20670938]
52 **Cochrane WA**, Payne WW, Simpkiss MJ, Woolf LI. Familial hypoglycemia precipitated by amino acids. *J Clin Invest* 1956; **35**: 411-422 [PMID: 13306783 DOI: 10.1172/JCI103292]
53 **Pace CS**, Stillings SN, Hover BA, Matschinsky FM. Electrical and secretory manifestations of glucose and amino acid interactions in rat pancreatic islets. *Diabetes* 1975; **24**: 489-496 [PMID: 1092581]
54 **Zammarchi E**, Filippi L, Novembre E, Donati MA. Biochemical evaluation of a patient with a familial form of leucine-sensitive hypoglycemia and concomitant hyperammonemia. *Metabolism* 1996; **45**: 957-960 [PMID: 8769351]
55 **Fahien LA**, MacDonald MJ, Kmiotek EH, Mertz RJ, Fahien CM. Regulation of insulin release by factors that also modify glutamate dehydrogenase. *J Biol Chem* 1988; **263**: 13610-13614 [PMID: 3047128]
56 **Chang TW**, Goldberg AL. The metabolic fates of amino acids and the formation of glutamine in skeletal muscle. *J Biol Chem* 1978; **253**: 3685-3693 [PMID: 649596]
57 **Stanley CA**, Fang J, Kutyna K, Hsu BY, Ming JE, Glaser B, Poncz M. Molecular basis and characterization of the hyperinsulinism/hyperammonemia syndrome: predominance of mutations in exons 11 and 12 of the glutamate dehydrogenase gene. HI/HA Contributing Investigators. *Diabetes* 2000; **49**: 667-673 [PMID: 10871207 DOI: 10.2337/diabetes.49.4.667]
58 **Kamoun P**, Rabier D, Saudubray JM. Muscular origin of hyperammonaemia. *Eur J Pediatr* 1998; **157**: 868 [PMID: 9809835]
59 **Treberg JR**, Clow KA, Greene KA, Brosnan ME, Brosnan JT. Systemic activation of glutamate dehydrogenase increases renal ammoniagenesis: implications for the hyperinsulinism/hyperammonemia syndrome. *Am J Physiol Endocrinol Metab* 2010; **298**: E1219-E1225 [PMID: 20332361 DOI: 10.1152/ajpendo.00028]
60 **Haigis MC**, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW, Guarente L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell* 2006; **126**: 941-954 [PMID: 16959573 DOI: 10.1016/j.cell.2006.06.057]
61 **Ahuja N**, Schwer B, Carobbio S, Waltregny D, North BJ, Castronovo V, Maechler P, Verdin E. Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. *J Biol Chem* 2007; **282**: 33583-33592 [PMID: 17715127 DOI: 10.1074/jbc.M705488200]
62 **Kapoor RR**, Flanagan SE, Fulton P, Chakrapani A, Chadefaux B, Ben-Omran T, Banerjee I, Shield JP, Ellard S, Hussain K. Hyperinsulinism-hyperammonaemia syndrome: novel mutations in the GLUD1 gene and genotype-phenotype correlations. *Eur J Endocrinol* 2009; **161**: 731-735 [PMID: 19690084 DOI: 10.1530/EJE-09-0615]
63 **Kapoor RR**, James C, Hussain K. Advances in the diagnosis and management of hyperinsulinemic hypoglycemia. *Nat Clin Pract Endocrinol Metab* 2009; **5**: 101-112 [PMID: 19165222 DOI: 10.1038/ncpendmet1046]
64 **Miki Y**, Taki T, Ohura T, Kato H, Yanagisawa M, Hayashi Y. Novel missense mutations in the glutamate dehydrogenase gene in the congenital hyperinsulinism-hyperammonemia syndrome. *J Pediatr* 2000; **136**: 69-72 [PMID: 10636977]
65 **De Lonlay P**, Benelli C, Fouque F, Ganguly A, Aral B, Dionisi-Vici C, Touati G, Heinrichs C, Rabier D, Kamoun P, Robert JJ, Stanley C, Saudubray JM. Hyperinsulinism and hyperammonemia syndrome: report of twelve unrelated patients. *Pediatr Res* 2001; **50**: 353-357 [PMID: 11518822]
66 **Bahi-Buisson N**, El Sabbagh S, Soufflet C, Escande F, Boddaert N, Valayannopoulos V, Bellané-Chantelot C, Lascelles K, Dulac O, Plouin P, de Lonlay P. Myoclonic absence epilepsy with photosensitivity and a gain of function mutation in glutamate dehydrogenase. *Seizure* 2008; **17**: 658-664 [PMID: 18321734 DOI: 10.1016/j.seizure.2008.01.005]
67 **Li C**, Matter A, Kelly A, Petty TJ, Najafi H, MacMullen C, Daikhin Y, Nissim I, Lazarow A, Kwagh J, Collins HW, Hsu BY, Nissim I, Yudkoff M, Matschinsky FM, Stanley CA. Effects of a GTP-insensitive mutation of glutamate dehydrogenase on insulin secretion in transgenic mice. *J Biol Chem* 2006; **281**: 15064-15072 [PMID: 16574664 DOI: 10.1074/jbc.M600994200]
68 **Vetterli L**, Carobbio S, Pournourmohammadi S, Martin-Del-Rio R, Skytt DM, Waagepetersen HS, Tamarit-Rodriguez J, Maechler P. Delineation of glutamate pathways and secretory responses in pancreatic islets with β-cell-specific abrogation of the glutamate dehydrogenase. *Mol Biol Cell* 2012; **23**: 3851-3862 [PMID: 22875990 DOI: 10.1091/mbc.E11-08-0676]
69 **Palladino AA**, Stanley CA. The hyperinsulinism/hyperammonemia syndrome. *Rev Endocr Metab Disord* 2010; **11**: 171-178 [PMID: 20936362]
70 **Arnoux JB**, de Lonlay P, Ribeiro MJ, Hussain K, Blankenstein O, Mohnike K, Valayannopoulos V, Robert JJ, Rahier J, Sempoux C, Bellanné C, Verkarre V, Aigrain Y, Jaubert F, Brunelle F, Nihoul-Fékété C. Congenital hyperinsulinism. *Early Hum Dev* 2010; **86**: 287-294 [PMID: 20550977]
71 **Avatapalle B**, Banerjee I, Malaiya N, Padidela R. Echocardiography monitoring for diazoxide induced pericardial effusion. *BMJ Case Rep* 2012; **2012**: [PMID: 22761225 DOI: 10.1136/bcr.03.2012.6110]
72 **Yang CS**, Wang ZY. Tea and cancer. *J Natl Cancer Inst* 1993; **85**: 1038-1049 [PMID: 8515490 DOI: 10.1093/jnci/85.13.1038]
73 **Li C**, Allen A, Kwagh J, Doliba NM, Qin W, Najafi H, Collins HW, Matschinsky FM, Stanley CA, Smith TJ. Green tea polyphenols modulate insulin secretion by inhibiting glutamate dehydrogenase. *J Biol Chem* 2006; **281**: 10214-10221 [PMID: 16476731 DOI: 10.1074/jbc.M512792200]
74 **Li C**, Li M, Chen P, Narayan S, Matschinsky FM, Bennett MJ, Stanley CA, Smith TJ. Green tea polyphenols control dysregulated glutamate dehydrogenase in transgenic mice by hijacking the ADP activation site. *J Biol Chem* 2011; **286**: 34164-34174 [PMID: 21813650 DOI: 10.1074/jbc.M111.268599]
75 **Li M**, Smith CJ, Walker MT, Smith TJ. Novel inhibitors complexed with glutamate dehydrogenase: allosteric regulation by control of protein dynamics. *J Biol Chem* 2009; **284**: 22988-23000 [PMID: 19531491 DOI: 10.1074/jbc.M109.020222]
76 **Huijmans JG**, Duran M, de Klerk JB, Rovers MJ, Scholte HR. Functional hyperactivity of hepatic glutamate dehydrogenase as a cause of the hyperinsulinism/hyperammonemia syndrome: effect of treatment. *Pediatrics* 2000; **106**: 596-600 [PMID: 10969108 DOI: 10.1542/peds.106.3.596]
77 **Daniotti M**, la Marca G, Fiorini P, Filippi L. New developments in the treatment of hyperammonemia: emerging use of carglumic acid. *Int J Gen Med* 2011; **4**: 21-28 [PMID:

21403788 DOI: 10.2147/IJGM.S10490]
78 **Fourtner SH**, Stanley CA, Kelly A. Protein-sensitive hypoglycemia without leucine sensitivity in hyperinsulinism caused by K(ATP) channel mutations. *J Pediatr* 2006; **149**: 47-52 [PMID: 16860127 DOI: 10.1016/j.jpeds.2006.02.033]
79 **Li C**, Nissim I, Chen P, Buettger C, Najafi H, Daikhin Y, Nissim I, Collins HW, Yudkoff M, Stanley CA, Matschinsky FM. Elimination of KATP channels in mouse islets results in elevated [U-13C]glucose metabolism, glutaminolysis, and pyruvate cycling but a decreased gamma-aminobutyric acid shunt. *J Biol Chem* 2008; **283**: 17238-17249 [PMID: 18445600 DOI: 10.1074/jbc.M709235200]
80 **Heslegrave AJ**, Hussain K. Novel insights into fatty acid oxidation, amino acid metabolism, and insulin secretion from studying patients with loss of function mutations in 3-hydroxyacyl-CoA dehydrogenase. *J Clin Endocrinol Metab* 2013; **98**: 496-501 [PMID: 23253615 DOI: 10.1210/jc.2012-3134]
81 **Prentki M**. New insights into pancreatic beta-cell metabolic signaling in insulin secretion. *Eur J Endocrinol* 1996; **134**: 272-286 [PMID: 8616523]
82 **Hussain K**, Clayton PT, Krywawych S, Chatziandreou I, Mills P, Ginbey DW, Geboers AJ, Berger R, van den Berg IE, Eaton S. Hyperinsulinism of infancy associated with a novel splice site mutation in the SCHAD gene. *J Pediatr* 2005; **146**: 706-708 [PMID: 15870679 DOI: 10.1016/j.jpeds.2005.01.032]
83 **Molven A**, Matre GE, Duran M, Wanders RJ, Rishaug U, Njølstad PR, Jellum E, Søvik O. Familial hyperinsulinemic hypoglycemia caused by a defect in the SCHAD enzyme of mitochondrial fatty acid oxidation. *Diabetes* 2004; **53**: 221-227 [PMID: 14693719 DOI: 10.2337/diabetes.53.1.221]
84 **Kapoor RR**, James C, Flanagan SE, Ellard S, Eaton S, Hussain K. 3-Hydroxyacyl-coenzyme A dehydrogenase deficiency and hyperinsulinemic hypoglycemia: characterization of a novel mutation and severe dietary protein sensitivity. *J Clin Endocrinol Metab* 2009; **94**: 2221-2225 [PMID: 19417036]
85 **Heslegrave AJ**, Kapoor RR, Eaton S, Chadefaux B, Akcay T, Simsek E, Flanagan SE, Ellard S, Hussain K. Leucine-sensitive hyperinsulinaemic hypoglycaemia in patients with loss of function mutations in 3-Hydroxyacyl-CoA Dehydrogenase. *Orphanet J Rare Dis* 2012; **7**: 25 [PMID: 22583614 DOI: 10.1186/1750-1172-7-25]
86 **Agren A**, Borg K, Brolin SE, Carlman J, Lundqvist G. Hydroxyacyl CoA dehydrogenase, an enzyme important in fat metabolism in different cell types in the islets of Langerhans. *Diabete Metab* 1977; **3**: 169-172 [PMID: 334589]
87 **Hardy OT**, Hohmeier HE, Becker TC, Manduchi E, Doliba NM, Gupta RK, White P, Stoeckert CJ, Matschinsky FM, Newgard CB, Kaestner KH. Functional genomics of the beta-cell: short-chain 3-hydroxyacyl-coenzyme A dehydrogenase regulates insulin secretion independent of K+ currents. *Mol Endocrinol* 2007; **21**: 765-773 [PMID: 17185391 DOI: 10.1210/me.2006-0411]
88 **Lantz KA**, Vatamaniuk MZ, Brestelli JE, Friedman JR, Matschinsky FM, Kaestner KH. Foxa2 regulates multiple pathways of insulin secretion. *J Clin Invest* 2004; **114**: 512-520 [PMID: 15314688 DOI: 10.1172/JCI21149]
89 **Filling C**, Keller B, Hirschberg D, Marschall HU, Jörnvall H, Bennett MJ, Oppermann U. Role of short-chain hydroxyacyl CoA dehydrogenases in SCHAD deficiency. *Biochem Biophys Res Commun* 2008; **368**: 6-11 [PMID: 18036338 DOI: 10.1016/j.bbrc.207.10.188]
90 **Sund NJ**, Vatamaniuk MZ, Casey M, Ang SL, Magnuson MA, Stoffers DA, Matschinsky FM, Kaestner KH. Tissue-specific deletion of Foxa2 in pancreatic beta cells results in hyperinsulinemic hypoglycemia. *Genes Dev* 2001; **15**: 1706-1715 [PMID: 11445544 DOI: 10.1101/gad.901601]
91 **Jeffery CJ**. Moonlighting proteins--an update. *Mol Biosyst* 2009; **5**: 345-350 [PMID: 19396370 DOI: 10.1039/b900658n]
92 **Stanley CA**. Two genetic forms of hyperinsulinemic hypoglycemia caused by dysregulation of glutamate dehydrogenase. *Neurochem Int* 2011; **59**: 465-472 [PMID: 21130127 DOI: 10.1016/j.neuint.2010.11.017]
93 **Zhang T**, Li C. Mechanisms of amino acid-stimulated insulin secretion in congenital hyperinsulinism. *Acta Biochim Biophys Sin* (Shanghai) 2013; **45**: 36-43 [PMID: 23212075 DOI: 10.1093/abbs/gms107]

P- Reviewer: Gómez-Sáez J, Junghyo J, Kietzmann T, Lehtonen SH
S- Editor: Wen LL **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.678

World J Diabetes 2014 October 15; 5(5): 678-688
ISSN 1948-9358 (online)

REVIEW

Hepatocyte growth factor, a biomarker of macroangiopathy in diabetes mellitus

Hiroyuki Konya, Masayuki Miuchi, Kahori Satani, Satoshi Matsutani, Taku Tsunoda, Yuzo Yano, Tomoyuki Katsuno, Tomoya Hamaguchi, Jun-Ichiro Miyagawa, Mitsuyoshi Namba

Hiroyuki Konya, Satoshi Matsutani, Yuzo Yano, Department of Internal Medicine, Ashiya Municipal Hospital, Ashiya, Hyogo 659-8502, Japan
Masayuki Miuchi, Kahori Satani, Taku Tsunoda, Jun-Ichiro Miyagawa, Mitsuyoshi Namba, Division of Diabetes, Endocrinology and Metabolism, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501, Japan
Tomoyuki Katsuno, Division of Innovative Diabetes Treatment, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501, Japan
Tomoya Hamaguchi, Division of Diabetes, Department of Internal Medicine, Itami City Hospital, Itami, Hyogo 664-8540, Japan
Author contributions: Konya H and Namba M involved in collecting the required publications about the review and editing the manuscript; Konya H wrote the manuscript; all authors organized the structure of the review.
Correspondence to: Hiroyuki Konya, MD, PhD, Department of Internal Medicine, Ashiya Municipal Hospital, 39-1, Asahigaoka-cho, Ashiya, Hyogo 659-8502, Japan. h-dyer@mvi.biglobe.ne.jp
Telephone: +81-797-312156 Fax: +81-797-228822
Received: December 27, 2013 Revised: March 1, 2014
Accepted: May 31, 2014
Published online: October 15, 2014

Abstract

Atherosclerotic involvements are an essential causal element of prospect in diabetes mellitus (DM), with carotid atherosclerosis (CA) being a common risk-factor for prospective crisis of coronary artery diseases (CAD) and/or cerebral infarction (CI) in DM subjects. From another point of view, several reports have supplied augmenting proof that hepatocyte growth factor (HGF) has a physiopathological part in DM involvements. HGF has been a mesenchymal-derived polyphenic factor which modulates development, motion, and morphosis of diverse cells, and has been regarded as a humor intermediator of epithelial-mesenchymal interplays. The serum concentrations of HGF have been elevated in subjects with CAD and CI, especially during the acute phase of both disturbances. In our study with 89 type 2 DM patients, the association between serum concentrations of HGF and risk-factors for macrovascular complications inclusive of CA were examined. The average of serum HGF levels in the subjects was more elevated than the reference interval. The serum HGF concentrations associated positively with both intimal-media thickness (IMT) (r = 0.24, P = 0.0248) and plaque score (r = 0.27, P = 0.0126), indicating a relationship between the elevated HGF concentrations and advancement of CA involvements. Multivariate statistical analysis accentuated that serum concentrations of HGF would be associated independently with IMT (standardized = 0.28, P = 0.0499). The review indicates what is presently known regarding serum HGF might be a new and meaningful biomarker of macroangiopathy in DM subjects.

Key words: Hepatocyte growth factor; Diabetes mellitus; Carotid atherosclerosis; Macroangiopathy; Biomarker

Core tip: Hepatocyte growth factor (HGF) has been a mesenchymal-derived polyphenic factor which modulates development, motion, and morphosis of diverse cells, and has been regarded as a humor intermediator of epithelial-mesenchymal interplays. The serum levels of HGF in diabetes mellitus (DM) subjects might be assayed by balancing of stimulators (hypertension, atheromatous arteriosclerosis, *etc*.) and suppressors (hyperglycemia, transforming growth factor-, angiotensin Ⅱ, *etc*.). The elevated serum level of HGF might have been regarded as an indicator of the DM involvements seriousness. Accordingly, the concentration of serum HGF might be a new and meaningful biomarker of macroangiopathy in DM subjects.

Konya H, Miuchi M, Satani K, Matsutani S, Tsunoda T, Yano Y, Katsuno T, Hamaguchi T, Miyagawa JI, Namba M. Hepatocyte

growth factor, a biomarker of macroangiopathy in diabetes mellitus. *World J Diabetes* 2014; 5(5): 678-688 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/678.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.678

INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disturbance and one of the principal chronic diseases internationally. The planetary number of diabetic (DM) patients is approximated at 382 million (mill) in 2013, and it is anticipated to be over 592 mill by the year 2035[1]. Close to 5.1 mill the dead in the 20-79 years aged group might be due to DM in 2013, elucidating 8.4% of the global all-cause deathrate[2]. In addition to the effect on the subjects' life quality, the microvascular [diabetic retinopathy (DR), diabetic nephropathy (DN), neuropathy] and macrovascular complicating diseases (coronary heart diseases, peripheral artery diseases, and stroke) of DM also increase the internal healthcare spendings. Approximated planetary healthcare expendings to care and preclude DM and its complicating diseases are anticipated to total leastwise 548 billion USD in 2013. By 2035, this number is proposed to surpass some 627 billion USD[3]. Worldwide, DM is probable to be the fifth leading killer[4].

DM individuals, both type 1 DM (T1DM) likewise T2DM, have an elevated hazard of growing endorgan dysfunction. In a clinical manner, the conception of DM cardiac myopathy is determined as cardiac ventricle damage that arises irrespective of hypertension (HTN) and coronary artery disease (CAD), namely as a discrete primitive disorder course that generates secondarily to a damage of metabolism and leads to morphological and functioning anomalies of the myocardia guiding to heart failure (HF). Human DM cardiac myopathy has been chiefly demonstrated by the damage of diastole, that might introduce the the damage of systole growing[5]. Intriguingly, solely roughly 30% of T2DM and T1DM subjects make grow DN, in contradistinction to DM cardiac myopathy that is existed in half of T2DM subjects and DR investigated in over 90% of T1DM individuals[6,7]. It suggests a distinct timecourse of DM endorgan disorder. Therefore, in a differential manner, respective cell types would be exact to hyperglycemia-caused disturbance possibly for sake of distinct expression or activeness of molecular factors would be in charge of damage activating and progression[8].

Atherosclerotic complicating diseases are an essential causal element of prognosis in T2DM, with carotid atherosclerosis (CA) being a common risk-factor for prospective crisis of CAD and/or cerebral infarction (CI)[9,10]. Some molecules, such as high-sensitivity C-reactive protein (hs-CRP) and interleukin-18, would have been presented to be atherosclerotic biomarkers[11,12]. Preclusion of DM and its involvements, early invention of disease stages, and interventions that would act in the presence of hyperglycemia to avoid, retard or inverse the involvements are the principal concerns. Biomarkers have been investigated for understanding the structures of the evolution and progress of DM involvements[13]. This review presents what is currently known regarding serum hepatocyte growth factor (HGF) level might be a new and meaningful biomarker of DM macroangiopathy.

PLEIOTROPHIC EFFECTS OF HGF

HGF has been a mesenchymal-derived polyphenic factor which modulates diverse cells development, motion, and morphosis and it is thought that HGF would be a body fluid intermediator of epithelial-mesenchymal interplays. HGF has been distinguished as a new element of the family of endothelium-specific growth factors and a topical HGF system, configured HGF and its particular receptor mesenchymal epithelial transition factor (c-MET, MET), would have been presented in blood vessel cells both *in vivo* and *in vitro*[14-17]. Additionally, there is the proof that HGF induces the security and/or restoration of vascular endothelical cells hurt by HTN, with elevated serum HGF concentrations happening dependent on endothelial cell dysfunction[18,19]. HGF has been a polyphenic cytokine related to tissue security and restoration of the vascular endothelia[13-18]. Furthermore, it has been demonstrated that HGF would have in vitro mitogenic action in cultivation systems, and is deemed to be a new angiogenetic growth factor[20] (Figure 1). Some of investigations have demonstrated that HGF/scatter factor (SF) is represented by smooth muscle cells (SMCs) but works on vascular endothelical cells, not SMCs in the artery wall[17]. Nevertheless, different investigations have suggested that SMCs can react to HGF/SF[15,16]. McKinnon *et al*[21] have restudied expression and action of HGF/SF and its receptor MET in artery SMC and vascular endothelical cell cultivations and in total arteries after superficial or deep damage or atherogenicity. High-density cultivations of SMCs brought about HGF/SF but did not express MET, meanwhile SMCs, at the leading-edge of damaged cultivations, expressed both ligand and receptor and displayed a conspicuous motion and development reaction to HGF/SF. In accordance with these outcomes, HGF/SF and MET expression was indiscernible in the media of undamaged carotid arteries but was caused after deep artery damage in areas of SMC migration in the neointima. In addition, strong MET expression was found in the SMCs of the atheromatous arteriosclerotic focuses of homozygous apoE(-/-) mice, meanwhile HGF/SF was expressed by macrophage-derived foam cells. These results showed that MET was caused in migrating and proliferating SMCs and that HGF/SF and MET were key agents of the SMC reaction in atherogenicity[21].

ANTI-APOPTOTIC ACTION OF HGF IN ENDOTHELIAL CELLS

It was focalized that the character of HGF would be a new, element of the angiogenetic proliferators[15,18].

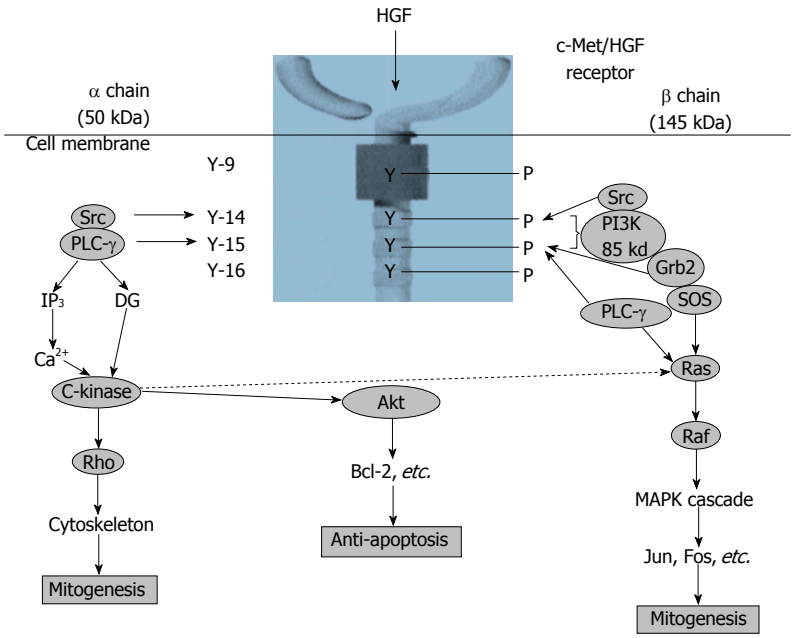

Figure 1 Signal transduction system of hepatocyte growth factor[20]. HGF: Hepatocyte growth factor; PI3K: Phosphatidylinositol 3-kinase; PLC-γ: Phospholipase C-γ; Grb2: Growth factor receptor-bound protein 2; SOS: Son of sevenless.

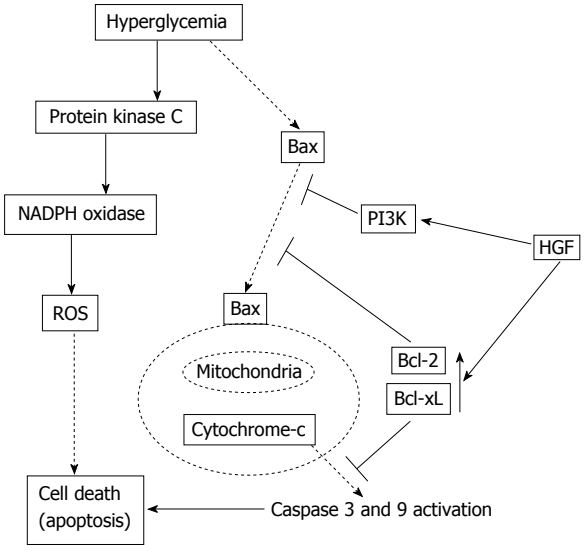

Figure 2 Potential mechanisms of anti-apoptotic effect of hepatocyte growth factor[33]. HGF: Hepatocyte growth factor; PI3K: Phosphatidylinositol 3-kinase; ROS: Reactive oxygen species.

Regional vascular HGF output was reduced by elevated glucose *via* the transforming growth factor-β (TGF-β) activating[22]. It was crucial that genetically modified HGF elevated bcl-2 protein without impacting bax protein and weakened the elevated glucose-caused caspase 3 and 9 activating[23]. The anti-apoptotic effect of HGF by bcl-2 initiation was possibly efficient against not merely elevated glucose conditions, but other stimulus related to activating of the mitochondrial-mediated apoptotic pathway, because HGF weakened caspase 3 activating stimulated by tumor necrosis factor-alpha by the phosphatidylinositol 3-kinase pathway, that was related to Akt activating[24]. These anti-apoptotic effects of HGF are not only unequaled as vascular endothelial growth factor (VEGF) and fibroblast growth factor but also demonstrated such effects. In addition, expression of VEGF and its receptor were reduced in the DM rats myocardia[25], as well as HGF[26]. Nonetheless, an unequalled latent mode of HGF is the capacity of immediate relationship between bcl-2 and MET due to bag-1 protein. The bag-1 protein has been accounted to interplay with the bcl-2 protein and to collaborate with the bcl-2 protein to inhibit apoptosis[27]. Of consequence, the bag-1 protein seems to reduce apoptosis by binding to bcl-2, the raf-1 protein kinase, and MET[28]. Besides, the conjunctive activating of these bcl-2-related genes might take part the apoptosis inhibition by HGF. It has been shown that bcl-2 affects antiapoptotic action by two modes: segregation of the executes of two major caspases-pro-caspase 9 and pro-caspase 8-and suppression of apoptogenic mitochondrial alterations, inclusive of cytochrome c secrete and loss, leading to apoptosis inductive factor secrete from isolated mitochondria[29,30]. In addition, it has been described that HGF could prevent against cell death by the phosphorylation of bad *via* phosphatidylinositol 3-kinase and augment bcl-xL[31], and bax translocation can be modulated by a configurational alteration leading to the exposition of its BH3 domain, and phosphatidylinositol 3-kinase precludes apoptosis by the depression of configurational alteration of the bax BH3 epitope[32]. These findings suggested that vascular endothelical cell death, particularly apoptosis, in hyperglycemia could be weakened by addition of growth factors, which would be potent anti-apoptotic factors (Figure 2)[33].

SERUM HGF CONCENTRATION IN T2DM PATIENTS

The previous studies showed that hyperglycemia reduced

regional HGF output in blood vessel unstriped muscle cells and vascular endothelical cells[22,34], Morishita *et al*[22] postulated that hyperglycemia influences HGF output in diverse apparatuses, such as the renal. If so, the serum level of HGF might be suppressed in DM. In a KKAy mice model of T2DM, the concentration of serum HGF was conspicuously decreased as compared to that in 14 wk of aged control mice[35], while renal and cardiac HGF concentration were remarkably decreased in KKAy mice as compared to those in C57BL mice. In this way, they moreover evaluated their hypothesis in human subjects in order to explore the association between the level of serum HGF and the severeness of T2DM. As supposed, the concentration of serum HGF was remarkably inversely correlated with HbA1c level[35]. In an interesting manner, the concentration of serum HGF in T2DM subjects was remarkably lower than that in non-DM subjects. There was no meaningful divergence in the serum HGF concentration between male and female subjects in either group. It is remarkable that there is a divergence between increased serum HGF in hypertensive (HTN) and decreased serum HGF in T2DM, whereas the tissue HGF levels are decreased in both diseases. The liver, lung and kidney are supposed to be major sources of serum HGF. High blood pressure (BP) in HTN patients does not cause injury to the liver or lung, while high blood glucose is known to influence the liver of such patients. Indeed, activation of serum TGF-β, a strong negative regulator of HGF, has been shown to be increased in T2DM patients[36]. In HTN, on the other hand, because the liver and lung are not injured by high BP, they can secrete HGF into serum in response to HTN damage. It is likely that this difference in the changes of serum HGF level between HTN and T2DM is due to the different influences exerted by high BP and high blood glucose on the major source of circulating HGF. In a contrasting manner, the concentration of serum HGF in T2DM subjects with HTN was markedly more elevated than that in the normal control subjects or that in T2DM subjects with no HTN.

Additionally, the concentration of serum HGF in all T2DM subjects was conspicuously correlated with systolic, but not with diastolic, BP. The concentration of serum HGF in T2DM subjects without HTN complications was markedly more elevated than that in the normal control subjects. The concentration of serum HGF in T2DM subjects with HTN involvements was higher than that in the other subjects. Nishimura *et al*[37] examined the association between the level of serum HGF and proliferative DM retinopathy (PDR), which is characterized by the major characteristic of retinal neovascularization. They found that the serum HGF concentration in T2DM individuals with no DR was more reduced than that in non DM individuals. Serum HGF concentration was elevated in PDR subjects who had not received photocoagulation, but not in those who had received photocoagulation. They concluded that the measurement of serum HGF may be helpful in predicting the presence of PDR in T2DM subjects. Afterwards, they reported that individuals with advanced grades of arteriosclerotic changes had higher serum HGF levels[38]. By contrast, they did not show a positive relationship between HTN and the level of serum HGF. As they included patients treated with antihypertensive drugs, it would be useful to assess the correlation between the level of serum HGF and BP of patients not treated with such drugs. It has also been reported that serum HGF was increased within 3 h after the beginning of pectoralgia in acute myocardial infarction (MI) subjects[39]. Attractively, increased HGF concentrations were conspicuously more common than those of creatine kinase (CK) within 3 h, and the increased level associated well with that of serum CK at 6-9 h after the beginning of acute MI. Therefore, HGF assay is a precise early checkup approach of the presence of arteriosclerotic lesions and acute MI. Serum HGF concentration may be a beneficial biomarker for investigating the cardiovascular disease development.

HGF is a member of the kringle proteins family, distinguished by a triple disulfide loop configuration (kringles) that communicates protein/protein and protein/cell interplay[40]. Consequently, HGF might serve a function in the modulation of thrombi and atheromatous arteriosclerosis. The kringle family to which HGF belongs contains tissue-plasminogen activator (t-PA), plasminogen, apolipoprotein (a) [Lp (a)] and urokinase. The effect of other factors associated with thrombi and atheromatous arteriosclerosis on the serum concentration of HGF was also evaluated, with the outcome that there was no remarkable relationship between the serum concentrations of HGF and total cholesterol. Likewise, the levels of t-PA, plasminogen activator inhibitor 1 and Lp (a) did not demonstrate any relationship with the concentration of serum HGF.

SERUM HGF CONCENTRATION IN T1DM PATIENTS

Nowak *et al*[41] hypothesized that the high level of HGF determined in T1DM subjects might be a significant DR progression biomarker and that the concentration of HGF might be a PDR risk indicator. Average levels of serum HGF in the control subjects were remarkably lower than in the T1DM subjects. They determined a meaning increment in the concentrations of serum HGF in T1DM subjects with PDR in comparison with the control subjects. Average concentrations of serum HGF were conspicuously higher in T1DM subjects with PDR than in T1DM subjects without DR. The concentration of HGF might be elevated in T1DM subjects with PDR, and levels increment with the DR progression, indicating that HGF takes on a role in the etiology of PDR in T1DM patients[41].

HGF AND CI AND CAD

The concentrations of serum HGF are elevated in sub-

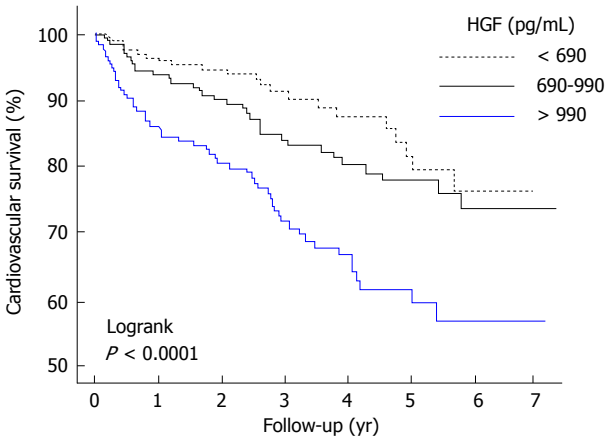

Figure 3 Kaplan-Meier survival curves in accordance with the tertiles of hepatocyte growth factor. The survival curves shows a worsened result for subjects with elevated hepatocyte growth factor (HGF) concentrations[45].

jects with CI and CAD, especially in the acute stage of both damages[39,42]. Besides, Nakamura *et al*[35] have shown that the concentrations of HGF were elevated in T2DM subjects who had HTN involvements such as arteriosclerosis. In addition, it has been described that the serum levels of HGF would be elevated in subjects during the beginning of acute MI and ischemic apoplexy[39,42].

Rajpathak *et al*[43] carried out a nested case-control study to constructively assessed the relationship between plasma HGF and ischemic apoplexy risk within the Women's Health Initiative Observational Study, a cohort of 50 to 79 years aged postmenopausal women. Base line plasma HGF concentrations were associated positively with body mass index (BMI), systolic BP, low-density lipoprotein cholesterol, insulin resistance, and inflammatory markers, such as CRP, and negatively with high-density lipoprotein cholesterol (HDL-C) (all $P < 0.05$). Base line plasma HGF concentrations were more elevated among cases than control subjects (geometric means, 601.8 *vs* 523.2 pg/mL; $P = 0.003$). Circulating plasma HGF levels are correlated with an elevated incidental ischemic apoplexy risk, extraneous to obesity and other cardiovascular disease risk-factors, amongst the 50 to 79 year aged postmenopausal women[43]. The white matter lesions (WML) existence is an essential predictive factor for the apoplexy onset. Increased levels of HGF are correlated with a high T2DM subjects death rate. The BMI was more elevated in the WML-positive subjects than that in the WML-negative subjects. Plasma concentrations of triglycerides were higher while HDL-C was more reduced in the WML-positive subjects than in the WML-negative subjects. Fasting plasma glucose ($P < 0.0001$), insulin levels ($P < 0.0001$), HOMA index ($P < 0.0001$) and HGF ($P < 0.0001$) levels were more elevated in the WML-positive subjects than in the WML-negative subjects. Multiple regression analysis showed that WML was independently prognosticated by the elevated HGF and insulin resistance ($P < 0.0001$ and $P < 0.0001$ respectively). The auxiliary investigation demonstrate that the WML existence was correlated with the increased HGF and insulin resistance in Japanese T2DM subjects[44].

Presently, the utilization of HGF as a biomarker of circulatory system disorder has been in the potent controversy as some reports showed elevated serum HGF level in HF subjects. Lamblin *et al*[45] studied the predictive value of 2 cytokines, HGF and, VEGF in subjects assessed for a decreased left ventricular ejection fraction (LVEF). Nevertheless, elevated concentrations of HGF were powerfully correlated with biomarkers of congestive HF severeness for example more elevated New York Heart Association class and more reduced LVEF, likewise clinical results inclusive of both cardiac and total deathrate (Figure 3). The relationship of HGF with harmful results continued multivariate statistical analysis that integrated latest style of risk-factors for example brain natriuretic peptide (BNP) and peak oxygen consume, a significant stage when evaluating the novel biomarker. Thoroughly, the concentrations of HGF would be more elevated in subjects with a heart trouble [1001 (741-1327) pg/mL] than in the subjects without it [773 (610-1045) pg/mL, $P < 0.000$]. Comparable outcomes would be determined when total deathrate was conceived. The concentrations of HGF would be more elevated in the subjects that deceased of any cause [940 (748-1306) pg/mL] than in subjects that would not. In an important way, the levels of HGF were intensely correlated with age, DM, and all biomarkers of congestive HF severeness. Accordingly, the survival curves suggested a worsened result for subjects with high HGF concentrations. In addition, Lamblin *et al*[46] investigated a first anterior Q-wave MI subjects. It was found that the plasma concentrations of HGF would be positively correlated with left ventricular (LV) volumes, wall motion systolic index, early transmitral velocity to mitral annular early diastolic velocity ratio, and BNP concentrations. Elevated concentrations of HGF would be correlated with more elevated CRP concentrations. Meanwhile, the concentrations of HGF were inversely correlated with LVEF. Multiple regression analysis demonstrated that both CRP and BNP were independently correlated with the concentrations of HGF at 3 and 12 mo. Subjects that deceased or were rehospitalised for HF during follow-up had more elevated concentrations of HGF at 1 mo, 3 mo, and 1 year after MI. Therefore, the circulating concentrations of HGF associated with all markers of LV remodeling after MI and would be correlated with rehospitalization for HF[46].

Susen *et al*[47] investigated the correlation between base line concentrations of the serum angiogenic growth factors, VEGF and HGF, and clinical result in 488 consecutive subjects related to elective percutaneous coronary revascularization (PCR) with no heparin pre-treatment. This primary endpoint, a complex of decease and MI, happened in 44 subjects at a median follow-up of 14.9 mo. At base line, the concentrations of HGF were in relation to CRP concentrations, DM, and late clinical unstability. HGF had a notable positive correlation ($P = 0.003$) with the primary endpoint in the univariate analysis. A same trend was found for VEGF ($P = 0.11$). The only

three variables remarkably correlated with the primary endpoint were HGF (P = 0.004), CRP (P = 0.007), and DM (P = 0.04) in the multivariate Cox model. It is demonstrated that an elevated serum HGF concentration is an independent predictive factor of clinical outcomes during follow-up and is associated with other surrogate markers of the atheromatous arteriosclerosis activeness in subjects, without heparin pre-treatment, related to PCR[47].

HGF would be a magnetic biochemical marker in congested HF subjects therefore it is augmented in the circumstance of cardiac muscle cell apoptosis and active tissue repair, whereby ascertaining patients that are at elevated hazard of harmful clinical results. Nevertheless, based off of obtainable proof, the the heart disorder pathogenesis should be assumed before utilizing HGF as a biochemical marker[48].

HGF AND DM CARDIAC MYOPATHY

The part of HGF/MET signalling in tissue of heart is chiefly attached to ischemic injury and little is recognized about its part in DM cardiac myopathy. Thus HGF brings about the vascular endothelical cells preservation or reparation and reduced serum and tissue concentrations of HGF would be referred for the advance of vascular endothelical cell injury caused by DM[49], the similar would be real for tissue of heart. Generally, elevated HGF would be supposed to be an involvements biomarker. Nevertheless, regional HGF output in blood vessel cells would be presented to be remarkably depressed by elevated D-glucose[50] that indicates reduced regional HGF generation might promote the atheromatous arteriosclerotic blood vessel alterations advance likewise cardiomyocytes damage in DM. Successively, an adaptative increment of HGF in progressed DM might promote the supposition that the levels of serum HGF are increased dependent on diverse apparatus damages.

Nakamura *et al*[49] discovered a serum level of HGF decrement in DM subjects with no HTN but an increment in subjects concerned about both DM likewise arterial HTN. In the latter group, the level of HGF successively elevated with the degree of HTN and it positively associated with systole BP in DM subjects. Furthermore, both clinical and animal experimental result indicated that the serum level of HGF was inversely associated with HbA1c in patients with no involvements, demonstrating that the damage of this vascular endothelical security in line with the DM seriousness. General HGF might affect in anagenesis as a humor intermediator, nevertheless it might be deficient to accelerate anagenesis, due to a decrement in regional HGF generation. Finally, the HGF/MET signalling would play an essential part in heart injury for example DM cardiac myopathy and precise discrimination of this part might ask for a new directions for agent exploitation and to assist better prospective DM care[8].

HGF AND THERAPEUTIC DRUG

Recently, HGF has been shown to be a downstream effector of peroxisome proliferator-activated receptor (PPAR)γ agonists[51]. Sanada *et al*[52,53] demonstrated that HGF exhibited anti-inflammatory and antioxidant effects using HGF transgenic mice. In particular, the fact that HGF has potent antifibrotic effects in both the heart and kidney through blockade of the profibrotic actions induced by angiotensin Ⅱ (Ang Ⅱ) and TGF-β1, and stimulation of degradation of fibrosis *via* matrix metalloproteinase activation is the center of interest[54-56]. In an interesting manner, amongst the accepted angiotensin receptor blockers (ARBs), irbesartan and telmisartan, so-called "metabosartans" [57], were presented to comprise a singular fraction of ARBs that can also be actuating PPARγ[58,59]. Indeed, telmisartan, reduced renal fibrosis and inflammation through the PPARγ-HGF pathway, independently of Ang Ⅱ type 1A receptor (AT1aR) blocking, in a unilateral ureteral obstruction model using AT1aR knockout (AT1aR-KO) mice[60].

Kusunoki *et al*[60] further investigated whether irbesartan has specific-organ protective effects *via* the PPARγ-HGF pathway independent of AT1aR blockade in a mouse fibrosis model, because, in large clinical trials such as the Irbesartan Microalbuminuria Type 2 Diabetes in Hypertensive Patients study and the Irbesartan Type Ⅱ Diabetic Nephropathy Trial, irbesartan demonstrated potent renoprotective effects irrespective of its hypotensive action[61,62].

"Aldosterone breakthrough" found in subjects accepting longterm care with angiotensin blocking is intensely correlated with elevated risk of LV hypertrophy, poor exercise capacity, refractory proteinuria, and decreasing glomerular filtration rate *via* the profibrotic effects of aldosterone. They used salt-sensitive HTN mediated by aldosterone and 1% NaCl infusion in AT1aR-KO mice, as this has been shown to induce severe cardiac fibrosis[63,64]. They demonstrated that irbesartan, which has not merely AT1aR- blockade actions, but PPARγ agonistic actions attended by HGF expression, suppressed organ injury by aldosterone and salt treatment[65]. Second-generation ARBs such as irbesartan, which has the double effects of AT1aR blocking and PPARγ activating, may have clinical merit for the care of HTN subjects with aldosterone breakthrough.

Calcium channel blockers are accounted to have protecting actions on the vascular endothelia *in vivo* and *in vitro*. Notably, nifedipine, amongst numerous calcium channel blockers, was demonstrated to ameliorate vascular endothelical damage in HTN subjects. Yamasaki *et al*[66] investigated the immediate actions of nifedipine on smoke-caused vascular endothelical damage, because tobacco use *per se* is a principal factor in vascular endothelical cells dysfunction, likewise HTN. They studied whether nifedipine would ameliorate endothelial action in 10 normotensive tobacco users with no atheromatous arteriosclerotic risk-factors. Nifedipine did not influence BP and cardiac rate of normotensive tobacco users. They determined forearm blood flow (FBF) by strain-gauge plethysmography after 2 and 4 wk of therapy. Alterations in vasorelaxant reaction to responsive hyperemia were

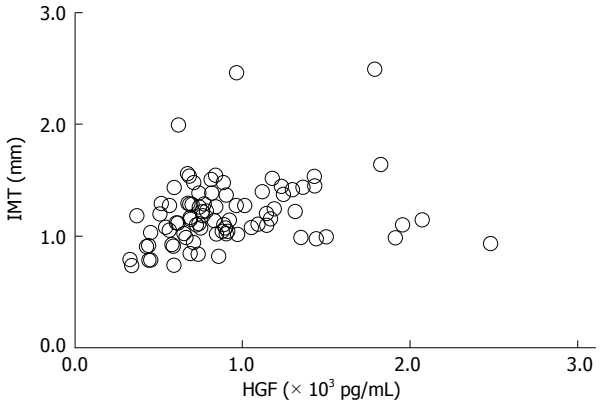

Figure 4 Relationship between serum hepatocyte growth factor and intimal-media thickness in type 2 diabetes mellitus subjects (r = 0.24, P = 0.0248)[69]. HGF: Hepatocyte growth factor.

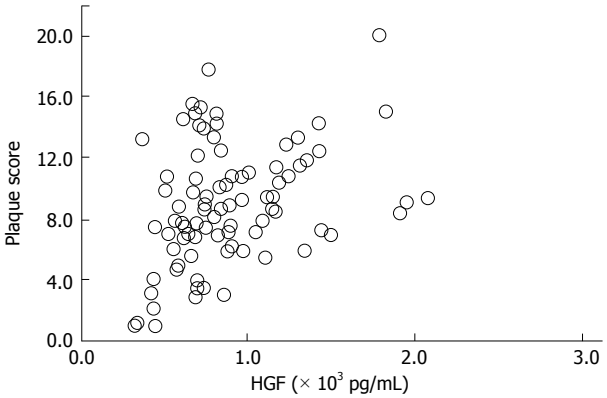

Figure 5 Relationship between serum hepatocyte growth factor and plaque score in type 2 diabetes mellitus subjects (r = 0.27, P = 0.0126)[69]. HGF: Hepatocyte growth factor.

conspicuously ameliorated in nifedipine-treated patients ($P < 0.05$), meanwhile there was no remarkable alteration in FBF reaction in controls. Furthermore, to investigate the machinery of the immediate actions of nifedipine on the endothelium, they focalized HGF, that is a new angiogenic growth factor with an antiapoptotic effect on vascular endothelical cells. Intriguingly, the serum level of HGF in tobacco users cured with nifedipine was markedly increased both at 2 and 4 wk ($P < 0.05$). Generally, these consequences indicated immediate actions of nifedipine in the endothelial damage amelioration in normotensive tobacco users. The increment in the serum level of HGF by nifedipine might bring about the vascular endothelical damage amelioration[66].

Makino *et al*[67] examined the action of calcium antagonist, benidipine, on endothelial mechanism in the essential HTN subjects, which induces endothelial damage. BP was decreased markedly. Endothelial mechanism was investigated applying FBF by strain-gauge plethysmography after 8 wk of therapy. Alterations in vasodilator reaction to responsive engorgement were notably ameliorated ($P < 0.01$), meanwhile the reaction to nitroglycerin was not altered, presenting the amelioration of endothelial mechanism. The level of serum HGF in patients cured with benidipine was grossly increased at 8 wk ($P < 0.05$). Intriguingly, an increment in the level of serum HGF by benidipine might bring about the amelioration of endothelial damage[67].

Takahashi *et al*[68] investigated whether lipid-lowering therapy (LLT) with statins would influence the leptin and angiogenic factors concentrations in CAD subjects. CAD subjects were randomised to 6 mo of intensive LLT with atorvastatin or moderate LLT with pravastatin. The plasma concentrations of leptin, Ang Ⅱ, HGF and VEGF were determined before statin treatment (baseline) and after 6 mo. Base line concentrations of leptin, Ang Ⅱ, HGF and VEGF were more elevated in the CAD subjects than in the non-CAD subjects (all $P < 0.05$). Intensive LLT reduced the concentrations of leptin, Ang Ⅱ, HGF and VEGF, while moderate LLT did not alter these concentrations. Their result displayed that LLT with atorvastatin reduces the leptin and angiogenic factors (HGF, VEGF) concentrations in CAD subjects, conceivably bringing about the favorable actions of LLT with atorvastatin in CAD[68].

HGF AND CA IN PATIENTS WITH T2DM

We conducted a clinical research to investigate the correlation between the serum HGF concentrations and the stage of CA in T2DM subjects[69]. The average level of serum HGF of T2DM patients in this clinical research was 895 +/- 408 pg/mL, a level notably more elevated than the reference values. The serum concentrations of HGF associated positively with both intimal-media thickness (IMT) (r = 0.24, P = 0.0248) and plaque score (PS) (r = 0.27, P = 0.0126) (Figures 4 and 5), indicating a correlation between the elevated HGF concentrations and development of atherosclerotic involvements.

Indeed, this was the first report presenting a notable relationship between the serum HGF concentrations and IMT and PS in T2DM subjects. Nevertheless, we failed to demonstrate a marked association between the concentrations of serum HGF and HbA1c. The clinical study outcome means the serum concentrations of HGF would be a beneficial biomarker of CA in T2DM subjects that is extraneous to entire glycemic control. Morishita *et al*[22] showed the elevated concentrations of glucose decreased the generation of HGF by vascular endothelical cells, conceivably as an outcome of apoptosis, in an in vitro study. In addition, these authors have indicated a inverse association between HGF and HbA1c in DM subjects without involvements[35]. Furthermore, the DECODE study presented that hyperglycemia after meal had an atherosclerotic action in T2DM subjects and impaired glucose tolerance subjects[70]. Collectively, these outcomes indicate additional investigations are certified to reveal the immediate or nonimmediate actions of control the level of blood glucose on both serum concentrations of HGF and the atherosclerotic involvements correlated with T2DM. It is indicated that these investigations should introduce supplemental scales like 1,5-an-

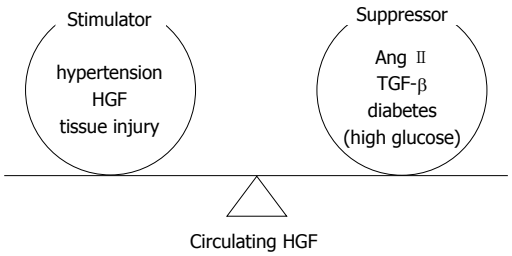

Figure 6 Determination of circulating hepatocyte growth factor level by the balance between stimulators and suppressors[19]. HGF: Hepatocyte growth factor; TGF: Transforming growth factor.

hydroglucitol and glucose level after meal. Nevertheless, using multivariate statistical analyses, we indicated that a positive relationship between the serum concentrations of HGF and IMT (standardized $\beta = 0.28$, $P = 0.0499$), we could not demonstrate any correlation between the serum concentrations of HGF and PS. The PS in the common carotid arteries (CCA) is considered as an indication of regional proliferating damages in large arteries, for instance atheromatic plaques. Since IMT and PS have discrete pathologic importance, we showed that serum HGF is a precise and characteristic biomarker for general endothelial cells proliferation. Although elevated serum concentrations of HGF would have been accounted in HTN subjects with DM[19], we could not show that the relationship was discovered between HGF and systolic BP. Contrarily, both IMT and PS associated positively with systolic BP. These outcomes would show that the concentrations of serum HGF might not be influenced by HTN intrinsically but might elevate as a secondary reaction to endothelial dysfunction that could occur during atherosclerotic progress. Hyperlipidemia, hyperglycemia and tobacco use are authenticated carotid atherosclerotic risk factors. In spite of these intense relationships, we could not show a correlation between the serum HGF concentrations and these three factors. It is potential that no correlation between serum HGF concentrations and hyperlipidemia and tobacco use was this result of the subject group not being classified in line with medical care with oral dyslipidemia therapeutic drugs, such as statin or tobacco use habit disturbance[71]. Many investigations have shown that the atherosclerotic progress in the CCA is a risk-factor for CI or MI[72,73]. IMT in those lacunar stroke subjects was not notably higher than in the no lacunar stroke subjects in our study. Contrastingly, PS in the lacunar stroke subject group was notably higher than in the no lacunar stroke group. It is demonstrated that PS is relevant to the lacunar stroke count[72], with Matsumori *et al*[42] also indicating the serum concentrations of HGF are elevated in CI subjects, especially in the preterm ischemic attack. It was discovered that both PS and IMT in ischemic heart disease (IHD) subjects would be notably more elevated than in those with no IHD. The relationship between CA and IHD has been accounted formerly[73] and moreover, it has been demonstrated that the serum levels of HGF would be elevated in acute MI subjects[39]. Our study of T2DM patients has indicated a positive relationship between the serum level of HGF and IMT and PS of the CCA. Additionally, IMT and PS would be ascertained as risk-factors for general atheromatous arteriosclerosis in both CI and CAD[69].

CONCLUSION

Actually, the serum level of HGF in DM subjects might be specified by balancing of stimulators (HTN, atheromatous arteriosclerosis, *etc*.) and suppressor (hyperglycemia, TGF-, Ang Ⅱ, *etc*.) (Figure 6)[8,19]. Accordingly, the increase of the serum HGF level might be regarded as an indicator of the DM involvements severeness. Therefore, serum concentration of HGF might be a beneficial biomarker of macroangiopathy in DM subjects.

REFERENCES

1 **International Diabetes Association**. The global burden, Diabetes and impaired glucose tolerance. IDF Diabetes Atlas Sixth edition, 2013: 32-34
2 **International Diabetes Association**. The global burden, Mortality. IDF Diabetes Atlas Sixth edition, 2013: 46-47
3 **International Diabetes Association**. The global burden, Health expenditure. IDF Diabetes Atlas Sixth edition, 2013: 48-49
4 **Roglic G**, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S, Connolly V, King H. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. *Diabetes Care* 2005; **28**: 2130-2135 [PMID: 16123478 DOI: 10.2337/diacare.28.9.2130]
5 **Bell DS**. Diabetic cardiomyopathy. *Diabetes Care* 2003; **26**: 2949-2951 [PMID: 14514607 DOI: 10.2337/diacare.26.10.2949]
6 **Kiencke S**, Handschin R, von Dahlen R, Muser J, Brunner-Larocca HP, Schumann J, Felix B, Berneis K, Rickenbacher P. Pre-clinical diabetic cardiomyopathy: prevalence, screening, and outcome. *Eur J Heart Fail* 2010; **12**: 951-957 [PMID: 20581103 DOI: 10.1093/eurjhf/hfq110]
7 **Parving HH**, Tarnow L, Rossing P. Genetics of diabetic nephropathy. *J Am Soc Nephrol* 1996; **7**: 2509-2517 [PMID: 8989728]
8 **Klimas J**. Potential target molecules in diabetic cardiomyopathy: hepatocyte growth factor (HGF) and ryanodine receptor 2 (RyR2). In: Milei J, Ambrosio J Cardiomyopathies. Rijeka: InTechOpen, 2013: 243-263 [DOI: 10.5772/55780]
9 **Folsom AR**, Chambless LE, Duncan BB, Gilbert AC, Pankow JS. Prediction of coronary heart disease in middle-aged adults with diabetes. *Diabetes Care* 2003; **26**: 2777-2784 [PMID: 14514579 DOI: 10.2337/diacare.26.10.2777]
10 **Shinoda-Tagawa T**, Yamasaki Y, Yoshida S, Kajimoto Y, Tsujino T, Hakui N, Matsumoto M, Hori M. A phosphodiesterase inhibitor, cilostazol, prevents the onset of silent brain infarction in Japanese subjects with Type II diabetes. *Diabetologia* 2002; **45**: 188-194 [PMID: 11935149 DOI: 10.1007/s00125-001-0740-2]
11 **Aso Y**, Okumura K, Takebayashi K, Wakabayashi S, Inukai T. Relationships of plasma interleukin-18 concentrations to hyperhomocysteinemia and carotid intimal-media wall thickness in patients with type 2 diabetes. *Diabetes Care* 2003; **26**: 2622-2627 [PMID: 12941729 DOI: 10.2337/diacare.26.9.2622]
12 **Ridker PM**, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998; **98**: 731-733 [PMID: 9727541]
13 **Aslan D**. Biomarkers for diabetic complications: The results of several clinical studies. *J Med Biochem* 2011; **30**: 207-212 [DOI: 10.2478/v10011-011-0024-4]
14 **Van Belle E**, Witzenbichler B, Chen D, Silver M, Chang L, Schwall R, Isner JM. Potentiated angiogenic effect of scatter factor/hepatocyte growth factor via induction of vascular

endothelial growth factor: the case for paracrine amplification of angiogenesis. *Circulation* 1998; **97**: 381-390 [PMID: 9468212 DOI: 10.1161/01.CIR.97.4.381]

15 **Nakamura Y**, Morishita R, Higaki J, Kida I, Aoki M, Moriguchi A, Yamada K, Hayashi S, Yo Y, Nakano H, Matsumoto K, Nakamura T, Ogihara T. Hepatocyte growth factor is a novel member of the endothelium-specific growth factors: additive stimulatory effect of hepatocyte growth factor with basic fibroblast growth factor but not with vascular endothelial growth factor. *J Hypertens* 1996; **14**: 1067-1072 [PMID: 8986905]

16 **Nakamura Y**, Morishita R, Higaki J, Kida I, Aoki M, Moriguchi A, Yamada K, Hayashi S, Yo Y, Matsumoto K. Expression of local hepatocyte growth factor system in vascular tissues. *Biochem Biophys Res Commun* 1995; **215**: 483-488 [PMID: 7487981 DOI: 10.1006/bbrc.1995.2490]

17 **Bussolino F**, Di Renzo MF, Ziche M, Bocchietto E, Olivero M, Naldini L, Gaudino G, Tamagnone L, Coffer A, Comoglio PM. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. *J Cell Biol* 1992; **119**: 629-641 [PMID: 1383237]

18 **Nakamura Y**, Morishita R, Nakamura S, Aoki M, Moriguchi A, Matsumoto K, Nakamura T, Higaki J, Ogihara T. A vascular modulator, hepatocyte growth factor, is associated with systolic pressure. *Hypertension* 1996; **28**: 409-413 [PMID: 8794825 DOI: 10.1161/01.HYP.28.3.409]

19 **Morishita R**, Moriguchi A, Higaki J, Ogihara T. Hepatocyte growth factor (HGF) as a potential index of severity of hypertension. *Hypertens Res* 1999; **22**: 161-167 [PMID: 10515437 DOI: 10.1291/hypres.22.161]

20 **Morishita R**, Aoki M, Yo Y, Ogihara T. Hepatocyte growth factor as cardiovascular hormone: role of HGF in the pathogenesis of cardiovascular disease. *Endocr J* 2002; **49**: 273-284 [PMID: 12201209 DOI: 10.1507/endocrj.49.273]

21 **McKinnon H**, Gherardi E, Reidy M, Bowyer D. Hepatocyte growth factor/scatter factor and MET are involved in arterial repair and atherogenesis. *Am J Pathol* 2006; **168**: 340-348 [PMID: 16400035 DOI: 10.2353/ajpath.2006.050379]

22 **Morishita R**, Nakamura S, Nakamura Y, Aoki M, Moriguchi A, Kida I, Yo Y, Matsumoto K, Nakamura T, Higaki J, Ogihara T. Potential role of an endothelium-specific growth factor, hepatocyte growth factor, on endothelial damage in diabetes. *Diabetes* 1997; **46**: 138-142 [PMID: 8971094]

23 **Nakagami H**, Morishita R, Yamamoto K, Taniyama Y, Aoki M, Yamasaki K, Matsumoto K, Nakamura T, Kaneda Y, Ogihara T. Hepatocyte growth factor prevents endothelial cell death through inhibition of bax translocation from cytosol to mitochondrial membrane. *Diabetes* 2002; **51**: 2604-2611 [PMID: 12145177 DOI: 10.2337/diabetes.51.8.2604]

24 **Nakagami H**, Morishita R, Yamamoto K, Taniyama Y, Aoki M, Matsumoto K, Nakamura T, Kaneda Y, Horiuchi M, Ogihara T. Mitogenic and antiapoptotic actions of hepatocyte growth factor through ERK, STAT3, and AKT in endothelial cells. *Hypertension* 2001; **37**: 581-586 [PMID: 11230338]

25 **Chou E**, Suzuma I, Way KJ, Opland D, Clermont AC, Naruse K, Suzuma K, Bowling NL, Vlahos CJ, Aiello LP, King GL. Decreased cardiac expression of vascular endothelial growth factor and its receptors in insulin-resistant and diabetic States: a possible explanation for impaired collateral formation in cardiac tissue. *Circulation* 2002; **105**: 373-379 [PMID: 11804995 DOI: 10.1161/hc0302.102143]

26 **Taniyama Y**, Morishita R, Hiraoka K, Aoki M, Nakagami H, Yamasaki K, Matsumoto K, Nakamura T, Kaneda Y, Ogihara T. Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat diabetic hind limb ischemia model: molecular mechanisms of delayed angiogenesis in diabetes. *Circulation* 2001; **104**: 2344-2350 [PMID: 11696476 DOI: 10.1161/hc4401.098470]

27 **Takayama S**, Sato T, Krajewski S, Kochel K, Irie S, Millan JA, Reed JC. Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. *Cell* 1995; **80**: 279-284 [PMID: 7834747 DOI: 10.1016/0092-8674(95)90410-7]

28 **Bardelli A**, Longati P, Albero D, Goruppi S, Schneider C, Ponzetto C, Comoglio PM. HGF receptor associates with the anti-apoptotic protein BAG-1 and prevents cell death. *EMBO J* 1996; **15**: 6205-6212 [PMID: 8947043]

29 **Jürgensmeier JM**, Xie Z, Deveraux Q, Ellerby L, Bredesen D, Reed JC. Bax directly induces release of cytochrome c from isolated mitochondria. *Proc Natl Acad Sci USA* 1998; **95**: 4997-5002 [PMID: 9560217]

30 **Narita M**, Shimizu S, Ito T, Chittenden T, Lutz RJ, Matsuda H, Tsujimoto Y. Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria. *Proc Natl Acad Sci USA* 1998; **95**: 14681-14686 [PMID: 9843949]

31 **Liu Y**. Hepatocyte growth factor promotes renal epithelial cell survival by dual mechanisms. *Am J Physiol* 1999; **277**: F624-F633 [PMID: 10516287]

32 **Gilmore AP**, Metcalfe AD, Romer LH, Streuli CH. Integrin-mediated survival signals regulate the apoptotic function of Bax through its conformation and subcellular localization. *J Cell Biol* 2000; **149**: 431-446 [PMID: 10769034 DOI: 10.1083/jcb.149.2.431]

33 **Nakagami H**, Kaneda Y, Ogihara T, Morishita R. Endothelial dysfunction in hyperglycemia as a trigger of atherosclerosis. *Curr Diabetes Rev* 2005; **1**: 59-63 [PMID: 18220582 DOI: 10.2174/1573399052952550]

34 **Araki S**, Shimada Y, Kaji K, Hayashi H. Apoptosis of vascular endothelial cells by fibroblast growth factor deprivation. *Biochem Biophys Res Commun* 1990; **168**: 1194-1200 [PMID: 2346483 DOI: 10.1016/0006-291X(90)91155-L]

35 **Nakamura S**, Morishita R, Moriguchi A, Yo Y, Nakamura Y, Hayashi S, Matsumoto K, Matsumoto K, Nakamura T, Higaki J, Ogihara T. Hepatocyte growth factor as a potential index of complication in diabetes mellitus. *J Hypertens* 1998; **16**: 2019-2026 [PMID: 9886892]

36 **Pfeiffer A**, Middelberg-Bisping K, Drewes C, Schatz H. Elevated plasma levels of transforming growth factor-beta 1 in NIDDM. *Diabetes Care* 1996; **19**: 1113-1117 [PMID: 8886558]

37 **Nishimura M**, Nakano K, Ushiyama M, Nanbu A, Ohtsuka K, Takahashi H, Yoshimura M. Increased serum concentrations of human hepatocyte growth factor in proliferative diabetic retinopathy. *J Clin Endocrinol Metab* 1998; **83**: 195-198 [PMID: 9435440]

38 **Nishimura M**, Ushiyama M, Nanbu A, Ohtsuka K, Takahashi H, Yoshimura M. Serum hepatocyte growth factor as a possible indicator of arteriosclerosis. *J Hypertens* 1997; **15**: 1137-1142 [PMID: 9350587]

39 **Matsumori A**, Furukawa Y, Hashimoto T, Ono K, Shioi T, Okada M, Iwasaki A, Nishio R, Sasayama S. Increased circulating hepatocyte growth factor in the early stage of acute myocardial infarction. *Biochem Biophys Res Commun* 1996; **221**: 391-395 [PMID: 8619866 DOI: 10.1006/bbrc.1996.0606]

40 **Nakano N**, Moriguchi A, Morishita R, Kida I, Tomita N, Matsumoto K, Nakamura T, Higaki J, Ogihara T. Role of angiotensin II in the regulation of a novel vascular modulator, hepatocyte growth factor (HGF), in experimental hypertensive rats. *Hypertension* 1997; **30**: 1448-1454 [PMID: 9403566]

41 **Nowak M**, Wielkoszyński T, Marek B, Kos-Kudła B, Swietochowska E, Siemińska L, Kajdaniuk D, Głogowska-Szelag J, Nowak K. A comparison of the levels of hepatocyte growth factor in serum in patients with type 1 diabetes mellitus with different stages of diabetic retinopathy. *Endokrynol Pol* 2008; **59**: 2-5 [PMID: 18335393]

42 **Matsumori A**, Takano H, Obata JE, Takeda S, Tsuyuguchi N, Ono K, Okada M, Miyamoto T, Ohnishi T, Daikuhara Y, Sasayama S. Circulating hepatocyte growth factor as a diagnostic marker of thrombus formation in patients with cerebral infarction. *Circ J* 2002; **66**: 216-218 [PMID: 11999653 DOI:

10.1253/circj.66.216]
43 **Rajpathak SN**, Wang T, Wassertheil-Smoller S, Strickler HD, Kaplan RC, McGinn AP, Wildman RP, Rosenbaum D, Rohan TE, Scherer PE, Cushman M, Ho GY. Hepatocyte growth factor and the risk of ischemic stroke developing among postmenopausal women: results from the Women's Health Initiative. *Stroke* 2010; **41**: 857-862 [PMID: 20203323]
44 **Anan F**, Masaki T, Jikumaru K, Iwao T, Eshima N, Saikawa T, Yoshimatsu H. Hepatocyte growth factor is a significant risk factor for white matter lesions in Japanese type 2 diabetic patients. *Eur J Clin Invest* 2010; **40**: 585-590 [PMID: 20497462 DOI: 10.1111/j.1365-2362.2010.02301.x]
45 **Lamblin N**, Susen S, Dagorn J, Mouquet F, Jude B, Van Belle E, Bauters C, de Groote P. Prognostic significance of circulating levels of angiogenic cytokines in patients with congestive heart failure. *Am Heart J* 2005; **150**: 137-143 [PMID: 16084160 DOI: 10.1016/j.ahj.2005.03.047]
46 **Lamblin N**, Bauters A, Fertin M, de Groote P, Pinet F, Bauters C. Circulating levels of hepatocyte growth factor and left ventricular remodelling after acute myocardial infarction (from the REVE-2 study). *Eur J Heart Fail* 2011; **13**: 1314-1322 [PMID: 21996026 DOI: 10.1093/eurjhf/hfr137]
47 **Susen S**, Sautière K, Mouquet F, Cuilleret F, Chmaït A, McFadden EP, Hennache B, Richard F, de Groote P, Lablanche JM, Dallongeville J, Bauters C, Jude B, Van Belle E. Serum hepatocyte growth factor levels predict long-term clinical outcome after percutaneous coronary revascularization. *Eur Heart J* 2005; **26**: 2387-2395 [PMID: 16105849 DOI: 10.1093/eurheartj/ehi436]
48 **Fountoulaki K**, Parissis J. Hepatocyte Growth Factor as a Prognostic Marker in Heart Failure: Promise and Challenges. *Cardiology* 2012; **121**: 237-239 [PMID: 22555339 DOI: 10.1159/000338158]
49 **Nakamura S**, Moriguchi A, Morishita R, Aoki M, Yo Y, Hayashi S, Nakano N, Katsuya T, Nakata S, Takami S, Matsumoto K, Nakamura T, Higaki J, Ogihara T. A novel vascular modulator, hepatocyte growth factor (HGF), as a potential index of the severity of hypertension. *Biochem Biophys Res Commun* 1998; **242**: 238-243 [PMID: 9439642 DOI: 10.1006/bbrc.1997.7800]
50 **Morishita R**, Aoki M, Hashiya N, Yamasaki K, Kurinami H, Shimizu S, Makino H, Takesya Y, Azuma J, Ogihara T. Therapeutic angiogenesis using hepatocyte growth factor (HGF). *Curr Gene Ther* 2004; **4**: 199-206 [PMID: 15180586 DOI: 10.2174/1566523043346453]
51 **Li Y**, Wen X, Spataro BC, Hu K, Dai C, Liu Y. hepatocyte growth factor is a downstream effector that mediates the antifibrotic action of peroxisome proliferator-activated receptor-gamma agonists. *J Am Soc Nephrol* 2006; **17**: 54-65 [PMID: 16291834 DOI: 10.1681/ASN.2005030257]
52 **Sanada F**, Taniyama Y, Azuma J, Iekushi K, Dosaka N, Yokoi T, Koibuchi N, Kusunoki H, Aizawa Y, Morishita R. Hepatocyte growth factor, but not vascular endothelial growth factor, attenuates angiotensin II-induced endothelial progenitor cell senescence. *Hypertension* 2009; **53**: 77-82 [PMID: 19047582]
53 **Sanada F**, Taniyama Y, Iekushi K, Azuma J, Okayama K, Kusunoki H, Koibuchi N, Doi T, Aizawa Y, Morishita R. Negative action of hepatocyte growth factor/c-Met system on angiotensin II signaling via ligand-dependent epithelial growth factor receptor degradation mechanism in vascular smooth muscle cells. *Circ Res* 2009; **105**: 667-75, 13 p following 675 [PMID: 19713535 DOI: 10.1161/CIRCRESAHA.109.202713]
54 **Iekushi K**, Taniyama Y, Azuma J, Sanada F, Kusunoki H, Yokoi T, Koibuchi N, Okayama K, Rakugi H, Morishita R. Hepatocyte growth factor attenuates renal fibrosis through TGF-β1 suppression by apoptosis of myofibroblasts. *J Hypertens* 2010; **28**: 2454-2461 [PMID: 20842048 DOI: 10.1097/HJH.0b013e32833e4149]
55 **Iekushi K**, Taniyama Y, Kusunoki H, Azuma J, Sanada F, Okayama K, Koibuchi N, Iwabayashi M, Rakugi H, Morishita R. Hepatocyte growth factor attenuates transforming growth factor-β-angiotensin II crosstalk through inhibition of the PTEN/Akt pathway. *Hypertension* 2011; **58**: 190-196 [PMID: 21670418 DOI: 10.1161/HYPERTENSIONAHA.111.173013]
56 **Okayama K**, Azuma J, Dosaka N, Iekushi K, Sanada F, Kusunoki H, Iwabayashi M, Rakugi H, Taniyama Y, Morishita R. Hepatocyte growth factor reduces cardiac fibrosis by inhibiting endothelial-mesenchymal transition. *Hypertension* 2012; **59**: 958-965 [PMID: 22392903 DOI: 10.1161/HYPERTENSIONAHA.111.183905]
57 **Nakagami H**, Kiomy Osako M, Nakagami F, Shimosato T, Minobe N, Moritani T, Shimamura M, Miyake T, Shimizu H, Takeya Y, Morishita R. Prevention and regression of non-alcoholic steatohepatitis (NASH) in a rat model by metabosartan, telmisartan. *Int J Mol Med* 2010; **26**: 477-481 [PMID: 20818485 DOI: 10.3892/ijmm_00000488]
58 **Benson SC**, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M, Qi N, Wang J, Avery MA, Kurtz TW. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension* 2004; **43**: 993-1002 [PMID: 15007034 DOI: 10.1161/01.HYP.0000123072.34629.57]
59 **Schupp M**, Janke J, Clasen R, Unger T, Kintscher U. Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-gamma activity. *Circulation* 2004; **109**: 2054-2057 [PMID: 15117841 DOI: 10.1161/01.CIR.0000127955.36250.65]
60 **Kusunoki H**, Taniyama Y, Azuma J, Iekushi K, Sanada F, Otsu R, Iwabayashi M, Okayama K, Rakugi H, Morishita R. Telmisartan exerts renoprotective actions via peroxisome proliferator-activated receptor-γ/hepatocyte growth factor pathway independent of angiotensin II type 1 receptor blockade. *Hypertension* 2012; **59**: 308-316 [PMID: 22252391 DOI: 10.1161/HYPERTENSIONAHA.111.176263]
61 **Parving HH**, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 2001; **345**: 870-878 [PMID: 11565519 DOI: 10.1056/NEJMoa011489]
62 **Lewis EJ**, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 2001; **345**: 851-860 [PMID: 11565517 DOI: 10.1056/NEJMoa011303]
63 **Kagiyama S**, Matsumura K, Fukuhara M, Sakagami K, Fujii K, Iida M. Aldosterone-and-salt-induced cardiac fibrosis is independent from angiotensin II type 1a receptor signaling in mice. *Hypertens Res* 2007; **30**: 979-989 [PMID: 18049031 DOI: 10.1291/hypres.30.979]
64 **Kagiyama S**, Matsumura K, Goto K, Otsubo T, Iida M. Role of Rho kinase and oxidative stress in cardiac fibrosis induced by aldosterone and salt in angiotensin type 1a receptor knockout mice. *Regul Pept* 2010; **160**: 133-139 [PMID: 19969025 DOI: 10.1016/j.regpep.2009.12.002]
65 **Kusunoki H**, Taniyama Y, Rakugi H, Morishita R. Cardiac and renal protective effects of irbesartan via peroxisome proliferator-activated receptorγ-hepatocyte growth factor pathway independent of angiotensin II Type 1a receptor blockade in mouse model of salt-sensitive hypertension. *J Am Heart Assoc* 2013; **2**: e000103 [PMID: 23608606]
66 **Yamasaki K**, Aoki M, Makino H, Hashiya N, Shimizu H, Ohishi M, Ogihara T, Morishita R. Effect of nifedipine on endothelial function in normotensive smokers: potential contribution of increase in circulating hepatocyte growth factor. *J Hum Hypertens* 2004; **18**: 701-705 [PMID: 15071489 DOI: 10.1038/sj.jhh.1001727]
67 **Makino H**, Aoki M, Hashiya N, Yamasaki K, Shimizu H, Miwa K, Ogihara T, Morishita R. A calcium-channel blocker, benidipine, improves forearm reactive hyperemia in patients

with essential hypertension. *Blood Press Suppl* 2005; **1**: 39-44 [PMID: 16060415 DOI: 10.1080/08038020510040612]

68 **Takahashi Y**, Satoh M, Tabuchi T, Nakamura M. Prospective, randomized, single-blind comparison of effects of 6 months' treatment with atorvastatin versus pravastatin on leptin and angiogenic factors in patients with coronary artery disease. *Heart Vessels* 2012; **27**: 337-343 [PMID: 21643812 DOI: 10.1007/s00380-011-0156-y]

69 **Satani K**, Konya H, Hamaguchi T, Umehara A, Katsuno T, Ishikawa T, Kohri K, Hasegawa Y, Suehiro A, Kakishita E, Namba M. Clinical significance of circulating hepatocyte growth factor, a new risk marker of carotid atherosclerosis in patients with Type 2 diabetes. *Diabet Med* 2006; **23**: 617-622 [PMID: 16759302 DOI: 10.1111/j.1464-5491.2006.01849.x]

70 Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. The DECODE study group. European Diabetes Epidemiology Group. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe. *Lancet* 1999; **354**: 617-621 [PMID: 10466661 DOI: 10.1016/S0140-6736(98)12131-1]

71 **Taniwaki H**, Kawagishi T, Emoto M, Shoji T, Kanda H, Maekawa K, Nishizawa Y, Morii H. Correlation between the intima-media thickness of the carotid artery and aortic pulse-wave velocity in patients with type 2 diabetes. Vessel wall properties in type 2 diabetes. *Diabetes Care* 1999; **22**: 1851-1857 [PMID: 10546019 DOI: 10.2337/diacare.22.11.1851]

72 **Hollander M**, Bots ML, Del Sol AI, Koudstaal PJ, Witteman JC, Grobbee DE, Hofman A, Breteler MM. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly: the Rotterdam study. *Circulation* 2002; **105**: 2872-2877 [PMID: 12070116]

73 **Mattace-Raso F**, van Popele NM, Schalekamp MA, van der Cammen TJ. Intima-media thickness of the common carotid arteries is related to coronary atherosclerosis and left ventricular hypertrophy in older adults. *Angiology* 2002; **53**: 569-574 [PMID: 12365865 DOI: 10.1177/000331970205300511]

P- Reviewer: Gómez-Sáez J, Lysy PAG **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.689

World J Diabetes 2014 October 15; 5(5): 689-696
ISSN 1948-9358 (online)

REVIEW

Advances in management of type 1 diabetes mellitus

Ravindranath Aathira, Vandana Jain

Ravindranath Aathira, Vandana Jain, Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India
Author contributions: Aathira R did the literature search and reviewed the relevant articles to contruct the body of the document; Jain V guided through the whole process and edited the final document.
Correspondence to: Vandana Jain, Additional Professor, Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India. drvandanajain@gmail.com
Telephone: +91-981-0167265 Fax: +91-011-26588663
Received: May 13, 2014 Revised: June 18, 2014
Accepted: July 17, 2014
Published online: October 15, 2014

Abstract

Treatment of type 1 diabetes mellitus has always posed a challenge to balance hyperglycemia control with hypoglycemia episodes. The quest for newer therapies is continuing and this review attempts to outline the recent developments. The insulin molecule itself has got moulded into different analogues by minor changes in its structure to ensure well controlled delivery, stable half-lives and lesser side effects. Insulin delivery systems have also consistently undergone advances from subcutaneous injections to continuous infusion to trials of inhalational delivery. Continuous glucose monitoring systems are also becoming more accurate and user friendly. Smartphones have also made their entry into therapy of diabetes by integrating blood glucose levels and food intake with calculated adequate insulin required. Artificial pancreas has enabled to a certain extent to close the loop between blood glucose level and insulin delivery with devices armed with meal and exercise announcements, dual hormone delivery and pramlintide infusion. Islet, pancreas-kidney and stem cells transplants are also being attempted though complete success is still a far way off. Incorporating insulin gene and secretary apparatus is another ambitious leap to achieve insulin independence though the search for the ideal vector and target cell is still continuing. Finally to stand up to the statement, prevention is better than cure, immunological methods are being investigated to be used as vaccine to prevent the onset of diabetes mellitus.

Key words: Type 1 diabetes advances; Insulin analogues; Closed loop system; Continuous glucose monitors; Insulin gene therapy

Core tip: As therapy of type 1 diabetes poses important challenges because of life long insulin dependence,multiple injections, excursions in glucose values and inability to simulate the pancreas, newer modalities of therapy are emerging. Hence, this is the right time to review developments in this front. This review conjures up recent advances in continuous glucose monitors, closed loop systems, insulin analogues, insulin gene therapy, transplantation and immunological vaccination.

Aathira R, Jain V. Advances in management of type 1 diabetes mellitus. *World J Diabetes* 2014; 5(5): 689-696 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/689.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.689

INTRODUCTION

The year 1923 is a watershed in the history of diabetes mellitus when insulin was discovered by Banting and Best[1]. Today the world has come a long way from that, but living with type 1 diabetes still remains akin to a tight rope walk, balancing between hyperglycemia and hypoglycemic episodes. Multiple injections, strict control on food and exercise are herculean tasks to deal with, especially in children. Hence, the need for better therapies is warranted and they have thus evolved from nascent stages to actual usage.

The incidence of type 1 diabetes varies among different countries, which reflects the roles played by genetic

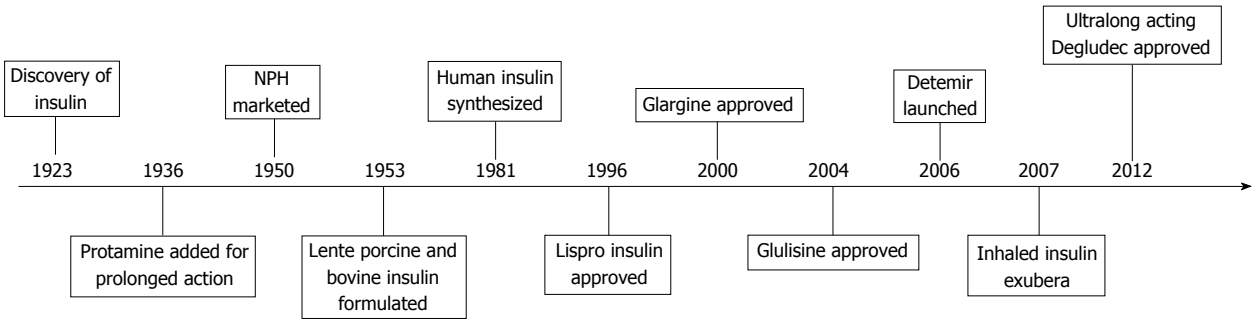

Figure 1 Time line of Insulin and its analogues. NPH: Neutral protamine hagedorn.

and environmental factors in the ultimate expression of the disease. It varies from 57.4 cases/100000 per year in Finland to 0.6 cases/100000 per year in India[2]. The fact that there is a rising trend in the number of children diagnosed to have type 1 diabetes is supported by a number of studies. Whether this can be attributed to an absolute increase in the incidence of the disease is still under speculation because the proportion of children with highest risk human leukocyte antigen haplotypes have decreased and hence, the changing environmental patterns may rather be uncovering the latent genetic factors to cause earlier expression of the disease[3]. The changing epidemiology is bringing more and more children to us to care for. Thus, unveiling newer and better therapies becomes an onus on us.

In this chapter, we shall be presenting a brief overview of the recent advances in the management of type 1 diabetes, including newer insulins, newer insulin delivery options, hypoglycemia prevention through use of technology and lastly, advances in the field of "curing" diabetes through transplant and gene therapy.

ADVANCES IN INSULIN

The quest for the ideal insulin has led to the discovery of a variety of analogues to match the mighty pancreas and yet, many lacunae are left to be filled. The timeline of important events in the history of insulin is presented in Figure 1.

Insulin analogues were designed to overcome the problems of poor stability and erratic absorption profile of the preceding generations of insulin.

Short acting insulin

Insulin lispro: Short acting insulin is necessary to deal with meal time hyperglycemia. Insulin Lispro which was approved in 1996 has rapid onset of action and shorter duration so that post prandial hypoglycaemia can be prevented. The inversion of proline at position 28 with lysine at position 29 allowed insulin to exist more in the monomeric form that is easily absorbed which could counteract meal time hyperglycemia without causing prolonged hypoglycaemia. The modification in the amino acid sequence did not alter the receptor binding and hence, is as effective as regular insulin[4].

Insulin aspart: Substituting proline at position 28 with aspartic acid formed insulin aspart which is also short acting due to absence of hexamer formation. Immunogenicity and teratogenicity profile was similar to regular insulin[5].

Insulin glulisine: This is the newest addition to the list of short acting insulin produced by substituting asparagine at position B3 by lysine and lysine at position B29 by glutamine. It is unique in action by causing phosphorylation of Insulin Receptor Substrate 2. Increased binding to insulin like growth factor (IGF) 1 receptor and mitogenic activity has however, raised concerns over its tumorigenic potential which needs further evaluation[6]. Food and drug administration (FDA) approval has been obtained for use of glulisine in children > 4 years.

Long acting insulin

Isophane, Lente and Ultralente failed to ensure long time control of glucose with minimum variations and hence, they made way for newer long acting insulins.

Insulin glargine: Amino acid alterations brought about a change in pH from 5.4 to 6.7 that made glargine poorly soluble at physiological pH. The stability of its hexameric structure prevents rapid absorption from subcutaneous tissue and its activity is maintained for 11 to 24 h. Glargine also has affinity to the IGF 1 receptor making it mitogenic, but the clinical significance of this finding is still questionable[7]. Safety in the pediatric age group has been established but due to the acidic pH burning sensation has been reported in some children.

Insulin detemir: Detemir binds reversibly to albumin and undergoes a slow release process as only free detemir is biologically active. Onset of action is within 1 to 2 h and lasts for 24 h. Peakless activity ensures stability[8]. Detemir shows more reproducible pharmacokinetics in children than glargine[9]. The United States FDA has approved the use of Detemir and Glargine only in children > 6 years.

Insulin albulin: As the name suggests, insulin albulin has been developed by directly fusing single human insulin gene to human albumin gene that makes this analogue long acting. The peakless effect makes albulin a potential

Figure 2 Dr. Arnold Kadish with the first insulin pump. (Courtesy: www.medscape.com).

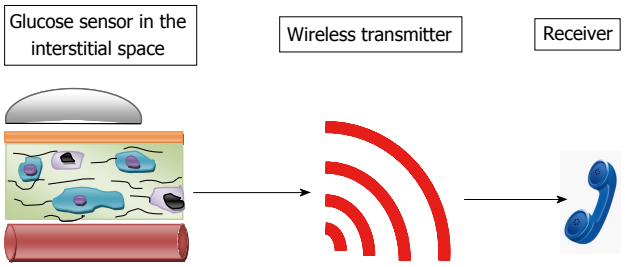

Figure 3 Schematic representation of continuous glucose monitors.

agent for long term glycemic control. The affinity of albulin to IGF 1 receptors is less compared to other analogues which makes albulin less likely to trigger mitogenesis[10]. Insulin albulin still has to evolve to enter clinical application.

Insulin degludec: Approved in 2012, Insulin degludec shows a flat profile upon injection with a half-life of 25 h, enabling once in 3 d injection. The dihexamers associate with each other to form multi hexamers that slowly form monomers and enter the bloodstream. When compared to other long acting insulins, degludec shows much lower variability in day to day glucose levels. Trials investigating degludec have also included children and adolescents. Nocturnal Hypoglycemia, which is the bottle neck in intensive glucose lowering, is reported to be up to 25% lower with degludec[11]. Increase in adverse cardiovascular events is a concern with degludec and use in pediatric age group is not yet approved.

Inhaled insulin

The search for alternative routes of delivery of insulin paved way to the discovery of inhaled insulin Exubera that was approved in 2006, but withdrawn from the market a year later due to poor sales. It was thought that the large surface area of the lungs would facilitate better absorption. However, bioavailability was found to be only 10% and so higher doses were required. Unpredictable absorption patterns that varied with age, respiratory tract infection and smoking form important hurdles for lungs to be the route of choice[12].

Despite the initial enthusiasm with oral insulin which was considered as the "holy grail" for treating diabetes, it remains an enigmatic target due to enzymatic digestion of insulin and inadequate intestinal absorption.

Buccal and skin patches are also candidate routes for delivering insulin that await further research.

INSULIN PUMPS

Parallel to the advancements in insulin, the modes of delivery also underwent considerable changes in the last 50 years. The first pump designed by Dr. Arnold Kadish in 1963 was bulky and had to be worn like a backpack as in Figure 2. It was replaced by the "big blue brick" model which again became obsolete due to inaccuracies. All the early models could only provide a single basal delivery rate and had to be programmed frequently. The technological boom that accompanied the dawn of the 20th century brought about further developments and today we have insulin pumps that are convenient, small, accurate and adjustable.

CONTINUOUS GLUCOSE MONITORS

Fear of hypoglycemia is recognised as the most important road block in the path to achieving good glycemic control. Continuous blood glucose monitoring system is an important aid in the management of type 1 diabetes and an essential prerequisite for closed loop systems. The superiority of Continuous glucose monitors (CGMs) over self-monitoring of glucose in reducing the time spent in hypoglycemia has been proven beyond doubt[13].

The basic structure of a CGM consists of a sensor, wireless transmitter and a receiver as in Figure 3.

Sensor provides real time blood glucose levels and typically consists of a membrane layer, electrode and enzyme matrix. It works on the same principle as the conventional glucose monitors using the glucose oxidase catalysed oxidation of glucose to produce hydrogen peroxide that generates an electric current at the electrode[14]. The membrane layer forms a barrier between the electrode and the surrounding tissues, which mandates adequate permeability to glucose and oxygen. Sensors are inserted subcutaneously and detect glucose concentration in the interstitial compartment. In the earlier versions, blood glucose values were stored and had to be downloaded to view the level of control retrospectively. The present CGMs have sensors that display the glucose values in real time which enables the user to take appropriate steps in case of skewed values. The CGMs are also equipped with systems that would alert the user when values are above or below the set thresholds. The receiver may either be a display device to be worn like a pager or may be connected to an insulin pump.

A drawback that has emerged with CGMs is bioinstability. Sensors become unstable secondary to inflammatory reaction, granuloma formation, blood clots, *etc*[15]. This brings about drifts in glucose values and a need for intermittent calibration with conventional blood glucose

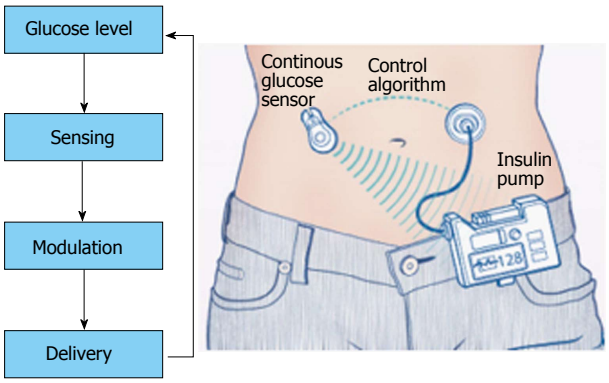

Figure 4 Principle of closed loop system.

measurements. Coating of the membrane layer with silicon oxide nanoparticles containing Polyethylene Glycol has been found to prevent bioinstability of sensors[16]. Further research is ongoing to discover the most appropriate material to coat the sensors. Another innovation that has been successful is replacement of electrochemical sensors with fluorescent sensors. When glucose binds to the receptors, the fluorophore fluoresces brightly. These sensors are highly accurate even with extreme values of glucose[17]. Despite these refinements, there are two important shortcomings with the CGMs. First, the interstitial glucose measurement does not exactly reflect the blood glucose concentration. Second is the time lag due to glucose transport to the interstitium and sensor processing. The CGMs lag behind blood glucose by an average of 4 to 10 min[18].

Another method of blood glucose monitoring that had emerged in 1999 was the Glucowatch Biographer. This device was worn like a wristwatch. It used the process of reverse iontophoresis to stimulate the secretion of subcutaneous fluid, and glucose content was measured using a biosensor unit. There was good correlation with the blood glucose monitoring devices[19]. However, skin irritation and false alarms were obstacles to the widespread clinical use of this device.

A recently developed non-invasive CGM device named HG1c uses the principle of Raman spectroscopy where a painless pulse of monochromatic light is transmitted into the skin, and the scattered light is detected for the determination of glucose levels. This device can be worn on the abdomen like a band and measures blood glucose levels every five minutes. The sensor transmits data to a smartphone which is also enabled with alarms during periods of glucose excursion[20]. A similar iPhone operating system-enabled smartphone-based Wireless Smart Gluco-Monitoring system has also been developed[21].

Many smartphone based glucose monitors and applications are helping to make the life of a diabetic patient easier. These allow the user to enter diabetes related data like carbohydrates and water consumed, insulin dose taken, duration of exercise, *etc*. Based on the information given these apps can also calculate the amount of insulin required. A device named Eyesense is under development which will be able to determine blood glucose level using a small photometer implanted in the interstitial fluid under the conjunctiva[21].

CLOSED LOOP SYSTEMS

The idea of closed loop systems came into vogue as the repeated discrete subcutaneous doses caused fluctuating insulin and in turn glucose levels. Blood glucose concentration stands on a delicate balance between caloric intake and expenditure which is modified by the insulin doses that necessarily do not mimic the original pancreatic secretion. As the CGMs started providing real time feedback of the glucose levels, the extreme variations were uncovered. The concept of artificial pancreas surfaced when CGMs were linked to insulin pumps as Continuous Subcutaneous Insulin Infusion gained acceptance from the 1990s[22]. The principle of closed loop systems is simple as shown in Figure 4.

In contrast to the pre-programmed insulin pumps, closed loop systems modulate insulin delivery at intervals of 1 to 15 min.

The characteristics that are desired in an ideal closed loop system would be the following[23]: (1) Response to glucose levels in a highly specific way; (2) Response within a timescale of minutes; (3) Monitoring within the visceral region; (4) Pulsatile output to avoid desensitization of insulin receptors; and (5) No chemical modification of insulin.

The backbone of the closed loop system is the control algorithm. Control algorithms direct insulin delivery as per glucose levels and account for measurement errors and kinetic delays.

There are two categories of control algorithms: (1) Proportional Integral Derivative (PID); and (2) Model Predictive Control (MPC).

PID

The schema of PID is given in Figure 5. The PID was one of the most initial algorithms developed for artificial pancreas. The proportional component detects deviations from target glucose, integral component measures the area under the curve between the measured and target levels and the derivative component assesses the rate of change of measured glucose levels. However, PID is rather a reactive algorithm which implies that skewed values of glucose cannot be prevented but can only be shortened in duration because the PID responds to observed glucose levels. Adding announced meals to the algorithm or patient directed insulin boluses can overcome hyperglycemia but hypoglycemic episodes may not be prevented.

MPC

This is a proactive algorithm because it can forecast the blood glucose values from the current concentration and is designed in such a way that it brings the forecasted glucose closer to the target glucose values. Based on the current glucose levels further insulin delivery is planned but after the first step is executed the system is reassessed and

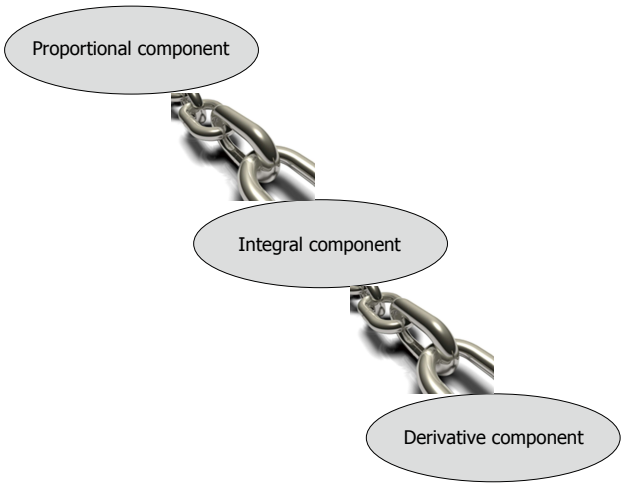

Figure 5 Components of proportional integral derivative.

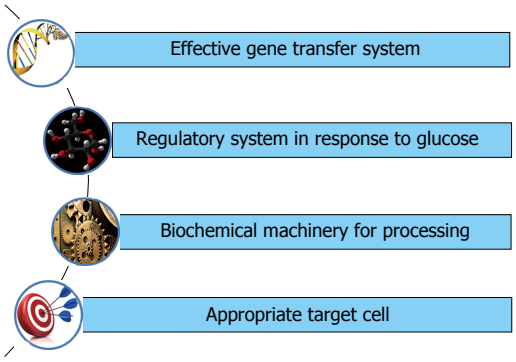

Figure 6 Requirements for insulin gene transfer.

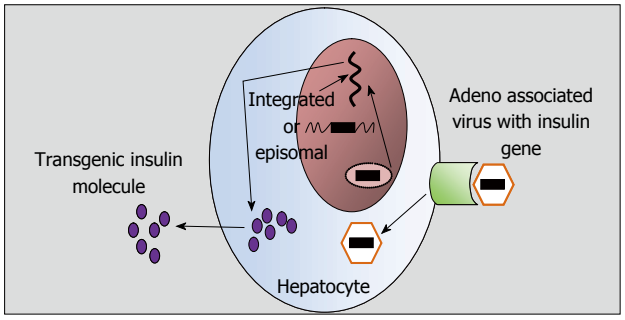

Figure 7 Insulin gene insertion with adeno associated virus.

further delivery is planned. This enables a step by step assessment and reaction, yet in a proactive manner. In this way MPC can prevent hypoglycemic episodes and reduce the time spent in hyperglycemia. MPC can efficiently deal with meals and exercise without any additional inputs[24]. MPC also has capabilities to learn the patient's routine to adjust the insulin delivery based on this information using the run to run control algorithms and also optimize according to circadian fluctuations[25].

Innovations in closed loop system

The inherent disadvantages of interstitial insulin infusion account for the delay in responding to post prandial hyperglycemia. Hence, systems have been developed for adding meal announcements to cause priming.

Intensification of insulin delivery saw hypoglycemia as the major barrier which induced development of dual hormonal pumps employing glucagon along with insulin. Glucagon has been the choice as it is a fast acting counter regulatory hormone to insulin and is found to be deficient in type 1 diabetes patients. Glucagon has enabled to close the glucose-insulin loop in the initial studies[26].

Intraportal or intraperitoneal insulin infusion to mimic the natural secretory pathway is another gate that has been opened for better control of blood sugar. However, the invasive procedure involved in placing the device and risks of infection are the hurdles to its more widespread usage[27].

"Low Glucose Suspend" is another feature to combat hypoglycemia as the pump would automatically stop insulin infusion for up to 2 h when hypoglycemia is detected which is of benefit especially during nocturnal hypoglycemic episodes[28].

Pramlintide is an amylin analogue that delays gastric emptying and reduces glucagon secretion. Pramlintide infusion along with insulin is found to enhance peripheral tissue sensitivity to insulin[29].

INSULIN GENE THERAPY

Gene therapy is the fancy word for most diseases without a cure and so it is for diabetes also. Insulin gene therapy envisages introduction of insulin secretory machinery into non beta cells. The requirements for insulin gene transfer are schematically represented in Figure 6.

Gene transfer system

Gene transfer can be achieved by viral or non viral vectors. Among non viral vectors direct injection of DNA, electroporation and gene gun methods were tried but gene expression was transient. Retro virus, adeno virus and adeno associated virus have been looked upon as the living carriers of the insulin gene (Figure 7). Problems are galore even with these viral vectors. Retro viral vectors integrate at random sites, have limited insertion capacity and infect only proliferating cells. Adenoviral vectors remain as extra- chromosomal DNA and sometimes activate cellular immune response to viral proteins.

Glucose responsive insulin production

Under normal circumstances insulin biosynthesis is regulated at the translational level which is rapid enough to react to physiological changes. Transcriptional control supplements the translational regulation. To ensure glucose responsiveness, glucose responsive promoters are linked to the insulin producing gene. However, introducing promoters alone may not be sufficient as translational regulation is difficult to be mimicked in a non-beta cell[30]; and since insulin release is controlled at the transcriptional level the rapidity of the response would

be compromised.

Biochemical machinery for processing

Proinsulin is converted to insulin by endoproteases PC1, PC2 and an exopeptidase, carboxypeptidase H which is another example of translational control[31]. In non beta cells the generic proprotein convertase Furin can cleave pro-insulin if appropriate cleavage sites are introduced by mutation but mutated pro- insulin may induce immune attack[32].

Appropriate target cell

An ideal target cell ought to have all beta cell characteristics but has to be free from immune attack. This statement seems utopian as the sophisticated machinery in the beta cell for insulin synthesis and release according to the metabolic needs is not to be easily found in any other cell type. Hepatocyte stood out as a good option as it is enabled with glucose sensing system and glucose regulated promoter. Unfortunately there are no processing enzymes and exocytosis system[33]. The pituitary cell on the other hand, has processing enzymes and exocytosis system but lacks glucose sensing system. Myocytes are also among candidate target cells. K cells, endocrine cells in the gut that secrete incretins, are endowed with glucose sensing system, glucose regulated promoter, exocytosis system and processing enzymes. Genetically engineered K cells have been shown to produce enough insulin in a glucose regulated manner in murine models though tumor cell lines were used. Though the ideal target non beta cell still remains elusive, the K cells form a promising option[34,35].

TRANSPLANTATION

Whole pancreas transplant

Despite developments in closed loop systems and encouraging results from insulin gene therapy, completely mimicking the beta cells still remained a distant dream. Thus, pancreas transplant was considered as a viable option. Whole pancreas transplant was tried initially in patients requiring kidney transplant but complications were galore like pseudocyst, fistula, thrombosis and pancreatitis. Moreover, transplanting the whole pancreas when the patients were only in need of the islets of Langerhans which constitute a meagre 2% of the pancreatic mass was like losing the battle for want of a horse shoe nail[36].

Islet cell transplant

In addition to transplanting only the endocrine component, islet cell transplantation is minimally invasive and is associated with lower morbidity. After pancreas retrieval, the islets are isolated and cultured which is the most formidable step in the whole procedure. The most commonly used anatomical site for islet transplant is the liver due to the convenience of access and good entrapment and engraftment in the sinusoids though spleen, renal capsule and the gonads have been tried[37]. Islet cell transplantation done in animals resulted in universal reversal of diabetes but reproduction of these results in human beings was a Himalayan task in the 1990s as only 11% achieved insulin independence. However, in 2009, the Collaborative Islet Transplant Registry reported that the overall incidence of sustained graft function was 77% after first 6 mo, 66% after 1 and 45% at 3 years[38]. Though independence from exogenous insulin can be achieved, extrapolation of results from studies done in adults to children with type 1 diabetes mellitus (T1DM) would be a precocious decision and awaits more research.

Stem cell therapy

The interest stem cell therapy created in almost all chronic diseases is also reverberating in type 1 diabetes. Generation of sufficient mass of beta cells, releasing insulin in response to physiological signals and protection from autoimmunity are the most important challenges. Stem cells can be converted to beta cells by sequential transient activation of specific transcription factors like Pa x 4, Nk x 6.1 and Nk x 2.2[39]. The possibility of teratogenicity with embryonal stem cells makes mesenchyme derived stem cells a better option. An alternative approach is by neogenesis of beta cells from mature beta cells with the use of GLP analogue (Exendin), Epidermal Growth Factor and gastrin. The common endodermal origin of pancreas, liver and small intestine allows trans-differentiation of any of these cell types to beta cells[40]. Trans-differentiation involves reprogramming mature cells by certain transcription factors into alternate developmental lineages.

IMMUNOLOGIC VACCINATION

The principle behind this model is to induce lymphocytes against a specific antigen in such a way that on encountering that particular epitope the lymphocytes would induce cytokines that suppress autoimmunity like interleukin 4 that are produced by Th1 cells. Insulin given orally and subcutaneously in mice models prevented T1DM[41]. Replicating these findings in humans will take time but these provide some light at the end of the tunnel.

CONCLUSION

Novel therapies are continuing to emerge for the ultimate cure of type 1 diabetes, but emulating the intricate control system of the beta cell that is tailor made for minute to minute control of blood sugar is a difficult goal to attain. We hope that sustained efforts toward this distant goal will provide the elixir for millions of children with T1DM.

Continuous glucose monitors have evolved from retrospective display to real time monitors enabled with alarms connected to smartphones and to more non-invasive methods. Closed loop systems have been undergoing developments to simulate the pancreas by incorporating better sensors, feedback, control algorithms and response. Newer insulin analogues have more predictable half-life

and activity. Inhalational, buccal and transdermal delivery routes are awaited for clinical application. Insulin independence is aimed at by incorporating insulin gene into non beta cells with reliable glucose response apparatus. Islet cell transplantation is also continually transforming to reach the point of complete cure. Immunological vaccination is in its nascent stages to prevent the occurrence of type 1 diabetes.

REFERENCES

1 **Karamitsos DT**. The story of insulin discovery. *Diabetes Res Clin Pract* 2011; **93** Suppl 1: S2-S8 [PMID: 21864746]

2 **Patterson CC**, Dahlquist GG, Gyürüs E, Green A, Soltész G. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. *Lancet* 2009; **373**: 2027-2033 [PMID: 19481249 DOI: 10.1016/S0140-6736(09)60568-7]

3 **Weets I**, De Leeuw IH, Du Caju MV, Rooman R, Keymeulen B, Mathieu C, Rottiers R, Daubresse JC, Rocour-Brumioul D, Pipeleers DG, Gorus FK. The incidence of type 1 diabetes in the age group 0-39 years has not increased in Antwerp (Belgium) between 1989 and 2000: evidence for earlier disease manifestation. *Diabetes Care* 2002; **25**: 840-846 [PMID: 11978678]

4 **Anderson JH**, Brunelle RL, Koivisto VA, Trautmann ME, Vignati L, DiMarchi R. Improved mealtime treatment of diabetes mellitus using an insulin analogue. Multicenter Insulin Lispro Study Group. *Clin Ther* 1997; **19**: 62-72 [PMID: 9083709]

5 **Raskin P**, Guthrie RA, Leiter L, Riis A, Jovanovic L. Use of insulin aspart, a fast-acting insulin analog, as the mealtime insulin in the management of patients with type 1 diabetes. *Diabetes Care* 2000; **23**: 583-588 [PMID: 10834413 DOI: 10.2337/diacare.23.5.583]

6 **Kurtzhals P**, Schäffer L, Sørensen A, Kristensen C, Jonassen I, Schmid C, Trüb T. Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use. *Diabetes* 2000; **49**: 999-1005 [PMID: 10866053 DOI: 10.2337/diabetes.49.6.999]

7 **Home P**. Insulin glargine: the first clinically useful extended-acting insulin in half a century? *Expert Opin Investig Drugs* 1999; **8**: 307-314 [PMID: 15992080 DOI: 10.1517/13543784.8.3.307]

8 **Home P**, Bartley P, Russell-Jones D, Hanaire-Broutin H, Heeg JE, Abrams P, Landin-Olsson M, Hylleberg B, Lang H, Draeger E. Insulin detemir offers improved glycemic control compared with NPH insulin in people with type 1 diabetes: a randomized clinical trial. *Diabetes Care* 2004; **27**: 1081-1087 [PMID: 15111525 DOI: 10.2337/diacare.27.5.1081]

9 **Danne T**, Datz N, Endahl L, Haahr H, Nestoris C, Westergaard L, Fjording MS, Kordonouri O. Insulin detemir is characterized by a more reproducible pharmacokinetic profile than insulin glargine in children and adolescents with type 1 diabetes: results from a randomized, double-blind, controlled trial. *Pediatr Diabetes* 2008; **9**: 554-560 [PMID: 18761644 DOI: 10.1111/j.1399-5448.2008.00443.x]

10 **Duttaroy A**, Kanakaraj P, Osborn BL, Schneider H, Pickeral OK, Chen C, Zhang G, Kaithamana S, Singh M, Schulingkamp R, Crossan D, Bock J, Kaufman TE, Reavey P, Carey-Barber M, Krishnan SR, Garcia A, Murphy K, Siskind JK, McLean MA, Cheng S, Ruben S, Birse CE, Blondel O. Development of a long-acting insulin analog using albumin fusion technology. *Diabetes* 2005; **54**: 251-258 [PMID: 15616036 DOI: 10.2337/diabetes.54.1.251]

11 **Heller S**, Buse J, Fisher M, Garg S, Marre M, Merker L, Renard E, Russell-Jones D, Philotheou A, Francisco AM, Pei H, Bode B. Insulin degludec, an ultra-longacting basal insulin, versus insulin glargine in basal-bolus treatment with mealtime insulin aspart in type 1 diabetes (BEGIN Basal-Bolus Type 1): a phase 3, randomised, open-label, treat-to-target non-inferiority trial. *Lancet* 2012; **379**: 1489-1497 [PMID: 22521071 DOI: 10.1016/S0140-6736(12)60204-9]

12 **Mandal TK**. Inhaled insulin for diabetes mellitus. *Am J Health Syst Pharm* 2005; **62**: 1359-1364 [PMID: 15972377 DOI: 10.2146/ajhp040249]

13 **Pickup JC**, Freeman SC, Sutton AJ. Glycaemic control in type 1 diabetes during real time continuous glucose monitoring compared with self monitoring of blood glucose: meta-analysis of randomised controlled trials using individual patient data. *BMJ* 2011; **343**: d3805 [PMID: 21737469 DOI: 10.1136/bmj.d3805]

14 **Mizutani F**, Yabuki S, Iijima S. Carbon paste electrode incorporated with cobalt(II) octaethoxyphthalocyanine for the amperometric detection of hydrogen peroxide. *Electroanalysis* 1995; **7**: 706-709 [DOI: 10.1002/elan.1140070802]

15 **Klueh U**, Liu Z, Feldman B, Henning TP, Cho B, Ouyang T, Kreutzer D. Metabolic biofouling of glucose sensors in vivo: role of tissue microhemorrhages. *J Diabetes Sci Technol* 2011; **5**: 583-595 [PMID: 21722574 DOI: 10.1177/193229681100500313]

16 **Lane JE**, Shivers JP, Zisser H. Continuous glucose monitors: current status and future developments. *Curr Opin Endocrinol Diabetes Obes* 2013; **20**: 106-111 [PMID: 23422244 DOI: 10.1097/MED.0b013e32835edb9d]

17 **Peyser T**, Zisser H, Khan U, Jovanovič L, Bevier W, Romey M, Suri J, Strasma P, Tiaden S, Gamsey S. Use of a novel fluorescent glucose sensor in volunteer subjects with type 1 diabetes mellitus. *J Diabetes Sci Technol* 2011; **5**: 687-693 [PMID: 21722584 DOI: 10.1177/193229681100500323]

18 **Wentholt IM**, Hart AA, Hoekstra JB, Devries JH. Relationship between interstitial and blood glucose in type 1 diabetes patients: delay and the push-pull phenomenon revisited. *Diabetes Technol Ther* 2007; **9**: 169-175 [PMID: 17425443 DOI: 10.1089/dia.2006.0007]

19 **Garg SK**, Potts RO, Ackerman NR, Fermi SJ, Tamada JA, Chase HP. Correlation of fingerstick blood glucose measurements with GlucoWatch biographer glucose results in young subjects with type 1 diabetes. *Diabetes Care* 1999; **22**: 1708-1714 [PMID: 10526740 DOI: 10.2337/diacare.22.10.1708]

20 **Lyandres O**, Yuen JM, Shah NC, VanDuyne RP, Walsh JT, Glucksberg MR. Progress toward an in vivo surface-enhanced Raman spectroscopy glucose sensor. *Diabetes Technol Ther* 2008; **10**: 257-265 [PMID: 18715199 DOI: 10.1089/dia.2007.0288]

21 **Joseph Tran BS**, Rosanna Tran BS, John R White Jr PA, Smartphone-Based Glucose Monitors and Applications in the Management of Diabetes: An Overview of 10 Salient "Apps" and a Novel Smartphone-Connected Blood Glucose Monitor. *Clinical Diabetes* 2012; **30**: 173 -178 [DOI: 10.2337/diaclin.30.4.173]

22 **Pickup J**, Keen H. Continuous subcutaneous insulin infusion at 25 years: evidence base for the expanding use of insulin pump therapy in type 1 diabetes. *Diabetes Care* 2002; **25**: 593-598 [PMID: 11874953 DOI: 10.2337/diacare.25.3.593]

23 **Adams G**, Clark J, Sahota T, Tanna S, Taylor MJ. Diabetes mellitus and closed-loop insulin delivery. *Biotechnol Genet Eng Rev* 2000; **17**: 455-496 [PMID: 11255678 DOI: 10.1080/02648725.2000.10648002]

24 **Bruttomesso D**, Farret A, Costa S, Marescotti MC, Vettore M, Avogaro A, Tiengo A, Man CD, Place J, Facchinetti A, Guerra S, Magni L, De Nicolao G, Cobelli C, Renard E, Maran A. Closed-Loop Artificial Pancreas Using Subcutaneous Glucose Sensing and Insulin Delivery and a Model Predictive Control Algorithm: Preliminary Studies in Padova and Montpellier. *J Diabetes Sci Technol* 2009; **3**: 1014-1021 [DOI: 10.1177/193229680900300504]

25 **Owens C**, Zisser H, Jovanovic L, Srinivasan B, Bonvin D, Doyle FJ. Run-to-run control of blood glucose concentrations for people with Type 1 diabetes mellitus. *IEEE Trans Biomed*

Eng 2006; **53**: 996-1005 [PMID: 16761826 DOI: 10.1109/TBME.2006.872818]
26 **El-Khatib FH**, Jiang J, Damiano ER. Adaptive closed-loop control provides blood-glucose regulation using dual subcutaneous insulin and glucagon infusion in diabetic Swine. *J Diabetes Sci Technol* 2007; **1**: 181-192 [PMID: 19888405 DOI: 10.1177/193229680700100208]
27 **Renard E**, Place J, Cantwell M, Chevassus H, Palerm CC. Closed-loop insulin delivery using a subcutaneous glucose sensor and intraperitoneal insulin delivery: feasibility study testing a new model for the artificial pancreas. *Diabetes Care* 2010; **33**: 121-127 [PMID: 19846796 DOI: 10.2337/dc09-1080]
28 **Choudhary P**, Shin J, Wang Y, Evans ML, Hammond PJ, Kerr D, Shaw JA, Pickup JC, Amiel SA. Insulin pump therapy with automated insulin suspension in response to hypoglycemia: reduction in nocturnal hypoglycemia in those at greatest risk. *Diabetes Care* 2011; **34**: 2023-2025 [PMID: 21868778 DOI: 10.2337/dc10-2411]
29 **Huffman DM**, McLean GW, Seagrove MA. Continuous subcutaneous pramlintide infusion therapy in patients with type 1 diabetes: observations from a pilot study. *Endocr Pract* 2009; **15**: 689-695 [PMID: 19546056 DOI: 10.4158/EP09044.ORR1]
30 **Goodge KA**, Hutton JC. Translational regulation of proinsulin biosynthesis and proinsulin conversion in the pancreatic beta-cell. *Semin Cell Dev Biol* 2000; **11**: 235-242 [PMID: 10966857 DOI: 10.1006/scdb.2000.0172]
31 **Hutton JC**. Insulin secretory granule biogenesis and the proinsulin-processing endopeptidases. *Diabetologia* 1994; **37** Suppl 2: S48-S56 [PMID: 7821740 DOI: 10.1007/BF00400826]
32 **Groskreutz DJ**, Sliwkowski MX, Gorman CM. Genetically engineered proinsulin constitutively processed and secreted as mature, active insulin. *J Biol Chem* 1994; **269**: 6241-6245 [PMID: 8119968]
33 **Nett PC**, Sollinger HW, Alam T. Hepatic insulin gene therapy in insulin-dependent diabetes mellitus. *Am J Transplant* 2003; **3**: 1197-1203 [PMID: 14510692 DOI: 10.1046/j.1600-6143.2003.00221.x]
34 **Corbett JA**. K cells: a novel target for insulin gene therapy for the prevention of diabetes. *Trends Endocrinol Metab* 2001; **12**: 140-142 [PMID: 11295563 DOI: 10.1016/S1043-2760(01)00400-3]
35 **Zhang Y**, Yao L, Shen K, Xu M, Zhou P, Yang W, Liu X, Qin X. Genetically engineered K cells provide sufficient insulin to correct hyperglycemia in a nude murine model. *Acta Biochim Biophys Sin* (Shanghai) 2008; **40**: 149-157 [PMID: 18235977 DOI: 10.1111/j.1745-7270.2008.00387.x]
36 **Jahansouz C**, Kumer SC, Ellenbogen M, Brayman KL. Evolution of β-Cell Replacement Therapy in Diabetes Mellitus: Pancreas Transplantation. *Diabetes Technol Ther* 2011; **13**: 395-418 [PMID: 21299398 DOI: 10.1089/dia.2010.0133]
37 **Bellin MD**, Kandaswamy R, Parkey J, Zhang HJ, Liu B, Ihm SH, Ansite JD, Witson J, Bansal-Pakala P, Balamurugan AN, Papas KK, Sutherland DE, Moran A, Hering BJ. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *Am J Transplant* 2008; **8**: 2463-2470 [PMID: 18808408 DOI: 10.1111/j.1600-6143.2008.02404]
38 **Vantyghem MC**, Kerr-Conte J, Arnalsteen L, Sergent G, Defrance F, Gmyr V, Declerck N, Raverdy V, Vandewalle B, Pigny P, Noel C, Pattou F. Primary graft function, metabolic control, and graft survival after islet transplantation. *Diabetes Care* 2009; **32**: 1473-1478 [PMID: 19638525 DOI: 10.2337/dc08-1685]
39 **Takeuchi H**, Nakatsuji N, Suemori H. Endodermal differentiation of human pluripotent stem cells to insulin-producing cells in 3D culture. *Sci Rep* 2014; **4**: 4488 [PMID: 24671046 DOI: 10.1038/srep04488]
40 **Berneman-Zeitouni D**, Molakandov K, Elgart M, Mor E, Fornoni A, Domínguez MR, Kerr-Conte J, Ott M, Meivar-Levy I, Ferber S. The temporal and hierarchical control of transcription factors-induced liver to pancreas transdifferentiation. *PLoS One* 2014; **9**: e87812 [PMID: 24504462 DOI: 10.1371/journal.pone.0087812.]
41 **Zhang ZJ**, Davidson L, Eisenbarth G, Weiner HL. Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc Natl Acad Sci USA* 1991; **88**: 10252-10256 [PMID: 1946445 DOI: 10.1073/pnas.88.22.10252]

P- Reviewer: Georgescu A, Romani A, Vorobjova T
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.697

World J Diabetes 2014 October 15; 5(5): 697-710
ISSN 1948-9358 (online)

REVIEW

Targeting inflammation in diabetes: Newer therapeutic options

Neeraj Kumar Agrawal, Saket Kant

Neeraj Kumar Agrawal, Saket Kant, Department of Endocrinology and Metabolism, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India
Author contributions: Agrawal NK and Kant S made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; drafting the article and revising it critically for important intellectual content; and final approval of the version to be published.
Correspondence to: Dr. Neeraj Kumar Agrawal, Associate Professor, Department of Endocrinology and Metabolism, Instiitute of Medical Sciences, Banaras Hindu University, Pandit Madan Mohan Malviya Rd, Varanasi 221005, India. drnkavns@gmail.com
Telephone: +91-941-5224741 Fax: +91-542-2367568
Received: December 27, 2013 Revised: April 24, 2014
Accepted: May 29, 2014
Published online: October 15, 2014

Abstract

Inflammation has been recognised to both decrease beta cell insulin secretion and increase insulin resistance. Circulating cytokines can affect beta cell function directly leading to secretory dysfunction and increased apoptosis. These cytokines can also indirectly affect beta cell function by increasing adipocyte inflammation.The resulting glucotoxicity and lipotoxicity further enhance the inflammatory process resulting in a vicious cycle. Weight reduction and drugs such as metformin have been shown to decrease the levels of C-Reactive Protein by 31% and 13%, respectively. Pioglitazone, insulin and statins have anti-inflammatory effects. Interleukin 1 and tumor necrosis factor-α antagonists are in trials and NSAIDs such as salsalate have shown an improvement in insulin sensitivity. Inhibition of 12-lipooxygenase, histone de-acetylases, and activation of sirtuin-1 are upcoming molecular targets to reduce inflammation. These therapies have also been shown to decrease the conversion of pre-diabetes state to diabetes. Drugs like glicazide, troglitazone, N-acetylcysteine and selective COX-2 inhibitors have shown benefit in diabetic neuropathy by decreasing inflammatory markers. Retinopathy drugs are used to target vascular endothelial growth factor, angiopoietin-2, various proteinases and chemokines. Drugs targeting the proteinases and various chemokines are pentoxifylline, inhibitors of nuclear factor-kappa B and mammalian target of rapamycin and are in clinical trials for diabetic nephropathy. Commonly used drugs such as insulin, metformin, peroxisome proliferator-activated receptors, glucagon like peptide-1 agonists and dipeptidyl peptidase-4 inhibitors also decrease inflammation. Anti-inflammatory therapies represent a potential approach for the therapy of diabetes and its complications.

Key words: Inflammation; Insulin resistance; Diabetes; Neuropathy; Retinopathy; Nephropathy

Core tip: The burden of diabetes and its complications is increasing worldwide. To control this pandemic, drugs targeting different areas of the pathogenesis of diabetes and its complications are needed. Inflammation plays a key role in the natural history of diabetes during the progression from pre-diabetes to diabetes, including decreased beta cell secretory capacity and insulin resistance. Insulin resistance is an important part of the metabolic syndrome and plays a role in the pathogenesis of various macrovascular complications. Drugs targeting inflammatory pathways represent a fresh approach in the treatment of diabetes and its complications.

Agrawal NK, Kant S. Targeting inflammation in diabetes: Newer therapeutic options. *World J Diabetes* 2014; 5(5): 697-710 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/697.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.697

INTRODUCTION

The incidence of both diabetes and obesity is increasing worldwide and approaching epidemic proportions. Inflammation has been recognised as a common mechanism in the pathophysiology of both these conditions. Inflammation increases insulin resistance and islet cell inflammation, which leads to defects in beta cell secretion both of which lead to diabetes. Inflammation may also be the underlying mechanism in the increased risk of cardiovascular disease in subjects with diabetes and/or obesity. Hence, targeting inflammation may be a new therapy in the already expanding options for the management of diabetes mellitus and its complications. There is concern over many drugs used for diabetes which increase cardiovascular morbidity and/or mortality. Targeting inflammation in diabetes will theoretically lead to better glycemic control, and decrease both micro- and macrovascular complications including cardiovascular complications. Most therapies for type 2 diabetes mellitus (T2DM) target insulin resistance and drugs targeting inflammation may be a paradigm shift, wherein earlier recognition of the inflammatory status of the predisposed individual with type 2 diabetes, or at risk for the development of type 2 diabetes, would be evaluated and appropriate therapy initiated.The aim of this review is to elaborate on the drugs targeting inflammation in diabetes and its complications. Both previous studies and upcoming targets including their molecular mechanisms will be discussed in the review.

Inflammation in diabetes

A number of studies have demonstrated that markers of inflammation correlate with incident diabetes. Total leucocyte count which is a surrogate marker of inflammation, and more specifically the neutrophil count in the higher quartiles of the normal range, correlates with worsening of insulin sensitivity, and incident diabetes[1] and cardiovascular disease[2]. This suggests that a simple surrogate marker such as total leucocyte count may be a marker of insulin resistance.

Insulin resistance has been defined as a state of inflammation involving both innate and adaptive immunity[3]. Islet cell inflammation as a result of an autoimmune phenomenon has already been recognised in T1DM and has been increasingly implicated in the pathogenesis of T2DM. In fact, obesity has also been seen to modify the development of T1DM. Small human studies have demonstrated that anti-inflammatory therapy has improved glycemia and beta cell function in T2DM[4,5]. Thus, inflammation is recognised as one of the important pathways in the pathogenesis of T2DM and its complications.

The major cell involved in inflammation and insulin resistance in T2DM is the adipocyte. Insulin regulates glucose uptake and triglyceride storage by adipocytes. The adipocytokines in turn also affect insulin secretion and insulin resistance[6,7]. The various adipocytokines, especially leptin, adiponectin, omentin, resistin, and visfatin may contribute to beta cell dysfunction by increasing insulin resistance. Adipose tissue also secretes dipeptidyl peptidase-4 (DPP-4) which enhances the degradation of glucagon like peptide-1 (GLP-1) and has an insulinotropic effect on beta cells[8].

Circulating cytokines can affect beta cell function directly and indirectly by increasing adipocyte inflammation. Cytokines including tumour necrosis factor-alpha (TNF-α), interleukin beta (IL-1β), and interferon-gamma (IFN-γ) disrupt the regulation of intracellular calcium in the beta cells and hence insulin release. In addition, TNF-α increases the expression of islet amyloid polypeptide (IAPP, amylin) in beta cells leading to their accelerated death[9]. IAPP expression and deposition induces and increases beta cell inflammation[10,11]. Glucotoxicity and especially lipotoxicity increase the local level of free fatty acids (FFA) in the islets, and long chain fatty acids, particularly palmitic acid, cause oxidative stress and jun N-terminal kinase (JNK) activation[12]. This further leads to increased IL-1β, TNF-α, chemokine (C-C motif) ligand 2 (CCL2), IL-6, chemokine (C-X-C motif) ligand 1 (CXCL1), and IL-8 production, and activated nuclear factor-kappa B (NF-κB) in human islets leading to islet cell dysfunction[13]. Overall, this leads to a vicious cycle of inflammation-induced beta cell dysfunction which in turn again increases inflammation.

Oxidative stress is another pathway that leads to inflammation through activation of JNK, NF-κB, and p38 mitogen-activated protein kinase (p38MAPK)[14]. Palmitic acid causes endoplasmic reticulum (ER) stress, oxidative stress, ceramide production, and JNK activation, all of which provoke inflammatory responses. Pancreatic islets have low antioxidant defence and are hence vulnerable to oxidative stress. There is differential regulation of oxidative stress genes in T2DM donors compared with control subjects, implicating oxidative stress in islet dysfunction[15]. Divalent metal transporter 1 is another factor that increases IL-1β-induced insulin resistance[16]. These findings suggest that oxidative stress is an important factor in the pathogenesis of T2DM.

Endoplasmic reticulum stress also leads to increased cytokine expression and NF-κB activation causing dysfunction of beta cells[17]. Infact, cyclopiazonic acid-induced ER stress has been shown to cause beta cell dysfunction through increased levels of cytokines and NF-κB expression[18]. The levels of thioredoxin-interacting protein (TXNIP) increase rapidly in islets during ER stress provoked by thapsigargin (depletes calcium stores in the ER). Up-regulation of TXNIP results in IL-1β and IL-6 production through initiation of the inflammasome[19,20]. TXNIP also leads to induction of oxidative stress through its interaction with thioredoxin, which is a critical redox protein in cells. TXNIP expression is regulated by glucose in human islets and plays a role in glucose-induced β cell death.Therefore, TXNIP may well be a key transducer of glucotoxicity, oxidative stress, and ER stress, feeding into various inflammatory pathways in islets.

The gut may also be involved in the development of

diabetes mellitus. Increased lipopolysaccharide absorption from the gut causes activation of toll like receptor 4 and NF-κB leading to decreased insulin gene expression and insulin secretion in rat and human islets[21]. There is data to suggest that colonization of the gut by specific bacterial species alters the development of autoimmunity in NOD mice and can modify the cytokine and chemokine profile leading to islet cell inflammation[22].

With all this in mind, the search for anti-inflammatory therapies for diabetes was started. Lifestyle modification and drugs already in use for the management of diabetes also have additional anti-inflammatory effects. In the Diabetes Prevention Program (DPP), weight reduction decreased the levels of C-Reactive Protein (CRP) by 31%, whereas metformin decreased CRP by only 13%[23]. Similar results have been observed with surgical weight loss procedures[24]. This implies that lifestyle interventions, even without drug therapy, can decrease insulin resistance; and decrease the progression of pre-diabetes states to T2DM and can decrease the progression of diabetes mellitus (DM) and its complications by decreasing inflammation. Drugs like thiazolidinedione for the same degree of glucose reduction have been shown to reduce markers of inflammation to a greater extent compared to other therapies[25]. This may be the result of peroxisome proliferator-activated receptor-γ (PPAR-γ) transrepression of inflammatory-response genes[26]. This demonstrates that a reduction in inflammation adds to the beneficial effects of these drugs, which are independent of the effect on glucose levels and thus is a direct effect.

Insulin therapy by itself over the short-term has been associated with a decrease in inflammation. This effect is mediated by the decreased activity of NF-κB which is the master transcriptional regulator of the inflammatory response[27]. However, this effect of insulin is temporary and/or requires higher doses of intravenous insulin[28]. This may be one of the additional advantages of adding insulin early in the course of T2DM and may delay the progression of DM and its complications.

One class of drugs used widely in diabetes mellitus that also have anti-inflammatory effects are statins. Statins inhibit hydroxymethylglutaryl-CoA reductase, and hence, cause a reduction in cholesterol levels. In addition, statins have also been shown to reduce the levels of CRP by 25%-30%[29]. This is a class effect of all statins and is not dose-dependent. The decrease in CRP levels does not correlate with the decrease in lipid levels, which implies that this effect is a direct effect of statins. CRP is an independent predictor of cardiovascular events. The Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin trial assessed the effect of rosuvastatin on the rates of primary cardiovascular events in subjects with high CRP concentrations, but without hyperlipidemia (CRP > 2 mg/L; low density lipoprotein (LDL) < 130 mg/dL)[30]. The CRP concentration was reduced by 37%, however, the LDL concentration was reduced by 50%, therefore, it is uncertain whether the effects of statins are truly mediated *via* the anti-inflammatory process or are the result of its lipid-lowering effect. In addition, incident T2DM increased in the statin-treated patients, an effect seen with other agents in the statin class[31]. This finding demonstrated a divide in the association between inflammation, diabetes, and cardiovascular disease, which may be explained by the potent effects of statins on lipids. Apart from CRP, statins do not have any effect on any other markers of inflammation such as fibrinogen.

NEWER THERAPEUTIC TARGETS

The following drugs are in trials for targeting inflammation and are not yet available as prescription drugs for diabetes.

Etanercept

Etanercept (934 amino acids, 150 kilo Dalton) is a dimeric fusion protein with an extracellular ligand binding domain of the Human Tumor Necrosis Factor Receptor (TNFR) linked to the Fc component of human IgG1. It is produced by a recombinant DNA technique in Chinese Hamster Ovary cells.

Blockade of TNF-α receptor has been shown to decrease insulin resistance in obese rats[32]. A trial of etanercept failed to improve insulin sensitivity in subjects with the metabolic syndrome despite lowering CRP[33]. This may have been due to the fact that the concentration of TNF-α intracellularly is almost twice that in the extracellular space, and it is the intracellular TNF-α that is responsible for insulin resistance *via* paracrine effects which were not blocked by etanercept.

Anakinra

Anakinra (153 amino acids, 17.3 kilo Dalton) is a non glycosylated form of the Human IL-1 Receptor antagonist (IL-1Ra) from which it differs only by the addition of a single methionine residue at the amino terminus. It is produced by a recombinant DNA technique in *E. coli*.

IL-1 contributes to impaired insulin secretion, decreased cell proliferation, and apoptosis of pancreatic β cells. The IL-1Ra is endogenously produced, and its concentrations are reduced in the pancreatic islets of patients with T2DM. Anakinra was studied in T2DM and showed promise in increasing beta cell secretory function, and reducing glycemia and markers of systemic inflammation[34]. Definitive conclusions on the possible clinical utility of IL-1Ra in the prevention of diabetes are awaited from the large ongoing Canakinumab Anti-inflammatory Thrombosis Outcomes Study phase Ⅲ clinical trial[35]. The study is being conducted in more than 40 countries around the world and is specifically testing whether blocking the pro-inflammatory cytokine IL-1β with canakinumab, as compared to placebo, can reduce rates of recurrent myocardial infarction, stroke, and cardiovascular death among patients with a history of myocardial infarction who remain at high risk due to a persis-

tent elevation of the inflammatory biomarker hsCRP (≥ 2 mg/L) despite best medical care.

Salsalates

Salsalates belong to the class of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) which exert their anti-inflammatory effect through inhibition of prostaglandin G/H synthase, or cyclooxygenase.These enzymes catalyse the transformation of arachidonic acid to prostaglandins and thromboxanes. NSAIDs also inhibit the expression of cell adhesion molecules, which play a role in targeting circulating cells to inflammatory sites and directly inhibit activation and function of neutrophils.

Trials with high dose salsalates in rodents[36] and in subjects with diabetes[37] have shown that salsalate by inhibiting the inhibitor of nuclear factor kappa-B kinase subunit beta decreases glucose intolerance and increases insulin sensitivity. In an open label study, salsalate, a prodrug form of salicylate, reduced fasting and post-challenge glucose levels and increased glucose utilization in euglycemic, hyperinsulinemic clamp studies[37]. Circulating FFAs were reduced and adiponectin levels were increased. In another study, salsalate, when compared with placebo, reduced fasting glucose by 13% ($P < 0.002$), glycemic response after an oral glucose challenge by 20% ($P = 0.004$), and glycated albumin by 17% ($P < 0.0003$). Although insulin levels were unchanged, fasting and oral glucose tolerance test and C-peptide levels decreased in the salsalate-treated subjects compared with placebo ($P < 0.03$), consistent with improved insulin sensitivity and a known effect of salicylates to inhibit insulin clearance. Adiponectin increased by 57% after salsalate treatment compared with placebo ($P < 0.003$). Additionally,within the group of salsalate-treated subjects, circulating levels of CRP were reduced by 34% ($P < 0.05$)[38]. These findings prove that salsalate reduces glycemia and may improve inflammatory cardiovascular risk indices in overweight individuals. These data support the hypothesis that sub-acute to chronic inflammation contributes to the pathogenesis of obesity-related dysglycemia and that targeting inflammation may provide a therapeutic option for diabetes prevention. However, the effects of salsalate on inflammation are controversial as shown by another study in which salsalate did not change flow mediated dilatation in peripheral conduit arteries in patients with T2DM despite lowering HbA1c. This finding suggests that salsalate does not have an effect on vascular inflammation[39].

Vitamin D

Calcitriol exerts regulatory effects on molecular pathways involved in inflammation, such as inhibition of PG synthesis and actions, inhibition of stress-activated kinase signaling and the resultant production of inflammatory cytokines,such as inhibition of NF-κB signaling and the production of pro-angiogenic factors. Clinical trials investigating the effects of vitamin D supplementation on serum levels of inflammatory markers have provided inconsistent results, with no evidence of effects in most trials, or effects on selected markers in a few other trials[40]. Similarly, available trials have shown no convincing benefits of vitamin D supplementation on plasma glucose levels and insulin resistance[41,42]. This systematic review and meta-analysis showed that vitamin D supplementation resulted in a small improvement in fasting glucose and insulin resistance in subjects with diabetes or impaired glucose tolerance, but no effect on glycated haemoglobin among those with diabetes. Hence, the role of vitamin D supplementation requires further well planned trials.

Chloroquine

Chloroquine is a weak base and carries a positive charge at acidic pH. It is this property of the drug that makes it selectively accumulate in lysosomes and generate a concentration gradient of a high order. This lysosomatotrophic action is responsible for the hepatic retention of insulin. Another action of the drug is decreased degradation of insulin in the muscle tissue.

A retrospective study suggested that the use of chloroquine to treat rheumatoid arthritis is associated with a lower incidence of T2DM[43]. However, this study included a specific group of patients who required the drug for another indication. Prospective studies of chloroquine are ongoing and the results are awaited.

Diacerin

Diacerin is a semi-synthetic anthraquinone derivative which directly inhibits IL-1 synthesis and release *in vitro* and downregulates IL-1 induced activities. It has been shown to possess a disease modifying effect in osteoarthritis.

In a randomized double-blind, placebo-controlled trial, 2-mo treatment of drug-naive T2DM patients with diacerin increased insulin secretion without changes in insulin sensitivity[44]. This implies a direct effect of the drug on beta cell function.

Other emerging therapies

Inhibition of 12-Lipo oxygenase: Twelve-Lipo oxygenase (12-LO) produces pro-inflammatory arachidonic acid products and is upregulated in islets of both T1DM and T2DM patients[45] leading to insulin resistance and islet cell dysfunction. Hyperglycemia and inflammatory cytokines increase the expression of 12-LO[45,46]. The activation of 12-LO has also been implicated in causing adipose tissue inflammation and insulin resistance. In NOD mice (T1DM model), Zucker diabetic fatty rats (T2DM model), and diet-induced obese mice (T2DM model) gene deletion and pharmacological suppression of 12-LO prevented the development of diabetes[47,48]. These findings point towards inhibition of 12-LO being a promising target in both T1DM and T2DM for decreasing insulin resistance, β cell dysfunction and cardiovascular complications.

Histone de-acetylases inhibition: Histone de-acetylases (HDAC) Ⅰ, ⅡA, ⅡB, Ⅲ and Ⅳ are involved in inflam-

matory responses in a variety of conditions including diabetes. HDAC inhibitors cause acetylation of the p65 subunit of NF-κB leading to its inhibition and hence a decrease in the inflammatory response. To date, there are no human data, however, animal data support the role of HDAC inhibition in β cell preservation. Linkage analysis has also revealed that a locus in 6q21, associated with both T1DM and T2DM, lies near HDAC2. Beta cell mass expansion has been observed with HDAC Ⅱ A inhibitors. In streptozotocin (STZ)-induced diabetes, ITF2357 an orally active inhibitor against class I and Ⅱ HDAC, leads to the prevention of diabetes[49].

Sirtuin 1: Sirtuin 1 (Sirt1) is a NAD^+-dependent HDAC class Ⅲ deacetylase. Some of the SIRT1 deacetylation substrates (PGc1a, FoXo, p53, and the p65 subunit of NF-κB (10,41-43 proteins) are central regulators of cellular metabolism, energy expenditure, inflammation and stress response pathways in the cell. These may be an additional target in reducing inflammation. Activation of Sirt1 may have an antiinflammatory role to play in the islets. Sirt1 overexpression prevents NF-κB mediated cytokine-induced β cell damage and its expression has been shown to be reduced in pancreatic islets after cytokine exposure[50]. Nicotinamide mononucleotide, a metabolite that augments sirtuin action, rescues islets from reduced insulin secretion after IL-1β and TNF-α exposure[51].

Identification of the targets of each class of HDAC in human islets under inflammatory conditions will aid in the therapeutic application of this emerging class of agents.

FAT-1 transgene: Long-chain n-3 PUFAs act directly by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism indirectly by altering the expression of inflammatory genes through effects on transcription factor activation. In addition, they increase anti-inflammatory mediators such as resolvins. Thus, n-3 PUFAs are potent anti-inflammatory agents. The FAT-1 transgenic mouse, which expresses the Caenorhabditis elegans *FAT-1* gene encoding an n-3 fatty acid desaturase that converts n-6 to n-3 fatty acids (which is absent in mammals) showed augmented production of n-3 polyunsaturated fatty acids. This has been shown to be protective against the development of diabetes after multiple low dose STZ injections, and displays lower levels of IL-1β, TNF-α, NF-κB and 12-HETE[52]. This may be an additional target for inflammation in T2DM.

Recent studies have indicated that ELF5A-1, an ancient and poorly understood protein, is an important regulator of cytokine release and signalling. This protein is the only protein which contains the unique amino acid, hypusine,which is a modified amino acid lysine residue. Hypusine modification by the inhibitory enzymes, deoxyhypusine synthase and deoxyhypusine hydroxylase, is required for ELF5A-1 action in cytokine signalling. Therefore, this modification may well be a new therapeutic target for preventing beta cell decline in the setting of diabetes inflammation[53]. Anti-inflammatory therapeutic targets have been used to decrease the conversion from prediabetes to diabetes and the progression of T2DM. Anti-inflammatory therapies have also been used as treatment modalities for the complications of T2DM and are detailed as follows.

Therapeutic treatments targeting inflammatory mediators in diabetic neuropathy

The various proposed mechanisms of diabetic neuropathy include increased reactive oxygen species production, increased protein glycosylation, neurovascular disturbances, and decreased neurotrophic support.Mouse models have shown that NF-κB activation is associated with diabetic neuropathy. Toll-like receptors can also activate NF-κB and lead to increased expression of cytokines and chemokines. The levels of pro-inflammatory cytokines, chemokines and TNF-α have been shown to be increased in mouse and human models, although the pathogenesis is not yet clear. Rodent studies revealed that increased COX-2 expression leads to a decrease in sensory and motor nerve conduction velocities (NCV), endoneurial blood flow, and intraepidermal nerve fiber density in diabetic mice compared to non-diabetic mice. This led to trials of COX-2 inhibitors and other anti-inflammatory drugs in diabetic neuropathy.

Monocytes from T2DM patients demonstrated increased expression of TNF-α, IL-1, IL-6, and IL-8 as compared to healthy controls and T1DM patients; treatment of these monocytes with 1,25-dihydroxyvitamin D3 downregulated the mRNAs of these cytokines[54]. The natural flavonoid, curcumin, led to a dose-dependent decrease in serum TNF-α levels and attenuated thermal hyperalgesia in STZ-treated mice[55,56]. The beneficial effect of this treatment was further enhanced by the use of insulin[57]. Other agents capable of preventing inflammatory-mediated events in rodent models include glicazide and troglitazone both of which attenuate TNF-α levels. Both of these treatments also prevented decreases in myelinated fiber area, fiber density, and the axon/myelin ratio in the tibial nerve of diabetic rats[58,59].

The anti-oxidant, N-acetylcysteine, dose-dependently decreased TNF-α levels[60] which translated into a decreased incidence or severity of neuropathy.

The expression of COX-2 is increased in the peripheral tissues of diabetic neuropathy models. Piroxicam statistically improved STZ-induced decreases in sensory neuron action potential amplitude[61]. The non-selective inhibitors, sulindac and indomethacin, decreased losses in sural and caudal sensory nerve conduction velocity of diabetic rodents compared to control mice[62,63]. Some non-selective COX inhibitors are effective treatment options, and flurbiprofen alone decreased motor NCV (MNCV). In fact, flurbiprofen treatment mimicked STZ-induced changes and did not reverse/alter STZ-induced changes on MNCV[64]. These findings indicate that COX-1 maintains neural function in rodents. Following this observation, studies were planned to assess the efficacy of COX-2 inhibitors. It was found that

celecoxib treatment prevented the decrease in MNCV and sensory nerve conduction velocity (slowing)[65], and meloxicam was shown to protect against MNCV slowing and endoneurial blood flow deficits in diabetic rodents. Intrathecal administration of COX-2 inhibitors led to a dose-dependent attenuation of mechanical behaviour[66]. Selective inhibition of COX-2 *via* pharmacological or gene inactivation played a preventive role in the increased TNF-α expression in the sciatic nerve of STZ-induced diabetic rodents[67]. However, clinical studies with these drugs are lacking. Only one study evaluating NSAID treatment in diabetic patients has been carried out, which demonstrated an improvement in the neuropathy score with ibruprofen and sulindac treatment compared to placebo[68]. However, these results should be interpreted with caution as no healthy age-matched controls were included. The study only compared responders with non-responders. NSAIDS are a double-edged sword in that their long-term use requires caution due to their well-known side effects. Although selective COX-2 inhibitors do not result in gastrointestinal side effects, cardiovascular side effects are a concern, especially in patients with a high risk for cardiovascular disease, of which subjects with DM form a part. However, it is clear that the agents targeting inflammation in diabetic neuropathy are effective only if targeted very early in the course of neuropathy. Evidence demonstrating their effectiveness after the development of diabetic neuropathy in reversing symptoms such as reductions in nerve conduction velocities or nociceptive behaviour is lacking. Larger studies investigating the time course of anti-inflammatory therapeutics should be planned. Current studies have demonstrated no reversal of diabetic neuropathy and the benefits observed only occur after a treatment period of at least 12 wk[69,70]. Overall, more studies are needed to validate these findings.

Therapeutic treatments targeting inflammatory mediators in diabetic retinopathy

Hyperglycemia increases advanced glycation endproduct (AGE) formation, reactive oxygen species and leads to nitric oxide synthatase dysregulation resulting in activation of NF-κB followed by an increase in cytokines (IL-1, IL-6, TNF-α), chemokines such as CCL-2, 58, 10, 12 and adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). This leads to activation of endothelial cells, recruitment of inflammatory cells, increased levels of vascular endothelial growth factor (VEGF) and Angiopoietin 2. These factors are involved in the pathogenesis of increased capillary permeability, capillary dropout and neo-vascularization.

The various therapies used as anti inflammatory therapies in diabetic retinopathy hence target VEGF, Angiopoietin 2, various proteinases and chemokines.

The most important factor, which has been extensively investigated in the alteration of the blood retinal barrier (BRB), is VEGF. Levels of VEGF are significantly elevated in patients with diabetic macular edema (DME) as compared to non-diabetic eye diseases[71,72]. VEGF is a potent vasoactive cytokine which increases vascular permeability. The major effect of VEGF is on endothelial tight junction proteins, leading to extravasation of fluid and hence retinal edema. It also induces the phosphorylation of VE-cadherin, occludin, and ZO-1, causing disruption of the barrier[73].

In addition, it also stimulates increased leukostasis in the microvasculature of the retina, which also leads to breakdown of the BRB[74,75].

Therefore, most of the clinical trials on retinopathy have targeted VEGF. Direct VEGF inhibitors include the anti-VEGF aptamer, pegaptanib, the monoclonal antibody fragment, ranibizumab, and the full length antibody bevacizumab. Other drugs include soluble VEGF receptor analogs, VEGF-Trap, small interfering RNAs (siRNAs) bevasiranib, and rapamycin (sirolimus). Some studies have shown that after two years, the mean change in the visual acuity letter score from baseline was 3.7 letters greater in the ranibizumab and prompt laser group, 5.8 letters greater in the ranibizumab and deferred laser group, and 1.5 letters worse in the triamcinolone and prompt laser group[76]. However, it is important that response to the anti-VEGF treatments in DME is variable, and is not as robust as in proliferative diabetic retinopathy or neovascular glaucoma. This implies that the pathogenesis of DME is multifactorial and anti-VEGF therapy is only one player in the overall pathogenesis.

Angiopoietins are another class of inflammatory growth factors that are important modulators of angiogenesis. The levels of angiopoietin-2 (Ang-2) are significantly elevated in patients with clinically significant macular edema[77], indicating that it alters the BRB. In another study increased expression of Ang-2 mRNA and protein has been demonstrated in the retina of diabetic animals[78]. Even in non-diabetic rats, intra-vitreal injection of Ang-2 led to a three-fold increase in retinal vascular permeability. Ang-2 also induces phosphorylation and loss of VE-cadherin[78]. Recent data have suggested that Ang-2 sensitizes endothelial cells to TNF-α-induced ICAM-1 expression and hence monocyte adhesion. This implies that Ang-2 is an autocrine regulator of endothelial cell inflammatory responses. Therefore, Ang-2 plays a permissive role in the augmentation of pro-inflammatory cytokines[79]. This molecule maybe an important therapeutic target in DME. Ang-2 inhibitors in various tumor models have been found to be effective in preventing tumor growth through the modulation of monocyte infiltration and angiogenesis[80]. Matrix metalloproteinases (MMPs) are major regulators of innate and acquired immunity[81]. Knockout mouse models have shown that these molecules play an important role in both acute and chronic inflammation[82]. It has also been shown that MMPs are important for the proteolytic alteration and hence activation of chemokines. They cleave many members of the CCL/monocyte chemoattractant protein (MCP) family of chemokines rendering them proactive,

which amplifies the inflammatory response. Furthermore, MMPs organise the recruitment of leukocytes as an essential component of tumor-associated inflammation[83]. It is now evident that MMPs also play an important role in the pathogenesis of diabetic retinopathy (DR). The vitreous level of proteinases, such as MMP9, are higher in diabetic subjects with DR than without DR[84]. Both MMP2 and MMP9 are elevated in the retina of animal models with early DR[85]. The retinal vascular permeability in diabetic animals is significantly increased which is a result of a decrease in cell-cell junctional protein and VE-cadherin. MMP inhibitors can decrease this vascular permeability[86]. This implies that the proteolytic degradation of VE-cadherin contributes to the BRB breakdown. This is evidence for the role of extracellular proteinases in the alteration of the BRB seen in DR[87]. Hyperglycemia can activate many soluble mediators such as AGE, reactive oxygen species (ROS), and inflammatory cytokines, which can increase MMP levels and activity in the diabetic state. Retinal inflammation leads to increased leukocyte infiltration in the retina, which by binding to endothelial cells activates cellular proteinases such as elastase, followed by removal of VE-cadherin and its associated protein from the cell surface, resulting in alterations in the endothelial monolayer[88]. These studies indicate an important role for these proteinases in DR.

The levels of many chemokines have been shown to be elevated in various studies.The most common chemokine found to be elevated in serum and vitreous is CCL2[89,90]. CCL2, also known as MCP-1, plays an important role in vascular inflammation by inducing leukocyte recruitment and activation. Hyperglycemia increases CCL2/MCP-1 generation in retinal vascular endothelial cells, pigmented epithelial cells and Muller's glial cells[91]. Furthermore, the gene polymorphism of CCL2 has been indicated as a potential risk factor for DR[92].

Studies have shown that genetic knockout of the CCL2 gene in diabetic mice plays a preventive role in alteration of the BRB[93], and that selective inhibition of the CCL2 gene can prevent alteration of the BRB in diabetes. Further studies using selective inhibitors of CCL2 and CCR2 are in progress.

Genistein, a tyrosine kinase inhibitor, has been shown to be effective in reducing diabetes-induced retinal inflammation by interfering with inflammatory signaling (ERK and P38 MAPKs) in activated microglia. This beneficial effect of genistein may represent a new intervention therapy for modulating early pathological pathways long before the occurrence of vision loss in diabetics[94].

Therapeutic treatments targeting inflammatory mediators in diabetic nephropathy

Inflammation activated by the metabolic, biochemical and haemodynamic derangements may play a key role in the development and progression of diabetic nephropathy. Cytokines such as IL-1, IL-6 and TNF-α stimulate the expression of cell adhesion molecules and profibrotic growth factors, increase endothelial permeability, promote mesangial proliferation, glomerular hypertrophy and the production of ROS. Chemokines like Protein kinase C (PKC)-dependent ICAM-1, VCAM-1 and MCP-1 facilitate leukocyte-endothelial adhesion and infiltration into diabetic kidneys. Adiponectin is protective in that it reduces oxidative stress, the production of TNF-α, and leukocyte-endothelial adhesion. Adiponectin has also been shown to interfere with receptor activation of platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Increased mammalian target of rapamycin (mTOR) activity has been shown to cause glomerular hypertrophy and hyperfiltration in diabetic subjects.

Adenosine is a potent autocrine anti-inflammatory and immunosuppressive molecule that is released from cells into the extracellular space at sites of inflammation and tissue injury. The levels of adenosine, an endogenous purine nucleoside, released from various tissues and organs are decreased in diabetic nephropathy (DN)[95]. DN was more severe in A_{2A} receptor knockout mice than in wild-type mice, which suggests that endogenous adenosine may contribute to kidney protection due to diabetes in a similar manner to that in kidney ischemia-reperfusion injury[96]. MCP-1/CCL2 inhibition by propagermanium ameliorated diabetic glomerulosclerosis and is another target for DN[97]. However, clinical inhibitors of CCL2 have shown only partial effects[98]. Even with CCL2 knockout, only a reduction in albuminuria was observed[99].

Pentoxifylline inhibits the expression of TNF-α mRNA levels[100]. In combination with angiotensin-converting enzyme inhibitors and AT1 receptor blockers (ARB), pentoxifylline decreased albuminuria in DN[101,102].

In a prospective, randomized, double-blind, placebo-controlled study, pentoxifylline (1200 mg daily) for 12 mo, in 34 patients with incipient or established DN had a reno-protective effect determined by a significant reduction in urinary albumin excretion in both incipient and established ($P < 0.01$) DN patients. This effect was attributed to a reduction in CRP, IL-6, TNF-α and serum leptin levels ($P < 0.01$)[103].

The results from 7 animal studies and 13 randomized controlled trials on diabetic kidney disease consistently demonstrated that short-term use of pentoxifylline produced a significant reduction in proteinuria and microalbuminuria in patients with diabetic and non-diabetic kidney diseases. The reports on long-term studies also showed that urinary protein excretion was considerably reduced in patients treated with pentoxifylline; however, as these results were mostly based on small clinical trials it is not clear whether the additive anti-proteinuric effect of pentoxifylline is sustained over time. Large scale clinical trials are needed to establish the long-term use of pentoxifylline as a pharmacological alternative for delaying or preventing the development of end-stage renal disease.

Adiponectin has been shown to suppress inflammatory markers including TNF-α, and receptor activation for PDGF, EGF and FGF. Adiponectin has also been shown to preserve nephrin, decrease the expression levels of TGF-β, and reduce albuminuria.

Inhibition of NF-κB in kidney using PPAR-γ[104],

ARB[105], or pentosan polysulfate[106] has been shown to ameliorate DN in animal models. However,the efficacy of inhibition of NF-κB in delaying progression of DN has not been reported.

HMG-CoA reductase inhibitors (statins) have a controversial role in DN. In a subanalysis of the Treating to New Targets study, treatment with 10 mg and 80 mg atorvastatin was found to increase estimated glomerular filtration rate (eGFR)[107], while in the Prevention of Renal and Vascular End-Stage Disease Intervention Trial, treatment with 40 mg pravastatin did not result in an increase in eGFR[108].

The mTOR is a serine/threonine kinase that mediates cell proliferation, survival, size, and mass[109]. Rapamycin decreases hyperglycemia-induced increase in mTOR activity and thus decreases renal changes in DN, including mesangial expansion and glomerular basement thickness[110]. Rapamycin also significantly reduces the influx of monocytes and macrophages associated with the progression of DN[111,112]. It has also been shown to decrease the release of pro-inflammatory cytokines or chemokines including MCP-1, regulate normal T cell expression and secreted, IL-8, and fractalkine[111,112]. Thus, rapamycin represents a new and valuable anti-inflammatory target in DN.

A recent study showed that aspirin decreased albuminuria in patients with DN[113]. In combination with AT1 receptor blockers (ARB) it led to a further decrease in the progression of DN and inflammatory markers compared to when used alone[114]. This effect of COX-2 inhibitors is postulated to occur as a result of the effects on renal hemodynamics and decrease in profibrotic cytokines[115]. However, in another study, treatment with 200 mg/d COX-2 inhibitor for six weeks did not decrease DN[116]. Thus, the overall data for COX-2 inhibitors in DN remains controversial.

PKC is induced by hyperglycemia and insulin resistance. This PKC activation then alters cell signaling molecules including inflammatory cytokines such as NF-κB, IL-6, TNF-α, and plasminogen activator-1 (PAI-1) in endothelial and mesangial cells[117-119]. Ruboxistaurin (RBX), a PKCβ isoform selective inhibitor, has been shown to prevent DN in rodent DN models by inhibiting mediators of extracellular matrix accumulation, TGF-β and amelioration of insulin signalling[120]. Diabetic PKCβ null mice showed decreased albuminuria and mesangial expansion[121]. A phase Ⅱ clinical trial with RBX significantly decreased albuminuria and maintained a stable eGFR[122]. Recently, it was shown that hyperglycemia itself can activate PKCβ isoforms, which increased the detrimental effects of Ang-2 on glomerular endothelial cells and decreased the glucagon-like peptide-1 (GLP-1) receptor, leading to resistance to GLP-1 treatment in DN[123]. Recent findings suggest that hyperglycemia also activates PKCβ and p38 mitogen-activated protein (MAPK) to increase Src homology-2 domain-containing phosphatase-1 and causes VEGF resistance and independent NF-κB activation to induce podocyte apoptosis in DN[124] which may be new targets of treatment.

Exogenous insulin has been shown to inhibit the activation of TNF-α in animal models[125]. Furthermore,insulin inhibits MCP-1 expression and activation of NF-κB in endothelial cells[126]. Recent studies in patients with T2DM have shown that insulin treatment decreases the expression of inflammatory cytokines, such as MCP-1, ICAM-1, soluble VCAM-1 (sVCAM-1), TNF-α, and IL-6[127,128].

Insulin can increase endothelial nitric oxide (NO) production by rapid post-translational mechanisms, mediated by the PI3K/Akt signaling pathway, leading to vasodilatation, an antithrombotic effect, and anti-inflammatory actions[129-131]. Insulin not only stimulates NO production, but also increases the expression of endothelial NO synthase (eNOS)[132]. Recent data indicate that vascular endothelial cell specific insulin receptor knockout mice had decreased eNOS expression in the aorta[133]. Thus, insulin resistance in vascular tissue could contribute to DN. However, to date, the efficacy of exogenous NO donor remains unclear. Insulin and metformin were studied in a trial for 14 wk. Despite substantially improving glucose control, neither insulin nor metformin reduced inflammatory biomarker levels including hsCRP, IL-6, and sTNFR2, which were the main effects evaluated in comparisons between the individual treatment groups (placebo metformin only; placebo metformin and insulin; active metformin only; or active metformin and insulin)[28].

PPARs regulate insulin sensitivity, lipid metabolism, adipogenesis and cell growth[134-137]. Recent studies indicated that a PPAR-γ agonist decreased the expression of inflammatory markers such as PAI-1, ICAM-1, and NF-κB in the kidney in DN and ameliorated renal function[138].

Analysis of the GLP-1 receptor (GLP-1R) has revealed its expression in endothelial cells and kidney[139,140]. In endothelial cells, GLP-1 inhibits the expression of TNF-α and VCAM-1[141]. GLP-1 acts on the glomerular endothelial cells and decreases the signaling pathway of Ang-2 at phospho-c-Raf (Ser338)/phospho-Erk1/2 *via* phospho-c-Raf (Ser259) activated by the cAMP/PKA pathway. Administration of GLP-1 in DN decreases inflammatory markers including PAI-1, CD68, IL-6, TNF-α, NF-κB, and CXCL2 in the kidney[117].

DPP-4 inhibitors provide vascular protection by increasing the bioavailability of GLP-1and its action. They have also been reported to decrease the levels of MCP-1. In addition, they have vasotropic actions and a possible reduction in DN[142]. A recent large phase Ⅲ study showed that linagliptin significantly reduced albuminuria in DN by 30%[143]. However, the role of DPP-4 inhibitors in the regulation of inflammatory cytokines and vasotropic actions remains largely unexplored and open to further trials.

DIABETES, THE METABOLIC SYNDROME AND NON-ALCOHOLIC FATTY LIVER DISEASE

Type 2 diabetes mellitus is part of the metabolic syn-

drome and non-alcoholic fatty liver disease (NAFLD) shares insulin resistance as a common pathophysiology with T2DM. More recently, NAFLD has been proposed, but not yet accepted, as a criterion for defining the metabolic syndrome[144]. Hepatic insulin resistance has a key role to play in the pathogenesis of NAFLD and adiponectin, an abundant adipocytokine, decreases both hepatic and systemic insulin resistance by decreasing inflammation[145]. Hence, adiponectin and its agonists may be promising targets to reduce both hepatic and systemic insulin resistance[146,147]. Exercise, in addition to its benefits in reducing weight and insulin resistance also reduces the levels of inflammatory cytokines implicated in diabetes-associated NAFLD[148]. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) have been used in NAFLD and lead to a significant reduction in the expression of pro-inflammatory molecules (TNF-α and IL-6) and of reactive oxygen species[149]. Inhibition of Bcl-2 (B-cell lymphoma 2), the first member of the Bcl-2 family of apoptosis regulatory proteins encoded by the *Bcl-2* gene, leads to intensification of inflammation in NAFLD[150]. Serum Bcl-2 concentrations in overweight-obese subjects with NAFLD have been shown to be reduced and may represent an additional target for therapy[151]. JNK, insulin resistance and inflammation represent possible links between NAFLD and coronary artery disease. There are few studies on anti-inflammatory drugs such as aspirin, anti-IL-6 receptors, immune-modulators (calcineurin inhibitors), substances which enhance the expression of heat shock proteins (which protect cells from endoplasmic reticulum stress-induced apoptosis), and anti-c-Jun amino-terminal kinases in NAFLD and these require further study[152]. Thus, NAFLD is a chronic low grade inflammation that leads to insulin resistance due to the increased levels of cytokines[153,154], and anti-inflammatory therapies may help decrease the burden of NAFLD and T2DM.

Thus, inflammation has a role to play both in the pathogenesis of diabetes and its complications and it represents a potential target for treatment in both diabetes and its complications.

REFERENCES

1 **Vozarova B**, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 2002; **51**: 455-461 [PMID: 11812755]

2 **Rana JS**, Boekholdt SM, Ridker PM, Jukema JW, Luben R, Bingham SA, Day NE, Wareham NJ, Kastelein JJ, Khaw KT. Differential leucocyte count and the risk of future coronary artery disease in healthy men and women: the EPIC-Norfolk Prospective Population Study. *J Intern Med* 2007; **262**: 678-689 [PMID: 17908163 DOI: 10.1111/j.1365-2796.2007.01864.x]

3 **Das A**, Mukhopadhyay S. The evil axis of obesity, inflammation and type-2 diabetes. *Endocr Metab Immune Disord Drug Targets* 2011; **11**: 23-31 [PMID: 21348821 DOI: 10.2174/187153011794982086]

4 **Larsen CM**, Faulenbach M, Vaag A, Vølund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* 2007; **356**: 1517-1526 [PMID: 17429083 DOI: 10.1056/NEJMoa065213]

5 **Goldfine AB**, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. *Ann Intern Med* 2010; **152**: 346-357 [PMID: 20231565 DOI: 10.7326/0003-4819-152-6-201003160-00004]

6 **Dunmore SJ**, Brown JE. The role of adipokines in β-cell failure of type 2 diabetes. *J Endocrinol* 2013; **216**: T37-T45 [PMID: 22991412 DOI: 10.1530/JOE-12-0278]

7 **Tsui H**, Paltser G, Chan Y, Dorfman R, Dosch HM. 'Sensing' the link between type 1 and type 2 diabetes. *Diabetes Metab Res Rev* 2011; **27**: 913-918 [PMID: 22069284]

8 **Lamers D**, Famulla S, Wronkowitz N, Hartwig S, Lehr S, Ouwens DM, Eckardt K, Kaufman JM, Ryden M, Müller S, Hanisch FG, Ruige J, Arner P, Sell H, Eckel J. Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. *Diabetes* 2011; **60**: 1917-1925 [PMID: 21593202 DOI: 10.2337/db10-1707]

9 **Cai K**, Qi D, Wang O, Chen J, Liu X, Deng B, Qian L, Liu X, Le Y. TNF-α acutely upregulates amylin expression in murine pancreatic beta cells. *Diabetologia* 2011; **54**: 617-626 [PMID: 21116608 DOI: 10.1007/s00125-010-1972-9]

10 **Montane J**, Klimek-Abercrombie A, Potter KJ, Westwell-Roper C, Bruce Verchere C. Metabolic stress, IAPP and islet amyloid. *Diabetes Obes Metab* 2012; **14** Suppl 3: 68-77 [PMID: 22928566 DOI: 10.1111/j.1463-1326.2012.01657.x]

11 **Masters SL**, Dunne A, Subramanian SL, Hull RL, Tannahill GM, Sharp FA, Becker C, Franchi L, Yoshihara E, Chen Z, Mullooly N, Mielke LA, Harris J, Coll RC, Mills KH, Mok KH, Newsholme P, Nuñez G, Yodoi J, Kahn SE, Lavelle EC, O'Neill LA. Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes. *Nat Immunol* 2010; **11**: 897-904 [PMID: 20835230 DOI: 10.1038/ni.1935]

12 **van Raalte DH**, Diamant M. Glucolipotoxicity and beta cells in type 2 diabetes mellitus: target for durable therapy? *Diabetes Res Clin Pract* 2011; **93** Suppl 1: S37-S46 [PMID: 21864750 DOI: 10.1016/S0168-8227(11)70012-2]

13 **Igoillo-Esteve M**, Marselli L, Cunha DA, Ladrière L, Ortis F, Grieco FA, Dotta F, Weir GC, Marchetti P, Eizirik DL, Cnop M. Palmitate induces a pro-inflammatory response in human pancreatic islets that mimics CCL2 expression by beta cells in type 2 diabetes. *Diabetologia* 2010; **53**: 1395-1405 [PMID: 20369226 DOI: 10.1007/s00125-010-1707-y]

14 **Lamb RE**, Goldstein BJ. Modulating an oxidative-inflammatory cascade: potential new treatment strategy for improving glucose metabolism, insulin resistance, and vascular function. *Int J Clin Pract* 2008; **62**: 1087-1095 [PMID: 18489578 DOI: 10.1111/j.1742-1241.2008.01789.x]

15 **Marselli L**, Thorne J, Dahiya S, Sgroi DC, Sharma A, Bonner-Weir S, Marchetti P, Weir GC. Gene expression profiles of Beta-cell enriched tissue obtained by laser capture microdissection from subjects with type 2 diabetes. *PLoS One* 2010; **5**: e11499 [PMID: 20644627 DOI: 10.1371/journal.pone.0011499]

16 **Hansen JB**, Tonnesen MF, Madsen AN, Hagedorn PH, Friberg J, Grunnet LG, Heller RS, Nielsen AØ, Størling J, Baeyens L, Anker-Kitai L, Qvortrup K, Bouwens L, Efrat S, Aalund M, Andrews NC, Billestrup N, Karlsen AE, Holst B, Pociot F, Mandrup-Poulsen T. Divalent metal transporter 1 regulates iron-mediated ROS and pancreatic β cell fate in response to cytokines. *Cell Metab* 2012; **16**: 449-461 [PMID: 23000401 DOI: 10.1016/j.cmet.2012.09.001]

17 **Sarkar SA**, Kutlu B, Velmurugan K, Kizaka-Kondoh S, Lee CE, Wong R, Valentine A, Davidson HW, Hutton JC, Pugazhenthi S. Cytokine-mediated induction of anti-apoptotic genes that are linked to nuclear factor kappa-B (NF-kappaB) signalling in human islets and in a mouse beta cell line. *Diabetologia* 2009; **52**: 1092-1101 [PMID: 19343319 DOI: 10.1007/

s00125-009-1331-x]
18 **Miani M**, Colli ML, Ladrière L, Cnop M, Eizirik DL. Mild endoplasmic reticulum stress augments the proinflammatory effect of IL-1β in pancreatic rat β-cells via the IRE1α/XBP1s pathway. *Endocrinology* 2012; **153**: 3017-3028 [PMID: 22529213 DOI: 10.1210/en.2011-2090]
19 **Oslowski CM**, Hara T, O'Sullivan-Murphy B, Kanekura K, Lu S, Hara M, Ishigaki S, Zhu LJ, Hayashi E, Hui ST, Greiner D, Kaufman RJ, Bortell R, Urano F. Thioredoxin-interacting protein mediates ER stress-induced β cell death through initiation of the inflammasome. *Cell Metab* 2012; **16**: 265-273 [PMID: 22883234 DOI: 10.1016/j.cmet.2012.07.005]
20 **Lerner AG**, Upton JP, Praveen PV, Ghosh R, Nakagawa Y, Igbaria A, Shen S, Nguyen V, Backes BJ, Heiman M, Heintz N, Greengard P, Hui S, Tang Q, Trusina A, Oakes SA, Papa FR. IRE1α induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. *Cell Metab* 2012; **16**: 250-264 [PMID: 22883233 DOI: 10.1016/j.cmet.2012.07.007]
21 **Amyot J**, Semache M, Ferdaoussi M, Fontés G, Poitout V. Lipopolysaccharides impair insulin gene expression in isolated islets of Langerhans via Toll-Like Receptor-4 and NF-κB signalling. *PLoS One* 2012; **7**: e36200 [PMID: 22558381 DOI: 10.1371/journal.pone.0036200]
22 **Atkinson MA**, Chervonsky A. Does the gut microbiota have a role in type 1 diabetes? Early evidence from humans and animal models of the disease. *Diabetologia* 2012; **55**: 2868-2877 [PMID: 22875196 DOI: 10.1007/s00125-012-2672-4]
23 **Haffner S**, Temprosa M, Crandall J, Fowler S, Goldberg R, Horton E, Marcovina S, Mather K, Orchard T, Ratner R, Barrett-Connor E. Intensive lifestyle intervention or metformin on inflammation and coagulation in participants with impaired glucose tolerance. *Diabetes* 2005; **54**: 1566-1572 [PMID: 15855347 DOI: 10.2337/diabetes.54.5.1566]
24 **Viardot A**, Lord RV, Samaras K. The effects of weight loss and gastric banding on the innate and adaptive immune system in type 2 diabetes and prediabetes. *J Clin Endocrinol Metab* 2010; **95**: 2845-2850 [PMID: 20375213 DOI: 10.1210/jc.2009-2371]
25 **Pfützner A**, Marx N, Lübben G, Langenfeld M, Walcher D, Konrad T, Forst T. Improvement of cardiovascular risk markers by pioglitazone is independent from glycemic control: results from the pioneer study. *J Am Coll Cardiol* 2005; **45**: 1925-1931 [PMID: 15963388 DOI: 10.1016/j.jacc.2005.03.041]
26 **Pascual G**, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature* 2005; **437**: 759-763 [PMID: 16127449 DOI: 10.1038/nature03988]
27 **Dandona P**, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S. Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab* 2001; **86**: 3257-3265 [PMID: 11443198 DOI: 10.1210/jc.86.7.3257]
28 **Pradhan AD**, Everett BM, Cook NR, Rifai N, Ridker PM. Effects of initiating insulin and metformin on glycemic control and inflammatory biomarkers among patients with type 2 diabetes: the LANCET randomized trial. *JAMA* 2009; **302**: 1186-1194 [PMID: 19755697 DOI: 10.1001/jama.2009.1347]
29 **Balk EM**, Lau J, Goudas LC, Jordan HS, Kupelnick B, Kim LU, Karas RH. Effects of statins on nonlipid serum markers associated with cardiovascular disease: a systematic review. *Ann Intern Med* 2003; **139**: 670-682 [PMID: 14568856 DOI: 10.7326/0003-4819-139-8-200310210-00011]
30 **Ridker PM**, Danielson E, Fonseca FA, Genest J, Gotto AM, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 2008; **359**: 2195-2207 [PMID: 18997196 DOI: 10.1056/NEJMoa0807646]
31 **Rajpathak SN**, Kumbhani DJ, Crandall J, Barzilai N, Alderman M, Ridker PM. Statin therapy and risk of developing type 2 diabetes: a meta-analysis. *Diabetes Care* 2009; **32**: 1924-1929 [PMID: 19794004]
32 **Hotamisligil GS**, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; **259**: 87-91 [PMID: 7678183 DOI: 10.1126/science.7678183]
33 **Bernstein LE**, Berry J, Kim S, Canavan B, Grinspoon SK. Effects of etanercept in patients with the metabolic syndrome. *Arch Intern Med* 2006; **166**: 902-908 [PMID: 16636217 DOI: 10.1001/archinte.166.8.902]
34 **Akash MS**, Shen Q, Rehman K, Chen S. Interleukin-1 receptor antagonist: a new therapy for type 2 diabetes mellitus. *J Pharm Sci* 2012; **101**: 1647-1658 [PMID: 22271340 DOI: 10.1002/jps.23057]
35 **Ridker PM**, Thuren T, Zalewski A, Libby P. Interleukin-1β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am Heart J* 2011; **162**: 597-605 [PMID: 21982649 DOI: 10.1016/j.ahj.2011.06.012]
36 **Yuan M**, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 2001; **293**: 1673-1677 [PMID: 11533494 DOI: 10.1126/science.1061620]
37 **Goldfine AB**, Silver R, Aldhahi W, Cai D, Tatro E, Lee J, Shoelson SE. Use of salsalate to target inflammation in the treatment of insulin resistance and type 2 diabetes. *Clin Transl Sci* 2008; **1**: 36-43 [PMID: 19337387 DOI: 10.1111/j.1752-8062.2008.00026.x]
38 **Fleischman A**, Shoelson SE, Bernier R, Goldfine AB. Salsalate improves glycemia and inflammatory parameters in obese young adults. *Diabetes Care* 2008; **31**: 289-294 [PMID: 17959861 DOI: 10.2337/dc07-1338]
39 **Goldfine AB**, Buck JS, Desouza C, Fonseca V, Chen YD, Shoelson SE, Jablonski KA, Creager MA. Targeting inflammation using salsalate in patients with type 2 diabetes: effects on flow-mediated dilation (TINSAL-FMD). *Diabetes Care* 2013; **36**: 4132-4139 [PMID: 24130358 DOI: 10.2337/dc13-0859]
40 **Chagas CE**, Borges MC, Martini LA, Rogero MM. Focus on vitamin D, inflammation and type 2 diabetes. *Nutrients* 2012; **4**: 52-67 [PMID: 22347618 DOI: 10.3390/nu4010052]
41 **Pittas AG**, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J, Balk EM. Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med* 2010; **152**: 307-314 [PMID: 20194237 DOI: 10.7326/0003-4819-152-5-201003020-00009.]
42 **George PS**, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. *Diabet Med* 2012; **29**: e142-e150 [PMID: 22486204 DOI: 10.1111/j.1464-5491.2012.03672.x]
43 **Wasko MC**, Hubert HB, Lingala VB, Elliott JR, Luggen ME, Fries JF, Ward MM. Hydroxychloroquine and risk of diabetes in patients with rheumatoid arthritis. *JAMA* 2007; **298**: 187-193 [PMID: 17622600 DOI: 10.1001/jama.298.2.187]
44 **Ramos-Zavala MG**, González-Ortiz M, Martínez-Abundis E, Robles-Cervantes JA, González-López R, Santiago-Hernández NJ. Effect of diacerein on insulin secretion and metabolic control in drug-naive patients with type 2 diabetes: a randomized clinical trial. *Diabetes Care* 2011; **34**: 1591-1594 [PMID: 21610123 DOI: 10.2337/dc11-0357]
45 **Dobrian AD**, Lieb DC, Cole BK, Taylor-Fishwick DA, Chakrabarti SK, Nadler JL. Functional and pathological roles of the 12- and 15-lipoxygenases. *Prog Lipid Res* 2011; **50**: 115-131 [PMID: 20970452 DOI: 10.1016/j.plipres.2010.10.005]

46 **Ma K**, Nunemaker CS, Wu R, Chakrabarti SK, Taylor-Fishwick DA, Nadler JL. 12-Lipoxygenase Products Reduce Insulin Secretion and {beta}-Cell Viability in Human Islets. *J Clin Endocrinol Metab* 2010; **95**: 887-893 [PMID: 20089617 DOI: 10.1210/jc.2009-1102]

47 **Sears DD**, Miles PD, Chapman J, Ofrecio JM, Almazan F, Thapar D, Miller YI. 12/15-lipoxygenase is required for the early onset of high fat diet-induced adipose tissue inflammation and insulin resistance in mice. *PLoS One* 2009; **4**: e7250 [PMID: 19787041 DOI: 10.1371/journal.pone.0007250]

48 **Tersey SA**, Carter, JD, Rosenberg, L, Taylor-Fishwick DA, Mirmira RG, Nadler JL.Amelioration of type 1 diabetes following treatment of non-obese diabetic mice with INGAP and lisofylline. *J Diabetes Mellitus* 2012; **2**: 251-257 [DOI: 10.4236/jdm.2012.22040]

49 **Christensen DP**, Dahllöf M, Lundh M, Rasmussen DN, Nielsen MD, Billestrup N, Grunnet LG, Mandrup-Poulsen T. Histone deacetylase (HDAC) inhibition as a novel treatment for diabetes mellitus. *Mol Med* 2011; **17**: 378-390 [PMID: 21274504 DOI: 10.2119/molmed.2011.00021]

50 **Lee JH**, Song MY, Song EK, Kim EK, Moon WS, Han MK, Park JW, Kwon KB, Park BH. Overexpression of SIRT1 protects pancreatic beta-cells against cytokine toxicity by suppressing the nuclear factor-kappaB signaling pathway. *Diabetes* 2009; **58**: 344-351 [PMID: 19008341 DOI: 10.2337/db07-1795]

51 **Caton PW**, Kieswich J, Yaqoob MM, Holness MJ, Sugden MC. Nicotinamide mononucleotide protects against pro-inflammatory cytokine-mediated impairment of mouse islet function. *Diabetologia* 2011; **54**: 3083-3092 [PMID: 21901281 DOI: 10.1007/s00125-011-2288-0]

52 **Bellenger J**, Bellenger S, Bataille A, Massey KA, Nicolaou A, Rialland M, Tessier C, Kang JX, Narce M. High pancreatic n-3 fatty acids prevent STZ-induced diabetes in fat-1 mice: inflammatory pathway inhibition. *Diabetes* 2011; **60**: 1090-1099 [PMID: 21330635 DOI: 10.2337/db10-0901]

53 **Maier B**, Tersey SA, Mirmira RG. Hypusine: a new target for therapeutic intervention in diabetic inflammation. *Discov Med* 2010; **10**: 18-23 [PMID: 20670594]

54 **Giulietti A**, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory. *Diabetes Res Clin Pract* 2007; **77**: 47-57 [PMID: 17112620 DOI: 10.1016/j.diabres.2006.10.007]

55 **Sharma S**, Kulkarni SK, Agrewala JN, Chopra K. Curcumin attenuates thermal hyperalgesia in a diabetic mouse model of neuropathic pain. *Eur J Pharmacol* 2006; **536**: 256-261 [PMID: 16584726 DOI: 10.1016/j.ejphar.2006.03.006]

56 **Li Y**, Zhang Y, Liu DB, Liu HY, Hou WG, Dong YS. Curcumin attenuates diabetic neuropathic pain by downregulating TNF-α in a rat model. *Int J Med Sci* 2013; **10**: 377-381 [PMID: 23471081 DOI: 10.7150/ijms.5224]

57 **Sharma S**, Chopra K, Kulkarni SK. Effect of insulin and its combination with resveratrol or curcumin in attenuation of diabetic neuropathic pain: participation of nitric oxide and TNF-alpha. *Phytother Res* 2007; **21**: 278-283 [PMID: 17199240 DOI: 10.1002/ptr.2070]

58 **Qiang X**, Satoh J, Sagara M, Fukuzawa M, Masuda T, Miyaguchi S, Takahashi K, Toyota T. Gliclazide inhibits diabetic neuropathy irrespective of blood glucose levels in streptozotocin-induced diabetic rats. *Metabolism* 1998; **47**: 977-981 [PMID: 9711995 DOI: 10.1016/S0026-0495(98)90354-7]

59 **Qiang X**, Satoh J, Sagara M, Fukuzawa M, Masuda T, Sakata Y, Muto G, Muto Y, Takahashi K, Toyota T. Inhibitory effect of troglitazone on diabetic neuropathy in streptozotocin-induced diabetic rats. *Diabetologia* 1998; **41**: 1321-1326 [PMID: 9833940 DOI: 10.1007/s001250051072]

60 **Sagara M**, Satoh J, Wada R, Yagihashi S, Takahashi K, Fukuzawa M, Muto G, Muto Y, Toyota T. Inhibition of development of peripheral neuropathy in streptozotocin-induced diabetic rats with N-acetylcysteine. *Diabetologia* 1996; **39**: 263-269 [PMID: 8721770 DOI: 10.1007/BF00418340]

61 **Parry GJ**, Kozu H. Piroxicam may reduce the rate of progression of experimental diabetic neuropathy. *Neurology* 1990; **40**: 1446-1449 [PMID: 2392233 DOI: 10.1212/WNL.40.9.1446]

62 **Zochodne DW**, Ho LT. The influence of indomethacin and guanethidine on experimental streptozotocin diabetic neuropathy. *Can J Neurol Sci* 1992; **19**: 433-441 [PMID: 1423041]

63 **Zochodne DW**, Ho LT. The influence of sulindac on experimental streptozotocin-induced diabetic neuropathy. *Can J Neurol Sci* 1994; **21**: 194-202 [PMID: 8000974]

64 **Pop-Busui R**, Marinescu V, Van Huysen C, Li F, Sullivan K, Greene DA, Larkin D, Stevens MJ. Dissection of metabolic, vascular, and nerve conduction interrelationships in experimental diabetic neuropathy by cyclooxygenase inhibition and acetyl-L-carnitine administration. *Diabetes* 2002; **51**: 2619-2628 [PMID: 12145179 DOI: 10.2337/diabetes.51.8.2619]

65 **Kellogg AP**, Wiggin TD, Larkin DD, Hayes JM, Stevens MJ, Pop-Busui R. Protective effects of cyclooxygenase-2 gene inactivation against peripheral nerve dysfunction and intraepidermal nerve fiber loss in experimental diabetes. *Diabetes* 2007; **56**: 2997-3005 [PMID: 17720896 DOI: 10.2337/db07-0740]

66 **Matsunaga A**, Kawamoto M, Shiraishi S, Yasuda T, Kajiyama S, Kurita S, Yuge O. Intrathecally administered COX-2 but not COX-1 or COX-3 inhibitors attenuate streptozotocin-induced mechanical hyperalgesia in rats. *Eur J Pharmacol* 2007; **554**: 12-17 [PMID: 17112505 DOI: 10.1016/j.ejphar.2006.09.072]

67 **Kellogg AP**, Cheng HT, Pop-Busui R. Cyclooxygenase-2 pathway as a potential therapeutic target in diabetic peripheral neuropathy. *Curr Drug Targets* 2008; **9**: 68-76 [PMID: 18220714 DOI: 10.2174/138945008783431691]

68 **Cohen KL**, Harris S. Efficacy and safety of nonsteroidal anti-inflammatory drugs in the therapy of diabetic neuropathy. *Arch Intern Med* 1987; **147**: 1442-1444 [PMID: 3115210 DOI: 10.1001/archinte.1987.00370080078016]

69 **Maeda K**, Yasuda H. [Diabetic neuropathy: clinical and experimental progress in its pathogenesis and treatment]. *Nihon Rinsho* 1999; **57**: 578-583 [PMID: 10199137]

70 **Boulton AJ**, Malik RA. Diabetic neuropathy. *Med Clin North Am* 1998; **82**: 909-929 [PMID: 9706126 DOI: 10.1016/S0025-7125(05)70029-8]

71 **Funatsu H**, Yamashita H, Ikeda T, Nakanishi Y, Kitano S, Hori S. Angiotensin II and vascular endothelial growth factor in the vitreous fluid of patients with diabetic macular edema and other retinal disorders. *Am J Ophthalmol* 2002; **133**: 537-543 [PMID: 11931788 DOI: 10.1016/S0002-9394(02)01323-5]

72 **Caldwell RB**, Bartoli M, Behzadian MA, El-Remessy AE, Al-Shabrawey M, Platt DH, Caldwell RW. Vascular endothelial growth factor and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. *Diabetes Metab Res Rev* 2003; **19**: 442-455 [PMID: 14648803 DOI: 10.1002/dmrr.415]

73 **Antonetti DA**, Barber AJ, Hollinger LA, Wolpert EB, Gardner TW. Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors. *J Biol Chem* 1999; **274**: 23463-23467 [PMID: 10438525 DOI: 10.1074/jbc.274.33.23463]

74 **Aiello LP**, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 1994; **331**: 1480-1487 [PMID: 7526212 DOI: 10.1056/NEJM199412013312203]

75 **Aiello LP**, Bursell SE, Clermont A, Duh E, Ishii H, Takagi C, Mori F, Ciulla TA, Ways K, Jirousek M, Smith LE, King GL. Vascular endothelial growth factor-induced retinal

permeability is mediated by protein kinase C in vivo and suppressed by an orally effective beta-isoform-selective inhibitor. *Diabetes* 1997; **46**: 1473-1480 [PMID: 9287049 DOI: 10.2337/diab.46.9.1473]

76 **Elman MJ**, Bressler NM, Qin H, Beck RW, Ferris FL, Friedman SM, Glassman AR, Scott IU, Stockdale CR, Sun JK. Expanded 2-year follow-up of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology* 2011; **118**: 609-614 [PMID: 21459214 DOI: 10.1016/j.ophtha.2010.12.033]

77 **Patel JI**, Hykin PG, Gregor ZJ, Boulton M, Cree IA. Angiopoietin concentrations in diabetic retinopathy. *Br J Ophthalmol* 2005; **89**: 480-483 [PMID: 15774928 DOI: 10.1136/bjo.2004.049940]

78 **Rangasamy S**, Srinivasan R, Maestas J, McGuire PG, Das A. A potential role for angiopoietin 2 in the regulation of the blood-retinal barrier in diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2011; **52**: 3784-3791 [PMID: 21310918 DOI: 10.1167/iovs.10-6386]

79 **Fiedler U**, Reiss Y, Scharpfenecker M, Grunow V, Koidl S, Thurston G, Gale NW, Witzenrath M, Rosseau S, Suttorp N, Sobke A, Herrmann M, Preissner KT, Vajkoczy P, Augustin HG. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. *Nat Med* 2006; **12**: 235-239 [PMID: 16462802 DOI: 10.1038/nm1351]

80 **Huang H**, Lai JY, Do J, Liu D, Li L, Del Rosario J, Doppalapudi VR, Pirie-Shepherd S, Levin N, Bradshaw C, Woodnutt G, Lappe R, Bhat A. Specifically targeting angiopoietin-2 inhibits angiogenesis, Tie2-expressing monocyte infiltration, and tumor growth. *Clin Cancer Res* 2011; **17**: 1001-1011 [PMID: 21233403 DOI: 10.1158/1078-0432.CCR-10-2317]

81 **Khokha R**, Murthy A, Weiss A. Metalloproteinases and their natural inhibitors in inflammation and immunity. *Nat Rev Immunol* 2013; **13**: 649-665 [PMID: 23969736 DOI: 10.1038/nri3499]

82 **Parks WC**, Wilson CL, López-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol* 2004; **4**: 617-629 [PMID: 15286728 DOI: 10.1038/nri1418]

83 **Kessenbrock K**, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010; **141**: 52-67 [PMID: 20371345]

84 **Jin M**, Kashiwagi K, Iizuka Y, Tanaka Y, Imai M, Tsukahara S. Matrix metalloproteinases in human diabetic and nondiabetic vitreous. *Retina* 2001; **21**: 28-33 [PMID: 11217926 DOI: 10.1097/00006982-200102000-00005]

85 **Giebel SJ**, Menicucci G, McGuire PG, Das A. Matrix metalloproteinases in early diabetic retinopathy and their role in alteration of the blood-retinal barrier. *Lab Invest* 2005; **85**: 597-607 [PMID: 15711567 DOI: 10.1038/labinvest.3700251]

86 **Navaratna D**, McGuire PG, Menicucci G, Das A. Proteolytic degradation of VE-cadherin alters the blood-retinal barrier in diabetes. *Diabetes* 2007; **56**: 2380-2387 [PMID: 17536065 DOI: 10.2337/db06-1694]

87 **Frey T**, Antonetti DA. Alterations to the blood-retinal barrier in diabetes: cytokines and reactive oxygen species. *Antioxid Redox Signal* 2011; **15**: 1271-1284 [PMID: 21294655 DOI: 10.1089/ars.2011.3906]

88 **Allport JR**, Muller WA, Luscinskas FW. Monocytes induce reversible focal changes in vascular endothelial cadherin complex during transendothelial migration under flow. *J Cell Biol* 2000; **148**: 203-216 [PMID: 10629229 DOI: 10.1083/jcb.148.1.203]

89 **Elner SG**, Elner VM, Jaffe GJ, Stuart A, Kunkel SL, Strieter RM. Cytokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. *Curr Eye Res* 1995; **14**: 1045-1053 [PMID: 8585935 DOI: 10.3109/02713689508998529]

90 **Abu el-Asrar AM**, Van Damme J, Put W, Veckeneer M, Dralands L, Billiau A, Missotten L. Monocyte chemotactic protein-1 in proliferative vitreoretinal disorders. *Am J Ophthalmol* 1997; **123**: 599-606 [PMID: 9152065]

91 **Bian ZM**, Elner VM, Yoshida A, Kunkel SL, Elner SG. Signaling pathways for glycated human serum albumin-induced IL-8 and MCP-1 secretion in human RPE cells. *Invest Ophthalmol Vis Sci* 2001; **42**: 1660-1668 [PMID: 11381075]

92 **Katakami N**, Matsuhisa M, Kaneto H, Matsuoka TA, Imamura K, Ishibashi F, Kanda T, Kawai K, Osonoi T, Kashiwagi A, Kawamori R, Shimomura I, Yamasaki Y. Monocyte chemoattractant protein-1 (MCP-1) gene polymorphism as a potential risk factor for diabetic retinopathy in Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract* 2010; **89**: e9-12 [PMID: 20488574 DOI: 10.1016/j.diabres.2010.04.006]

93 **Rangasamy S**, McGuire PG, Das A. Diabetic retinopathy and inflammation: novel therapeutic targets. *Middle East Afr J Ophthalmol* 2012; **19**: 52-59 [PMID: 22346115 DOI: 10.4103/0974-9233.92116]

94 **Ibrahim AS**, El-Shishtawy MM, Peña A, Liou GI. Genistein attenuates retinal inflammation associated with diabetes by targeting of microglial activation. *Mol Vis* 2010; **16**: 2033-2042 [PMID: 21042558]

95 **Elsherbiny NM**, Al-Gayyar MM. Adenosine receptors: new therapeutic targets for inflammation in diabetic nephropathy. *Inflamm Allergy Drug Targets* 2013; **12**: 153-161 [PMID: 23621447 DOI: 10.2174/1871528111312030001]

96 **Awad AS**, Huang L, Ye H, Duong ET, Bolton WK, Linden J, Okusa MD. Adenosine A2A receptor activation attenuates inflammation and injury in diabetic nephropathy. *Am J Physiol Renal Physiol* 2006; **290**: F828-F837 [PMID: 16332931 DOI: 10.1152/ajprenal.00310.2005]

97 **Kanamori H**, Matsubara T, Mima A, Sumi E, Nagai K, Takahashi T, Abe H, Iehara N, Fukatsu A, Okamoto H, Kita T, Doi T, Arai H. Inhibition of MCP-1/CCR2 pathway ameliorates the development of diabetic nephropathy. *Biochem Biophys Res Commun* 2007; **360**: 772-777 [PMID: 17631861 DOI: 10.1016/j.bbrc.2007.06.148]

98 **Chow FY**, Nikolic-Paterson DJ, Ozols E, Atkins RC, Rollin BJ, Tesch GH. Monocyte chemoattractant protein-1 promotes the development of diabetic renal injury in streptozotocin-treated mice. *Kidney Int* 2006; **69**: 73-80 [PMID: 16374426 DOI: 10.1038/sj.ki.5000014]

99 **Chow FY**, Nikolic-Paterson DJ, Ma FY, Ozols E, Rollins BJ, Tesch GH. Monocyte chemoattractant protein-1-induced tissue inflammation is critical for the development of renal injury but not type 2 diabetes in obese db/db mice. *Diabetologia* 2007; **50**: 471-480 [PMID: 17160673 DOI: 10.1007/s00125-006-0497-8]

100 **Han J**, Thompson P, Beutler B. Dexamethasone and pentoxifylline inhibit endotoxin-induced cachectin/tumor necrosis factor synthesis at separate points in the signaling pathway. *J Exp Med* 1990; **172**: 391-394 [PMID: 2358784 DOI: 10.1084/jem.172.1.391]

101 **Harmankaya O**, Seber S, Yilmaz M. Combination of pentoxifylline with angiotensin converting enzyme inhibitors produces an additional reduction in microalbuminuria in hypertensive type 2 diabetic patients. *Ren Fail* 2003; **25**: 465-470 [PMID: 12803510 DOI: 10.1081/JDI-120021159]

102 **Navarro JF**, Mora C, Muros M, García J. Additive antiproteinuric effect of pentoxifylline in patients with type 2 diabetes under angiotensin II receptor blockade: a short-term, randomized, controlled trial. *J Am Soc Nephrol* 2005; **16**: 2119-2126 [PMID: 15917336 DOI: 10.1681/ASN.2005010001]

103 **Leyva-Jiménez R**, Rodríguez-Orozco AR, Ortega-Pierres LE, Ramírez-Enríquez J, Gómez-García A, Alvarez-Aguilar C. [Effect of pentoxifylline on the evolution of diabetic nephropathy]. *Med Clin* (Barc) 2009; **132**: 772-778 [PMID: 19464709 DOI: 10.1016/j.medcli.2008.05.024]

104 **Makino H**, Miyamoto Y, Sawai K, Mori K, Mukoyama M, Nakao K, Yoshimasa Y, Suga S. Altered gene expression related to glomerulogenesis and podocyte structure in early

diabetic nephropathy of db/db mice and its restoration by pioglitazone. *Diabetes* 2006; **55**: 2747-2756 [PMID: 17003339 DOI: 10.2337/db05-1683]

105 **Kamal F**, Yanakieva-Georgieva N, Piao H, Morioka T, Oite T. Local delivery of angiotensin II receptor blockers into the kidney passively attenuates inflammatory reactions during the early phases of streptozotocin-induced diabetic nephropathy through inhibition of calpain activity. *Nephron Exp Nephrol* 2010; **115**: e69-e79 [PMID: 20424485 DOI: 10.1159/000313832]

106 **Wu J**, Guan TJ, Zheng S, Grosjean F, Liu W, Xiong H, Gordon R, Vlassara H, Striker GE, Zheng F. Inhibition of inflammation by pentosan polysulfate impedes the development and progression of severe diabetic nephropathy in aging C57B6 mice. *Lab Invest* 2011; **91**: 1459-1471 [PMID: 21808238 DOI: 10.1038/labinvest.2011.93]

107 **Shepherd J**, Kastelein JJ, Bittner V, Deedwania P, Breazna A, Dobson S, Wilson DJ, Zuckerman A, Wenger NK. Intensive lipid lowering with atorvastatin in patients with coronary heart disease and chronic kidney disease: the TNT (Treating to New Targets) study. *J Am Coll Cardiol* 2008; **51**: 1448-1454 [PMID: 18402899 DOI: 10.1016/j.jacc.2007.11.072]

108 **Brouwers FP**, Asselbergs FW, Hillege HL, de Boer RA, Gansevoort RT, van Veldhuisen DJ, van Gilst WH. Long-term effects of fosinopril and pravastatin on cardiovascular events in subjects with microalbuminuria: Ten years of follow-up of Prevention of Renal and Vascular End-stage Disease Intervention Trial (PREVEND IT). *Am Heart J* 2011; **161**: 1171-1178 [PMID: 21641365 DOI: 10.1016/j.ahj.2011.03.028]

109 **Lieberthal W**, Levine JS. The role of the mammalian target of rapamycin (mTOR) in renal disease. *J Am Soc Nephrol* 2009; **20**: 2493-2502 [PMID: 19875810 DOI: 10.1681/ASN.2008111186]

110 **Nagai K**, Matsubara T, Mima A, Sumi E, Kanamori H, Iehara N, Fukatsu A, Yanagita M, Nakano T, Ishimoto Y, Kita T, Doi T, Arai H. Gas6 induces Akt/mTOR-mediated mesangial hypertrophy in diabetic nephropathy. *Kidney Int* 2005; **68**: 552-561 [PMID: 16014032 DOI: 10.1111/j.1523-1755.2005.00433.x]

111 **Sakaguchi M**, Isono M, Isshiki K, Sugimoto T, Koya D, Kashiwagi A. Inhibition of mTOR signaling with rapamycin attenuates renal hypertrophy in the early diabetic mice. *Biochem Biophys Res Commun* 2006; **340**: 296-301 [PMID: 16364254 DOI: 10.1016/j.bbrc.2005.12.012]

112 **Yang Y**, Wang J, Qin L, Shou Z, Zhao J, Wang H, Chen Y, Chen J. Rapamycin prevents early steps of the development of diabetic nephropathy in rats. *Am J Nephrol* 2007; **27**: 495-502 [PMID: 17671379 DOI: 10.1159/000106782]

113 **Hopper AH**, Tindall H, Davies JA. Administration of aspirin-dipyridamole reduces proteinuria in diabetic nephropathy. *Nephrol Dial Transplant* 1989; **4**: 140-143 [PMID: 2496357]

114 **Mulay SR**, Gaikwad AB, Tikoo K. Combination of aspirin with telmisartan suppresses the augmented TGFbeta/smad signaling during the development of streptozotocin-induced type I diabetic nephropathy. *Chem Biol Interact* 2010; **185**: 137-142 [PMID: 20223228 DOI: 10.1016/j.cbi.2010.03.008]

115 **Cheng HF**, Wang CJ, Moeckel GW, Zhang MZ, McKanna JA, Harris RC. Cyclooxygenase-2 inhibitor blocks expression of mediators of renal injury in a model of diabetes and hypertension. *Kidney Int* 2002; **62**: 929-939 [PMID: 12164875 DOI: 10.1046/j.1523-1755.2002.00520.x]

116 **Sinsakul M**, Sika M, Rodby R, Middleton J, Shyr Y, Chen H, Han E, Lehrich R, Clyne S, Schulman G, Harris R, Lewis J. A randomized trial of a 6-week course of celecoxib on proteinuria in diabetic kidney disease. *Am J Kidney Dis* 2007; **50**: 946-951 [PMID: 18037095 DOI: 10.1053/j.ajkd.2007.09.005]

117 **Mima A**, Hiraoka-Yamomoto J, Li Q, Kitada M, Li C, Geraldes P, Matsumoto M, Mizutani K, Park K, Cahill C, Nishikawa S, Rask-Madsen C, King GL. Protective effects of GLP-1 on glomerular endothelium and its inhibition by PKCβ activation in diabetes. *Diabetes* 2012; **61**: 2967-2979 [PMID: 22826029 DOI: 10.2337/db11-1824]

118 **Pieper GM**. Activation of nuclear factor-kappaB in cultured endothelial cells by increased glucose concentration: prevention by calphostin C. *J Cardiovasc Pharmacol* 1997; **30**: 528-532 [PMID: 9335415 DOI: 10.1097/00005344-199710000-00019]

119 **Yerneni KK**, Bai W, Khan BV, Medford RM, Natarajan R. Hyperglycemia-induced activation of nuclear transcription factor kappaB in vascular smooth muscle cells. *Diabetes* 1999; **48**: 855-864 [PMID: 10102704 DOI: 10.2337/diabetes.48.4.855]

120 **Mima A**, Ohshiro Y, Kitada M, Matsumoto M, Geraldes P, Li C, Li Q, White GS, Cahill C, Rask-Madsen C, King GL. Glomerular-specific protein kinase C-β-induced insulin receptor substrate-1 dysfunction and insulin resistance in rat models of diabetes and obesity. *Kidney Int* 2011; **79**: 883-896 [PMID: 21228767 DOI: 10.1038/ki.2010.526]

121 **Ohshiro Y**, Ma RC, Yasuda Y, Hiraoka-Yamamoto J, Clermont AC, Isshiki K, Yagi K, Arikawa E, Kern TS, King GL. Reduction of diabetes-induced oxidative stress, fibrotic cytokine expression, and renal dysfunction in protein kinase Cbeta-null mice. *Diabetes* 2006; **55**: 3112-3120 [PMID: 17065350 DOI: 10.2337/db06-0895]

122 **Gilbert RE**, Kim SA, Tuttle KR, Bakris GL, Toto RD, McGill JB, Haney DJ, Kelly DJ, Anderson PW. Effect of ruboxistaurin on urinary transforming growth factor-beta in patients with diabetic nephropathy and type 2 diabetes. *Diabetes Care* 2007; **30**: 995-996 [PMID: 17229944 DOI: 10.2337/dc06-2079]

123 **Filippatos TD**, Elisaf MS. Effects of glucagon-like peptide-1 receptor agonists on renal function. *World J Diabetes* 2013; **4**: 190-201 [PMID: 24147203 DOI: 10.4239/wjd.v4.i5.190]

124 **Mima A**, Kitada M, Geraldes P, Li Q, Matsumoto M, Mizutani K, Qi W, Li C, Leitges M, Rask-Madsen C, King GL. Glomerular VEGF resistance induced by PKCδ/SHP-1 activation and contribution to diabetic nephropathy. *FASEB J* 2012; **26**: 2963-2974 [PMID: 22499584 DOI: 10.1096/fj.11-202994]

125 **Satomi N**, Sakurai A, Haranaka K. Relationship of hypoglycemia to tumor necrosis factor production and antitumor activity: role of glucose, insulin, and macrophages. *J Natl Cancer Inst* 1985; **74**: 1255-1260 [PMID: 3889459 DOI: 10.1093/jnci/74.6.1255]

126 **Aljada A**, Ghanim H, Saadeh R, Dandona P. Insulin inhibits NFkappaB and MCP-1 expression in human aortic endothelial cells. *J Clin Endocrinol Metab* 2001; **86**: 450-453 [PMID: 11232040 DOI: 10.1210/jc.86.1.450]

127 **Antoniades C**, Tousoulis D, Marinou K, Papageorgiou N, Bosinakou E, Tsioufis C, Stefanadi E, Latsios G, Tentolouris C, Siasos G, Stefanadis C. Effects of insulin dependence on inflammatory process, thrombotic mechanisms and endothelial function, in patients with type 2 diabetes mellitus and coronary atherosclerosis. *Clin Cardiol* 2007; **30**: 295-300 [PMID: 17551966 DOI: 10.1002/clc.20101]

128 **Ye SD**, Zheng M, Zhao LL, Qian Y, Yao XM, Ren A, Li SM, Jing CY. Intensive insulin therapy decreases urinary MCP-1 and ICAM-1 excretions in incipient diabetic nephropathy. *Eur J Clin Invest* 2009; **39**: 980-985 [PMID: 19663918 DOI: 10.1111/j.1365-2362.2009.02203.x]

129 **Mima A**. Inflammation and oxidative stress in diabetic nephropathy: new insights on its inhibition as new therapeutic targets. *J Diabetes Res* 2013; **2013**: 248563 [PMID: 23862164 DOI: 10.1155/2013/248563]

130 **Montagnani M**, Chen H, Barr VA, Quon MJ. Insulin-stimulated activation of eNOS is independent of Ca2+ but requires phosphorylation by Akt at Ser (1179). *J Biol Chem* 2001; **276**: 30392-30398 [PMID: 11402048 DOI: 10.1074/jbc.M103702200]

131 **Kuboki K**, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, Feener EP, Herbert TP, Rhodes CJ, King GL. Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo : a specific vascular action of insulin. *Circulation* 2000; **101**: 676-681 [PMID: 10673261 DOI: 10.1161/01.CIR.101.6.676]

132 **Vicent D**, Ilany J, Kondo T, Naruse K, Fisher SJ, Kisanuki

YY, Bursell S, Yanagisawa M, King GL, Kahn CR. The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *J Clin Invest* 2003; **111**: 1373-1380 [PMID: 12727929 DOI: 10.1172/JCI15211]

133 **Rask-Madsen C**, Li Q, Freund B, Feather D, Abramov R, Wu IH, Chen K, Yamamoto-Hiraoka J, Goldenbogen J, Sotiropoulos KB, Clermont A, Geraldes P, Dall'Osso C, Wagers AJ, Huang PL, Rekhter M, Scalia R, Kahn CR, King GL. Loss of insulin signaling in vascular endothelial cells accelerates atherosclerosis in apolipoprotein E null mice. *Cell Metab* 2010; **11**: 379-389 [PMID: 20444418 DOI: 10.1016/j.cmet.2010.03.013]

134 **Guan Y**, Breyer MD. Peroxisome proliferator-activated receptors (PPARs): novel therapeutic targets in renal disease. *Kidney Int* 2001; **60**: 14-30 [PMID: 11422732 DOI: 10.1046/j.1523-1755.2001.00766.x]

135 **Desvergne B**, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 1999; **20**: 649-688 [PMID: 10529898 DOI: 10.1210/edrv.20.5.0380]

136 **Willson TM**, Lambert MH, Kliewer SA. Peroxisome proliferator-activated receptor gamma and metabolic disease. *Annu Rev Biochem* 2001; **70**: 341-367 [PMID: 11395411 DOI: 10.1146/annurev.biochem.70.1.341]

137 **Kota BP**, Huang TH, Roufogalis BD. An overview on biological mechanisms of PPARs. *Pharmacol Res* 2005; **51**: 85-94 [PMID: 15629253 DOI: 10.1016/j.phrs.2004.07.012]

138 **Ko GJ**, Kang YS, Han SY, Lee MH, Song HK, Han KH, Kim HK, Han JY, Cha DR. Pioglitazone attenuates diabetic nephropathy through an anti-inflammatory mechanism in type 2 diabetic rats. *Nephrol Dial Transplant* 2008; **23**: 2750-2760 [PMID: 18388116 DOI: 10.1093/ndt/gfn157]

139 **Park CW**, Kim HW, Ko SH, Lim JH, Ryu GR, Chung HW, Han SW, Shin SJ, Bang BK, Breyer MD, Chang YS. Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in db/db mice. *J Am Soc Nephrol* 2007; **18**: 1227-1238 [PMID: 17360951 DOI: 10.1681/ASN.2006070778]

140 **Erdogdu O**, Nathanson D, Sjöholm A, Nyström T, Zhang Q. Exendin-4 stimulates proliferation of human coronary artery endothelial cells through eNOS-, PKA- and PI3K/Akt-dependent pathways and requires GLP-1 receptor. *Mol Cell Endocrinol* 2010; **325**: 26-35 [PMID: 20452396 DOI: 10.1016/j.mce.2010.04.022]

141 **Kodera R**, Shikata K, Kataoka HU, Takatsuka T, Miyamoto S, Sasaki M, Kajitani N, Nishishita S, Sarai K, Hirota D, Sato C, Ogawa D, Makino H. Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. *Diabetologia* 2011; **54**: 965-978 [PMID: 21253697 DOI: 10.1007/s00125-010-2028-x]

142 **Liu WJ**, Xie SH, Liu YN, Kim W, Jin HY, Park SK, Shao YM, Park TS. Dipeptidyl peptidase IV inhibitor attenuates kidney injury in streptozotocin-induced diabetic rats. *J Pharmacol Exp Ther* 2012; **340**: 248-255 [PMID: 22025647 DOI: 10.1124/jpet.111.186866]

143 **Groop PH**, Cooper ME, Perkovic V, Emser A, Woerle HJ, von Eynatten M. Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. *Diabetes Care* 2013; **36**: 3460-3468 [PMID: 24026560 DOI: 10.2337/dc13-0323]

144 **Tarantino G**, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? *World J Gastroenterol* 2013; **19**: 3375-3384 [PMID: 23801829 DOI: 10.3748/wjg.v19.i22.3375]

145 **Finelli C**, Tarantino G. What is the role of adiponectin in obesity related non-alcoholic fatty liver disease? *World J Gastroenterol* 2013; **19**: 802-812 [PMID: 23430039 DOI: 10.3748/wjg.v19.i6.802]

146 **Massip-Salcedo M**, Zaouali MA, Padrissa-Altés S, Casillas-Ramirez A, Rodés J, Roselló-Catafau J, Peralta C. Activation of peroxisome proliferator-activated receptor-alpha inhibits the injurious effects of adiponectin in rat steatotic liver undergoing ischemia-reperfusion. *Hepatology* 2008; **47**: 461-472 [PMID: 18098300 DOI: 10.1002/hep.21935]

147 **Walter R**, Wanninger J, Bauer S, Eisinger K, Neumeier M, Weiss TS, Amann T, Hellerbrand C, Schäffler A, Schölmerich J, Buechler C. Adiponectin reduces connective tissue growth factor in human hepatocytes which is already induced in non-fibrotic non-alcoholic steatohepatitis. *Exp Mol Pathol* 2011; **91**: 740-744 [PMID: 21946149 DOI: 10.1016/j.yexmp.2011.09.006]

148 **Finelli C**, Tarantino G. Have guidelines addressing physical activity been established in nonalcoholic fatty liver disease? *World J Gastroenterol* 2012; **18**: 6790-6800 [PMID: 23239917 DOI: 10.3748/wjg.v18.i46.6790]

149 **Di Minno MN**, Russolillo A, Lupoli R, Ambrosino P, Di Minno A, Tarantino G. Omega-3 fatty acids for the treatment of non-alcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 5839-5847 [PMID: 23139599 DOI: 10.3748/wjg.v18.i41.5839]

150 **Panasiuk A**, Dzieciol J, Panasiuk B, Prokopowicz D. Expression of p53, Bax and Bcl-2 proteins in hepatocytes in non-alcoholic fatty liver disease. *World J Gastroenterol* 2006; **12**: 6198-6202 [PMID: 17036395]

151 **Tarantino G**, Scopacasa F, Colao A, Capone D, Tarantino M, Grimaldi E, Savastano S. Serum Bcl-2 concentrations in overweight-obese subjects with nonalcoholic fatty liver disease. *World J Gastroenterol* 2011; **17**: 5280-5288 [PMID: 22219597 DOI: 10.3748/wjg.v17.i48.5280]

152 **Tarantino G**, Caputi A. JNKs, insulin resistance and inflammation: A possible link between NAFLD and coronary artery disease. *World J Gastroenterol* 2011; **17**: 3785-3794 [PMID: 21987620 DOI: 10.3748/WJG.V17.I33.3785]

153 **Tarantino G**, Savastano S, Colao A. Hepatic steatosis, low-grade chronic inflammation and hormone/growth factor/adipokine imbalance. *World J Gastroenterol* 2010; **16**: 4773-4783 [PMID: 20939105 DOI: 10.3748/WJG.V16.I38.4773]

154 **Di Minno MN**, Tufano A, Rusolillo A, Di Minno G, Tarantino G. High prevalence of nonalcoholic fatty liver in patients with idiopathic venous thromboembolism. *World J Gastroenterol* 2010; **16**: 6119-6122 [PMID: 21182227 DOI: 10.3748/WJG.V16.I48.6119]

P- Reviewer: Bandeira E, Paraskevas KI, Tarantino G
S- Editor: Ji FF **L- Editor**: Webster JR **E- Editor**: Liu SQ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.711

World J Diabetes 2014 October 15; 5(5): 711-716
ISSN 1948-9358 (online)

HLA alleles may serve as a tool to discriminate atypical type 2 diabetic patients

Mariana Fernández, Matías Fabregat, Gerardo Javiel, Adriana Mimbacas

Mariana Fernández, Matías Fabregat, Gerardo Javiel, Adriana Mimbacas, Biodiversity and Genetic Department, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, CP 11600, Uruguay
Gerardo Javiel, Unit of Diabetes Hospital Pasteur, ASSE-Ministry of Public Health, Montevideo, CP 11400, Uruguay
Gerardo Javiel, Diabetologyc Service of Private Health Center, Centro de Asistencia del Sindicato Médico del Uruguay (CASMU), Montevideo, CP 11600, Uruguay
Author contributions: Fernández M and Fabregat M performed the experiment and were also involved in editing the manuscript; Javiel G and Mimbacas A co-ordinated and provided the collection of all human material in addition to providing financial support; Fernández M and Mimbacas A designed the study.
Correspondence to: Adriana Mimbacas, PhD, Biodiversity and Genetic Department, Instituto de Investigaciones Biológicas Clemente Estable, Avenida Italia 3318, Montevideo, CP 11600, Uruguay. amimbacas@iibce.edu.uy
Telephone: +598-2-4861417 Fax: +598-2-4875548
Received: February 27, 2014 Revised: May 1, 2014
Accepted: July 18, 2014
Published online: October 15, 2014

Abstract

AIM: To investigate whether the presence of human leukocyte antigen (HLA) marker could add new information to discriminated atypical diabetic type 2 patients.

METHODS: We analyzed 199 patients initially diagnosed as type 2 diabetes who are treated in special care diabetes clinics (3rd level). This population was classified in "atypical" (sample A) and "classic" (sample B) according to HLA typing. We consider "classic patient" when has absence of type 1 diabetes associated HLA alleles and no difficulties in their diagnosis and treatments. By the other hand, we considered "atypical patient" when show type 1 diabetes associated HLA alleles and difficulties in their diagnosis and treatments. The standard protocol Asociacion Latinoamericana de Diabetes 2006 was used for patients follow up. To analyze differences between both populations in paraclinical parameters we used unpaired *t* tests and contingence tables. Bivariate and multivariate analyses were carried out using the SPSS software program. In all studies we assume differences statistically significant, with a *P*-value < 0.05 corrected and 95%CI.

RESULTS: The typing HLA in the "atypical" populations show that 92.47% patients presented at list one type 1 diabetes associated HLA alleles (DQB1*0201-0302 and DR 3-4) and 7.53% had two of its. The results showed for categorical variables (family history, presence or absence of hypertension and/or dyslipidemia, reason for initial consultation) the only difference found was at dyslipidemia (OR = 0.45, 0.243 < OD < 0.822 (*P* < 0.001). In relation to continuous variables we found significant differences between atypical *vs* classic only in cholesterol (5.07 ± 1.1 *vs* 5.56 ± 1.5, *P* < 0.05), high density lipoproteins (1.23 ± 0.3 *vs* 1.33 ± 0.3, *P* < 0.05) and low density lipoproteins (2.86 ± 0.9 *vs* 3.38 ± 1.7, *P* < 0.01). None of the variables had discriminating power when logistic regression was done.

CONCLUSION: We propose an algorithm including HLA genotyping as a tool to discriminate atypical patients, complementing international treatment guidelines for complex patients.

Key words: Atypical diabetes; Clinical algorithm; Immunity molecular marker

Core tip: There are evidences that exists a lot of patients who were diagnosed as type 2 diabetics but present difficult management, don't have good responses to treatment and don't achieve the metabolic goals. We include the study of human leukocyte antigen markers typically associated whit type 1 diabetes to characterize these patients. This paper provides information about the possibility of incorporate a standardized molecular

diagnosis in the clinical practice to identify complex or atypical type 2 diabetic patient.

Fernández M, Fabregat M, Javiel G, Mimbacas A. HLA alleles may serve as a tool to discriminate atypical type 2 diabetic patients. *World J Diabetes* 2014; 5(5): 711-716 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/711.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.711

INTRODUCTION

Diabetes mellitus is a chronic disease that requires ongoing medical care to prevent acute complications and reduce the risk of long-term complications. While recognizing two major groups of diabetic patients, type 1 and 2, the clinical presentation and disease progression vary considerably in both types of diabetes. However, ADA Position Statement establishes that there are patients who cannot be classified as type 1 or type 2[1]. The true diagnosis may be more obvious only over time. There is growing evidence that emphasize the existence of a significant overlap between diabetes type 1 and 2[2-12].

Despite the increasing incidence of the disease and the efforts made to establish diagnostic guidelines some patients do not qualify strictly into the given definitions.

Such patients which can be simultaneously classified in more than one group significantly complicate the medical treatment. They generally require the assistance of a multidisciplinary team in second or third level centers. It is in these patients considered "atypical", where it is necessary to deepen the diagnosis with other complementary examinations with additional technologies. In these cases the classical diagnostic markers and risk factors analysis for various chronic complications, are not sufficient by themselves for a clinical differentiation. In a previous paper we found a high proportion of type 2 diabetes patients who presented HLA susceptibility alleles for type 1 diabetes[13]. Therefore, we propose to add the usage of a molecular marker (HLA) to the international standard criteria

According to the ADA type 1 diabetes is strongly associated with specific HLA groups while in type 2 diabetes does not exist this association[14]. Of all of the type 1 diabetes associated genes and regions revealed by different studies, the HLA association remains the strongest by far, with reported ORs ranging from 0.02 to .11 for specific DR-DQ haplotypes[15,16].

The presence of these genetic variants in patients diagnosed as type 2 let us assign them the "atypical" label. We propose this clinical, biochemical and molecular study to keep deepening in the characterization of HLA as a tool for their differentiation.

In this paper we pretend to provide the Clinicians with a tool to identify those patients at atypical presentation in whom the algorithms have not been useful. We present the basis for a possible new algorithm that can contribute to the early identification of these problematic patients.

MATERIALS AND METHODS

Population design

We analyzed a population of 199 patients seen in 3rd level Clinics for Diabetes from two centers: public (Pasteur Hospital) and private initially diagnosed with type 2 diabetes[14]. For the preparation of this study were considered only those patients receiving comprehensive care of their diabetes, following a nutritional plan and presenting a good adherence to physical activity according to their functional ability within the recommendations of Asociacion Latinoamericana de Diabetes (ALAD)/ADA and medicated with one or more oral antidiabetic drugs. In turn, this population was classified based on the presence or absence of type 1 diabetes HLA susceptibility alleles described in the Uruguayan population[13].

Sample A: 93 "atypical" patients that met the following inclusion criteria: (1) Patients who had good adherence to the treatment; (2) They fulfilled the objectives of education and nutrition plans according to international guidelines; (3) Present doubts on classification of diabetic type and/or no good therapeutic response (two consecutive measurements of glicated hemoglobin within three months not reduced in 1.5%[17]) to ADA, ALAD algorithms; and (4) Patients with susceptibility HLA alleles for autoimmune disease. We considered DQB1*0201-0302 and DR 3-4 as susceptible ones in the Uruguayan population[18].

Sample B: 106 "classic" patients fulfilling the same requirements a, b of sample A but which do not have diagnostic doubts, responded to treatment and do not present HLA alleles associated with autoimmune disease.

Patient of both samples who had other endocrine disorders or tumors were excluded.

All subjects were interviewed by medical doctors following ALAD guidelines on diagnosis treatment and control of type 2 diabetes with evidence-based medicine[19].

All patients were assessed for the following items: (1) Family history of diabetes; (2) Personal history: chronological age, age at diagnosis, time of evolution; (3) Motive of initial consultation: patients were categorized into five groups: incidental finding by fasting glucose, oral glucose tolerance test, presence of typical symptoms, acute debut with ketoacidosis without precipitating cause, and patients referred by other specialists for the presence of complications; (4) Presence or absence of classical risk factors associated with type 2 diabetes (hypertension and/or dyslipidemia); (5) Body mass index (BMI) was calculated and categorized according to the World Health Organization[20]: overweight (25-29.9 kg/m^2) and obesity ($\geq$ 30 kg/m^2); and (6) Clinical evaluation and metabolic parameters: glicated hemoglobin, cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides (TG), TG/HDL ratio as insulin-resistance index (> 3)[21,22]. To analyze levels of

Table 1 Reference values (mmol/L) of parameters stratified

Dyslipidemia parameters	Desirable	Limit	Abnormal
Total cholesterol	< 5.2	5.2-6.19	> 6.2
HDL	> 1.2	1.2-0.9	< 0.9
LDL	< 3.4	3.4-4.0	> 4.1
Triglycerides	2.3	2.3-2.99	> 3.0

HDL: High density lipoproteins; LDL: Low density lipoproteins.

Table 2 Phenotypic classification of dyslipidemia

	Total Cholesterol	LDL	Triglycerides	HDL
Hypercholesterolemia	⩾ 6.2	⩾ 4.1	< 2.3	
Combined hyperlipidemia		⩾ 4.1	⩾ 2.3	
Hipo alfa lipoproteinemia		> 4	< 2.3	< 1

Reference values (mmol/L). HDL: High density lipoproteins; LDL: Low density lipoproteins.

Table 3 Clinical characteristics expressed by media and standard deviation

	Sample A n = 93	Sample B n = 106	P value
Age (yr)	62.01 ± 11.65	66.02 ± 9.55	0.060
Age onset (yr)	47.18 ± 12.61	49.54 ± 10.13	0.131
Years of evolution	16.41 ± 9.72	15.45 ± 9.22	0.528
BMI (kg/mts^2)	32.07 ± 5.26	31.45 ± 5.95	0.430
HbA1c (%)[1]	8.31 ± 1.87	8.16 ± 1.65	0.545
Total cholesterol (nmol/L)	5.07 ± 1.1	5.56± 1.5	0.010[a]
HDL (nmol/L)	1.23 ± 0.3	1.33 ± 0.3	0.010[a]
LDL (nmol/L)	2.86 ± 0.9	3.38 ± 1.7	0.009[b]
Triglycerides (nmol/L)	2.29 ± 1.4	2.81 ± 0.9	0.864
TG/HDL	2.10 ± 1.5	1.96 ± 2.0	0.572

[1]At beginning of the study. Sample A *vs* Sample B: [a]$P < 0.05$, [b]$P < 0.01$ for all parameters. BMI: Body mass index; LDL: Low-density lipoprotein-cholesterol; HDL: High-density lipoprotein-cholesterol; TG/HDL: Insulin resistance index; HbA1c (%): Glycated hemoglobin percentage.

Table 4 χ^2 analysis

	OR	95%CI	P value
Dyslipidemia	0.45	0.24-0.82	< 0.01
Total cholesterol	0.48	0.27-0.84	< 0.01
HDL	1.84	1.04-3.23	< 0.05
LDL	0.50	0.28-0.91	< 0.05

LDL: Low-density lipoprotein-cholesterol; HDL: High-density lipoprotein-cholesterol.

dyslipidemia, both samples were stratified according to the 2° Dyslipidemia Consensus in Uruguay (Table 1)[23]. We analyzed the phenotypic classification of dyslipidemia respect to Table 2[24].

Molecular analysis

DNA was obtained from peripheral blood using standard (phenol/chloroform) technique. The HLA typing was performed by reverse ASO technique (Innogenetics Ltd, Belgium, UE).

All patients gave written informed consent and the study protocol was approved by the Ethical Committee of Ministry of Public Health and the corresponding Ethical Committee of each participant Institution.

Statistical analysis

Continuous variables were expressed as the means and standard deviations. Differences between groups were determined by unpaired t tests after checking the normal distribution or converted to normalize of the data. Categorical variables were described using proportion and 2 × 2 contingence table. Bivariate and multivariate analyses were based on dependent variables (two categories sample A, sample B). Logistic regression with all variables was done. All tests were carried out using the SPSS software program. In all studies we assume differences statistically significant, with a P-value < 0.05 corrected and 95%CI.

RESULTS

Population characterizes

The total population consisted of 94 women (47.24%) and 105 men (52.76%). The gender distribution was similar in samples A and B. In the statistical analysis of categorical variables (family history, presence or absence of hypertension and/or dyslipidemia, reason for initial consultation) the only difference found was at dyslipidemia (ODDs 0.45, CI: 0.243-0.822 ($P < 0.001$)). In relation to values of cholesterol, HDL, LDL and TG, only the last parameter not showed statistical differences (Table 3). Subsequently each of these variables was analyzed, separating into classes in accordance to the Uruguayan Dyslipidemia Consensus. Sample A showed a higher proportion of normal values for cholesterol and LDL (55.9% *vs* 37.7%, 70.7% *vs* 54.8%, respectively). In relation to dyslipidemia phenotypic classification, hypercholesterolemia was the only parameters statistically significant: 12.3% atypical patients *vs* 2.2%, classic patients with ODDs 0.07 (CI: 0.009-0.54).

Furthermore, we found that only part of the patients from the sample A (atypical) presented classical risk factors associated with type 2 diabetes (hypertension and/or dyslipidemia).

Analyzing the qualitative variables (Table 4) the only difference found was also in the lipid profile. In relation to BMI no difference between both samples were observed. It is important to point out those only 4 individuals in sample A had a normal weight in spite of having HLA alleles associated with type 1 diabetes.

None of the variables had discriminating power when logistic regression was done. The P value of the χ^2 test was > 0.05.

HLA marker

The typing HLA in the "atypical" populations show that

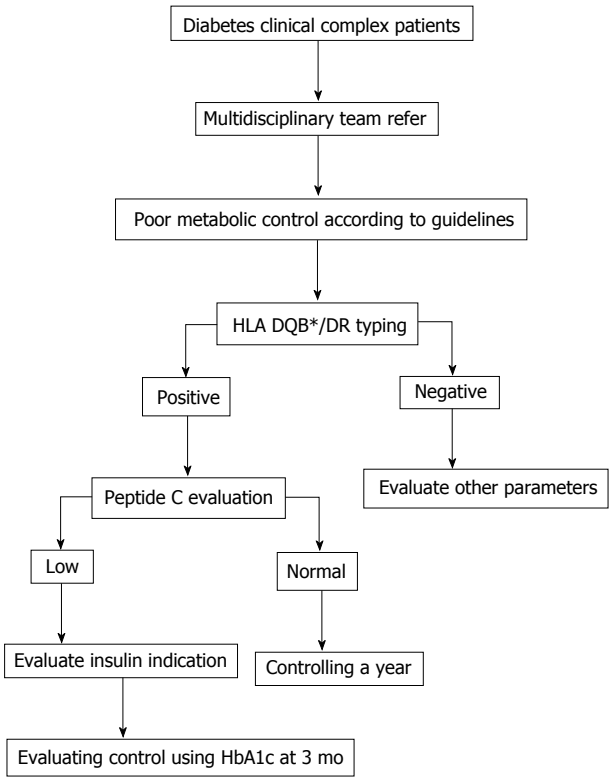

Figure 1 Algorithm for complex diabetes patients with difficulties in diagnosis, evolution, poor therapeutic response where international algorithms have been fulfilled. HbA1c: Glycated hemoglobin.

92.47% patients presented at list one type 1 diabetes associated HLA alleles (DQB1*0201-0302 and DR 3-4) and 7.53% had two of its.

DISCUSSION

The usual elements that are taken into consideration in the diagnosis and treatment of atypical diabetic patients are not sufficient for identify individuals considered "atypical" for presentation, evolution and/or poor therapeutic response according to international guidelines. For this reason, we investigate whether the inclusion of an immunity molecular marker would provide conclusive information that helps the Clinician with an appropriate individualized therapeutic classification in this group of patients.

According to the consensus this marker differentiates two major types of diabetes. Type 1 diabetes is strongly associated with HLA while type 2 diabetes is not[14]. Our study demonstrated that the clinical, biochemical and molecular-genetic characterization of atypical patient population and their comparison with classic type 2 diabetes patients showed that although a few differences were found to be statistically significant, they are not individually sufficient to clarify the situation of each patient. We propose here to add the usage of HLA typing to the international standard criteria.

Despite the enormous efforts that have been made to identify gene variants associated with type 2 diabetes, until present no one has fulfill the expectations to prevent or improve the treatment of diabetes. The addition of the genotypic variants risk score to clinical prediction models, only moderately (minimally) improve the statistical results[25,26]. In a previous paper analyzing the genotype-phenotype relation, observed the existence of a high proportion of patients that despite being classified as type 2 diabetes according to the diagnostic guidelines, they presented HLA alleles strongly associated with type 1 diabetes[27].

The observed statically differences in the lipid profiles of atypical patients are insufficient to define changes in classification, treatment and/or monitoring. In these complex patients usual clinical markers used for diagnosis and for the risk factors analysis for various complications were not sufficient by themselves to differentiate classic type 2 diabetics.

BMI is usually considered as an important marker to differentiate between types of diabetes but, no differences were observed between classical and atypical patients. As in these patients a fast increment of the obesity rate has been observed, the presence of this factor has been considered as an important factor in reducing the described differences between type 1 and 2 diabetes[12]. The presences of overweight or obesity would induce the Clinician not to look for the presence of HLA susceptibility to autoimmune disease. In fact, in the sample of atypical patients only 4 of them had normal weight despite having HLA alleles associated with type 1 diabetes. This finding is not consistent with international classifications where, although there may be exceptions, defines the patient with type 2 diabetes as overweight or with abdominal fat distribution without autoimmunity, while rarely type 1 diabetics are obese[1].

Based on these data, we believe that this molecular marker analysis provide valuable data to clarify these patients. It is also clear that the mere presence of molecular marker is not indicative of the evolution of each patient's disease or how pancreatic reserve presents in each individual.

From the results, we consider that the study should be complemented with the search for other clinical or evolution markers to enable an accurate differentiation. Dosage of peptide C could be a very good parameter to evaluate the stage of beta cell. This factor was not included in this study because it is not standardized in Uruguay.

At present, we have not enough evidence to answer a crucial question on these atypical patients, at what point the genetic study should be done? (1) to debut; (2) after adopt changes in lifestyle and no achieve control objectives were observed; (3) after 6 mo of no response to treatment plan indicated by international guidelines; and (4) at any time of evolution. We think that is important know the genotype of the patient when, after adjusting nutritional plan and changes in lifestyle, no clinical improvements were observed. This question should be answered with new evidence that address the issues raised in this work.

Here, we simply propose a new tool for the Clinician. We are aware that the genetic typing of HLA is a costly analysis but, the information presented here justifies its implementation in a very specific group of patients. From our point of view, the addition of such study to the actually used algorithm would clearly help to Clinicians in making a different evaluation of atypical patients (Figure 1).

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

COMMENTS

Background

The type 2 diabetes is a pathology that represents serious challengers for the clinicians. Now a day more and more patient cannot be classified in neither of the type 1 or type 2 diabetes. Their clinical presentation, evolution and difficulty in achieving therapeutic goals make them atypical patients. According to previous data most atypical patients, classically diagnosed as type 2 diabetes, show type 1 diabetes associated human leukocyte antigen (HLA) alleles. These genetics variants can appear like markers for atypical type 2 diabetic patients.

Research frontiers

The HLA marker was classically associated whit type 1 diabetes. Their presence in diabetic type 2 patient is reported in about 10%. This study show that type 2 diabetic patients with the same adherence to indications and treatments will have a different develop of their disease when have type 1 diabetes associated HLA alleles. The authors try to demonstrate that this marker is a potentially way to differentiate patient who will be out of guise of treatments, in response to drugs and in achieve metabolic goals.

Innovations and breakthroughs

Recent reports have highlighted the importance of improve the knowledge of type 2 diabetes, their etiology, diagnosis and treatments. The global grow tendency of this pathology and the difficulties observed in this treatments makes experts check over algorithm for a good follow up of this patients. This is the first study to report a standardized marker to include in the algorithm in order to identify uncharacteristically type 2 diabetics.

Applications

By understanding how the development of the type 2 diabetes in atypical patients is the authors have to recognize them early. This study may represent a future strategy for discriminates them and use the guides of treatments in an individualized way.

Terminology

HLA marker implicates genetics variants which had being studding since a lot of year for their associated to autoimmunity showed for type 1 diabetes. There are susceptibility and protectant alleles. Non-surprisingly, these variants were reported in type 2 diabetes but are unknown there influence in the development of the pathology.

Peer review

Overall an interesting manuscript, which helps to shed some discriminatory light on a growing sub-population of diabetic patients who cannot be readily classified as type 1 or type 2 based upon their medical history and metabolic profile.

REFERENCES

1 **American Diabetes Association**. Standards of medical care in diabetes--2012. *Diabetes Care* 2012; **35** Suppl 1: S11-S63 [PMID: 22187469 DOI: 10.2337/dc12-s011]

2 **Naik RG**, Brooks-Worrell BM, Palmer JP. Latent autoimmune diabetes in adults. *J Clin Endocrinol Metab* 2009; **94**: 4635-4644 [PMID: 19837918 DOI: 10.1210/jc.2009-1120]

3 **Libman IM**, Becker DJ. Coexistence of type 1 and type 2 diabetes mellitus: "double" diabetes? *Pediatr Diabetes* 2003; **4**: 110-113 [PMID: 14655267 DOI: 10.1034/j.1399-5448.2003.00012.x]

4 **Kolb H**, Mandrup-Poulsen T. An immune origin of type 2 diabetes? *Diabetologia* 2005; **48**: 1038-1050 [PMID: 15864529 DOI: 10.1007/s00125-005-1764-9]

5 **Tuomi T**. Type 1 and type 2 diabetes: what do they have in common? *Diabetes* 2005; **54** Suppl 2: S40-S45 [PMID: 16306339 DOI: 10.2337/diabetes.54.suppl_2.S40]

6 **Pozzilli P**, Buzzetti R. A new expression of diabetes: double diabetes. *Trends Endocrinol Metab* 2007; **18**: 52-57 [PMID: 17208448 DOI: 10.1016/j.tem.2006.1]

7 **Pozzilli P**, Guglielmi C, Pronina E, Petraikina E. Double or hybrid diabetes associated with an increase in type 1 and type 2 diabetes in children and youths. *Pediatr Diabetes* 2007; **8** Suppl 9: 88-95 [PMID: 17991137 DOI: 10.1111/j.1399-5448.2007.00338.x]

8 **Mimbacas A**, Javiel G. Diabetes type 1 and type 2: What is behind a classification? In: Chih-Pin Liu eds. Type 1 Diabetes - Complications, Pathogenesis, and Alternative Treatments, 2011: 287-304

9 **Mimbacas A**, Vitarella G, Souto J, Reyes AL, Farias J, Fernandez M, Fabregat M, Javiel G. The phenotype masks the genotype: a possible new Diabetes expression. *J Pediatr Genet* 2012; **2**: 131–134

10 **Stone MA**, Camosso-Stefinovic J, Wilkinson J, de Lusignan S, Hattersley AT, Khunti K. Incorrect and incomplete coding and classification of diabetes: a systematic review. *Diabet Med* 2010; **27**: 491-497 [PMID: 20536944 DOI: 10.1111/j.1464-5491.2009.02920.x]

11 **Inzucchi SE**, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**: 1364-1379 [PMID: 22517736 DOI: 10.2337/dc12-0413]

12 **NHS**. Royal College of General Practitioners. Available from: URL: http: //www.webicina.com/

13 **Mimbacas A**, García L, Zorrilla P, Acosta M, Airaudo C, Ferrero R, Pena A, Simonelli B, Soto E, Vitarella G, Fernandez J, Javiel G. Genotype and phenotype correlations in diabetic patients in Uruguay. *Genet Mol Res* 2009; **8**: 1352-1358 [PMID: 19937591 DOI: 10.4238/vol8-4gmr667]

14 **American Diabetes Association**. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2011; **34**: s62-S69 [DOI: 10.2337/dc11-S062]

15 **Noble JA**, Erlich HA. Genetics of type 1 diabetes. *Cold Spring Harb Perspect Med* 2012; **2**: a007732 [PMID: 22315720 DOI: 10.1101/cshperspect.a007732]

16 **Erlich H**, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, Mychaleckyj JC, Todd JA, Bonella P, Fear AL, Lavant E, Louey A, Moonsamy P. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. *Diabetes* 2008; **57**: 1084-1092 [PMID: 18252895 DOI: 10.2337/db07-1331]

17 **Nathan DM**, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2009; **32**: 193-203 [PMID: 18945920 DOI: 10.2337/dc08-9025]

18 **Mimbacas A**, Pérez-Bravo F, Hidalgo PC, Javiel G, Pisciottano C, Grignola R, Jorge AM, Gallino JP, Gasagoite J, Cardoso H. Association between diabetes type 1 and DQB1 alleles in a case-control study conducted in Montevideo, Uruguay. *Genet Mol Res* 2003; **2**: 29-35 [PMID: 12917799]

19 **Asociacion Latinoamericana de Diabetes**. 2012 Guias ALAD de diagnóstico, control y tratamiento de la diabetes tipo 2.

Available from: URL: http: //issuu.com/alad-diabetes/docs/guias_alad_2012
20 **World Health Organization**. Global Database on Body Mass Index an interactive surveillance tool for monitoring nutrition transition. 2011. Available from: URL: http: //apps.who.int/bmi/index.jsp
21 **McLaughlin T**, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med* 2003; **139**: 802-809 [PMID: 14623617 DOI: 10.7326/0003-4819-139-10-200311180-00007]
22 **Marotta T**, Russo BF, Ferrara LA. Triglyceride-to-HDL-cholesterol ratio and metabolic syndrome as contributors to cardiovascular risk in overweight patients. *Obesity* (Silver Spring) 2010; **18**: 1608-1613 [PMID: 20019684 DOI: 10.1038/oby.2009.446]
23 2° Dislipidemia Consensus in Uruguay SUDEAT (Sociedad Uruguaya de Artereosclerrosis). 2° Consenso uruguayo sobre dislipidemias. El Taller de Willy, Eds. Montevideo, Uruguay, 1998
24 **Lismann S**. Tratamiento actual de las dislipemia. Available from: URL: http: //www.intramed.net /UserFiles/Files/dislip.pdf
25 **Lyssenko V**, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tuomi T, Berglund G, Altshuler D, Nilsson P, Groop L. Clinical risk factors, DNA variants, and the development of type 2 diabetes. *N Engl J Med* 2008; **359**: 2220-2232 [PMID: 19020324 DOI: 10.1056/NEJMoa0801869]
26 **Vassy JL**, Meigs JB. Is genetic testing useful to predict type 2 diabetes? *Best Pract Res Clin Endocrinol Metab* 2012; **26**: 189-201 [PMID: 22498248 DOI: 10.1016/j.beem.2011.09.002]
27 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2004; **27** Suppl 1: S5-S10 [PMID: 14693921]

P- Reviewer: Romani A, Velasco I, Zhao JB **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.717

World J Diabetes 2014 October 15; 5(5): 717-723
ISSN 1948-9358 (online)

RETROSPECTIVE STUDY

Taste sensitivity, nutritional status and metabolic syndrome: Implication in weight loss dietary interventions

Simona Bertoli, Monica Laureati, Alberto Battezzati, Valentina Bergamaschi, Emanuele Cereda, Angela Spadafranca, Laila Vignati, Ella Pagliarini

Simona Bertoli, Alberto Battezzati, Emanuele Cereda, Angela Spadafranca, Laila Vignati, International Center for the Assessment of Nutritional Status, Department of Food, Environmental and Nutritional Sciences, University of Milan, 20133 Milano, Italy
Monica Laureati, Valentina Bergamaschi, Ella Pagliarini, Department of Food, Environmental and Nutritional Sciences, University of Milan, 20133 Milano, Italy
Author contributions: Bertoli S and Pagliarini E contributed to study design; Laureati M, Bergamaschi V, Cereda E, Spadafranca A and Vignati L contributed to data collection; Cereda E contributed to data analyses; Bertoli S, Laureati M, Battezzati A, Cereda E and Pagliarini E contributed to data interpretation; Laureati M and Cereda E contributed to manuscript drafting; Bertoli S, Battezzati A and Pagliarini E contributed to critical revision of the manuscript; all the authors significantly contributed to the work, read and approved the final version of the manuscript.
Correspondence to: Simona Bertoli, MD, PhD, International Center for the Assessment of Nutritional Status, Department of Food, Environmental and Nutritional Sciences, University of Milan, Via Botticelli 21, 20133 Milan, Italy. simona.bertoli@unimi.it
Telephone: +39-02-50316079 Fax: +39-02-50316077
Received: December 13, 2013 Revised: June 3, 2014
Accepted: June 20, 2014
Published online: October 15, 2014

Abstract

AIM: We investigated the relationship between taste sensitivity, nutritional status and metabolic syndrome and possible implications on weight loss dietary program.

METHODS: Sensitivity for bitter, sweet, salty and sour tastes was assessed by the three-Alternative-Forced-Choice method in 41 overweight (OW), 52 obese (OB) patients and 56 normal-weight matched controls. OW and OB were assessed also for body composition (by impedence), resting energy expenditure (by indirect calorimetry) and presence of metabolic syndrome (MetS) and were prescribed a weight loss diet. Compliance to the weight loss dietary program was defined as adherence to control visits and weight loss $\geqslant$ 5% in 3 mo.

RESULTS: Sex and age-adjusted multiple regression models revealed a significant association between body mass index (BMI) and both sour taste ($P < 0.05$) and global taste acuity score (GTAS) ($P < 0.05$), with lower sensitivity with increasing BMI. This trend in sensitivity for sour taste was also confirmed by the model refitted on the OW/OB group while the association with GTAS was marginally significant ($P = 0.06$). MetS+ subjects presented higher thresholds for salty taste when compared to MetS- patients while no significant difference was detected for the other tastes and GTAS. As assessed by multiple regression model, the association between salty taste and MetS appeared to be independent of sex, age and BMI. Patients continuing the program ($n = 37$) did not show any difference in baseline taste sensitivity when compared to drop-outs ($n = 29$). Similarly, no significant difference was detected between patients reporting and not reporting a weight loss $\geqslant$ 5% of the initial body weight. No significant difference in taste sensitivity was detected even after dividing patients on the basis of nutritional (OW and OB) or metabolic status (MetS+ and MetS-).

CONCLUSION: There is no cause-effect relationship between overweight and metabolic derangements. Taste thresholds assessment is not useful in predicting the outcome of a diet-induced weight loss program.

Key words: Taste sensitivity; Nutritional status; Obesity; Metabolic syndrome; Weight loss dietary intervention

Core tip: This paper analyzed for the first time the relationship between taste sensitivity, nutritional status and

metabolic syndrome parameters and its effects on the success of weight loss dietary program. We found that taste sensitivity appears related to weight excess and to metabolic syndrome only in the case of salty taste, while there is no implication related to a weight loss program.

Bertoli S, Laureati M, Battezzati A, Bergamaschi V, Cereda E, Spadafranca A, Vignati L, Pagliarini E. Taste sensitivity, nutritional status and metabolic syndrome: Implication in weight loss dietary interventions. *World J Diabetes* 2014; 5(5): 717-723 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/717.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.717

INTRODUCTION

The prevalence of obesity has grown in parallel with the worldwide rise in metabolic syndrome and diabetes becoming a global public health problem that threatens the economies of all nations. Obesity is fuelled by individual factors, nutrition transition and increasingly sedentary lifestyles that lead to excess caloric intake[1]. Among individual factors, taste sensitivity plays an important role in food preferences, choices, and thus consumption[2]. Taste sensitivity can be defined as the minimum concentration at which the subject is able to perceive a specific taste quality, such as sweet, sour, salty and bitter[2]. A growing literature suggested that the ability to taste phenylthiocarbamide/6-n-propylthiouracil (PROP), synthetic compounds identified as major ligands for bitter-taste-receptor genes (TAS2R38), influences dietary behaviour[3,4]. In particular variation in taste sensitivity to bitter has been associated with differences in preferences for and selection of bitter fruits and vegetables, as well as sweet foods, added fats, spicy foods, and alcoholic beverages[5-7]. Past studies failed to show any association between sweet thresholds and nutritional status[8-10], while more recent studies described a difference between overweight and normal-weight subjects[11,12]. In particular, it has been shown that PROP phenotype is related to body mass index (BMI) in females and that sweet (sucrose) as well as salty (sodium chloride) taste sensitivity are lower in young overweight/obese individuals compared with normal weight controls[13]. This suggests that overweight and obese subjects may have a reduced or distorted sensory sensitivity that might increase the desire and ingestion of food, thus leading to excessive energy intake and weight gain[14]. A recent neuroimaging study seems to support this hypothesis showing that gustatory stimulation induced differential fMRI brain activation patterns in obese patients compared to healthy control subjects[15]. Moreover, a possible interaction between tasting profile such as sweet liking or supertasting status with metabolic syndrome has been suggested in adolescence[16] and more recently in the adults[17]. Finally, other investigators have reported that taste sensitivity may be affected by short-term caloric deprivation in both overweight and lean subjects, with lower thresholds of perception in fasted state than in satiated state[18,19]. Thus, it could be suggested an implication for taste sensitivity also in diet-induced weight loss program. However, evidence in regard to this issue is still in lack.

The purposes of the current study were to investigate: (1) the relationship between nutritional status and taste sensitivity; (2) the relationship between metabolic syndrome parameters and taste sensitivity; and (3) to investigate if sensory acuity could predict the outcome of a diet-induced weight loss program.

MATERIALS AND METHODS

The present study was performed in adherence to the principles established by the Declaration of Helsinki, after the protocol was approved by the local Institutional Ethics Committee. Every patient was asked for informed consent before all the assessments were made.

Forty-one overweight (OW; F:M, 34:7) and 52 obese (OB; F:M, 32:20) patients, admitted to the International Center for the Assessment of Nutritional Status (Università degli Studi di Milano, Italy) only for weight and dietetic concern, and 56 healthy normal-weight (F:M, 36:20) volunteers were recruited. Major study inclusion criteria were age < 65 years (range: 18-64), euthyroidism, no diabetes, no alcohol drinking, no diet to lose weight in the last 6 mo, no restrained eating behaviour and absence of well-established dysgeusia. Binge eating disorder was also excluded according to current diagnostic criteria[20]. On the same day, all the patients underwent a full nutritional assessment and taste sensitivity analysis in fasting state.

Nutritional assessment and presence of metabolic syndrome

Nutritional assessment was performed after 8-12 h of fasting and included: (1) Medical history and physical examination, including blood pressure measurement; (2) Anthropometric evaluation by collecting body weight (to the nearest 0.1 kg) and standing height (to the nearest 0.1 cm) through the same calibrated scale provided of a telescopic vertical steel stadiometer (SECA 220; Germany) and kept the patient dressing only underwear. BMI was derived accordingly [weight (kg)/height (m^2)]. Waist circumference was also measured (to the nearest 0.5 cm) at the midpoint between the iliac crest and the last rib[21]; (3) Body composition by a four-polar impedence meter (BIA; Human IM Scan, DS-Medigroup, Milan, Italy). Whole-body resistance was measured on the left side of the body at frequency of 50 kHz (R_{50}) following international guidelines and fat free mass was calculated using the formula for healthy adults proposed by Deurenberg *et al*[22]. Percentage of body fat mass (BF%) was derived accordingly; (4) Resting energy expenditure (REE) assessment by indirect calorimetry (Sensor Medics Vmax-29N; Anaheim, CA). Concentrations of carbon dioxide and oxygen were measured with the ventilated-hood

Table 1 Compounds used to elicit the 4 basic tastes with relevant dilution step and concentration range

Taste	Compound	Dilution step	Concentration range (g/L)
Sweet	Sucrose	3	1.23-100.00
Bitter	Caffeine	0.2 log	0.16-1.00
Salty	Sodium cloride	3.5	0.50-75.00
Sour	Citric acid	3.5	0.33-50.00

technique. Therefore, gas concentrations were used to determine REE with the Weir equation[23]; (5) Venous blood sampling in fasted state for the evaluation of glucose, high density lipoproteins (HDL) and triglycerides; and (6) Dietary recall by the same well trained dietician to evaluate eating behaviour, eating habits and food preferences which were almost taken into account during diet preparation.

Weight loss program was based on hypocaloric balanced diet providing at least the 90% of measured REE. Energy intake was provided for the 55.3% ± 0.6% by carbohydrates (simple carbohydrates < 15%), 23.8% ± 1.7% by lipids (satured fat < 7%) and 20.9% ± 1.7% by protein. Three-five servings of fruit and vegetables were daily advised; the source of protein intake was dependent on the frequencies of consumption of meat (2 times/wk), fish (4 times/wk), legumes (4 times/wk), eggs (1 time/wk), low-fat cheese (1-2 time/wk), low-fat ham (1-2 time/wk). Olive oil is indicated as the main culinary lipid. Dietary cholesterol was lower than 200 mg/die and fibre intake was about 30 g. Follow-up evaluations to check for compliance and weight loss were set after one and three months since the inception of the dietary program. During control visits an expert dietician measured body weight, fat mass and carried out a careful interview focused on the adherence to prescribed diet.

The updated criteria from the International Diabetes Federation[24] were used to define metabolic syndrome (MetS+). That is to say, subjects had to have ≥ 3 of the following: (1) waist circumference > 94 cm in men and >88 cm in women; (2) serum triglyceride ≥ 150 mg/dL; (3) HDL-cholesterol < 40 mg/dL in men and < 50 mg/dL in women; (4) blood pressure ≥ 130/85 mmHg; and (5) fasting plasma glucose level ≥ 100 mg/dL. Participants treated with antihypertensive or triglyceride-lowering medications were considered as hypertensive or hypertriglyceridemic, respectively.

Subjects in the control group were not evaluated for waist circumference, body composition and REE.

Taste sensitivity analysis

Taste sensitivity determination was performed at the sensory laboratory of the Department of Food, Environmental and Nutritional Sciences (DeFENS- Università degli Studi di Milano) designed according to ISO guidelines[25]. Participants were asked not to smoke, eat or drink anything except water before the test.

Recognition taste thresholds were evaluated by means of the three-alternative-forced-choice method[26]. Sucrose, caffeine, sodium chloride and citric acid were used to elicit sweet, bitter, salty and sour tastes, respectively. For each compound, five concentrations were prepared in mineral water. Concentration range of each taste stimulus was chosen on the basis of threshold values reported in the literature[27,28]. Concentration ranges were established in order that the lowest concentration was clearly below and the highest concentration clearly above the level at which subjects are able to detect or recognize the stimulus. A preliminary test was carried out to adjust concentration ranges since in some cases subjects occasionally recognized the lowest concentration or did not recognize the highest concentration of the stimuli. The final ranges of concentration (expressed in g/L) and dilution factors used to elicit the four basic tastes are reported in Table 1. The solutions were prepared the same day of the session and tested at room temperature. For each basic taste participants were presented with 5 triads of samples marked with three-digit numbers. Each triad consisted of one cup containing the stimulus and two cups containing an equal volume of blank (mineral water). The 5 triads proceeded from weaker to progressively stronger concentration, with the position of the cup containing the stimulus randomized over trials and assessors. For each triad, participants were instructed to indicate which sample was different from the other two[26]. If assessors were uncertain, they were instructed to guess (forced choice procedure). At the beginning of each session, and before each triad, the assessors were instructed to rinse their mouth with mineral water. Data were self-recorded by the subjects on paper sheets.

The individual threshold for each sensory stimulus was calculated as the geometric mean of the concentration at which the last miss occurred and the next higher concentration that was correctly recognized[26]. In addition, from the above mentioned threshold values, an individual global taste acuity score (GTAS) was determined, as recently reported by Monneuse *et al*[12]. For every basic taste we divided patients into tertiles according to taste sensitivity threshold data. We attributed the score 3, 2 and 1 to increasing threshold values and the sum of these scores defined the GTAS. Therefore, the higher the GTAS the higher the acuity.

Weight loss program outcomes

Compliance to the program was defined as adherence to control visits and weight loss ≥ 5% in 3 mo.

Statistical analysis

Variables were presented as frequencies or percentages if categorical (sex, smoking and menopause status, metabolic syndrome) and as mean ± SD if continuous (age, BMI, body fat mass, waist, taste thresholds). As preliminary results indicated that data on tastes sensitivity were not normally distributed, values were log-transformed to achieve a near-Gaussian distribution. Categorical variables were compared by χ^2 test and comparison between groups for continuous variables was performed by Student *t*-test

Table 2 Features of the population according to weight status

	Controls (n = 56)	Overweight (n = 41)	Obese (n = 52)	P
Sex (M:F)	20:36	7:34	20:32	0.061
Age (yr)	41.6 ± 12.3	46.9 ± 11.5	45.8 ± 11.6	0.060
Range	24-66	20-64	19-64	
Current smoking (n)	30 (53.6)	22 (53.7)	33 (63.4)	0.511
Menopause (n)	15 (41.7)	16 (47.0)	15 (46.9)	0.404
BMI (kg/m^2)	22.1 ± 1.7	27.9 ± 1.6	34.8 ± 4.6	<0.001
Body fat mass (%)	-	45.6 ± 5.2	47.6 ± 5.1	0.054
Waist (cm)	-	91.5 ± 7.5	106.2 ± 18.2	<0.001
Metabolic syndrome (n)	0 (0)	8 (19.5)	25 (48.1)	0.004
Taste thresholds				
Sweet (log g/L)	0.74 ± 0.44	0.78 ± 0.40	0.85 ± 0.48	0.418
Salty (log g/L)	0.23 ± 0.54	0.13 ± 0.48	0.36 ± 0.58	0.099
Sour (log g/L)	-0.21 ± 0.54	-0.34 ± 0.40	-0.05 ± 0.67	0.105
Bitter (log g/L)	-0.34 ± 0.35	-0.21 ± 0.29	-0.24 ± 0.30	0.151
GTAS	8.0 ± 1.9	8.0 ± 1.6	7.3 ± 2.1	0.132

Data are reported as mean ± SD or counts (%). P values according to χ^2 or parametric tests (ANOVA analysis), where appropriate. GTAS: Global Taste Acuity Score; BMI: Body mass index; M:F: Male:Female.

Table 3 Multiple regression model between taste sentitivity and nutri-metabolic parameters

	Sour	Bitter	Salty	BMI	BF%[1]	Waist[1]	MetS[1]	MetS criteria[1]
Sour	-	-	-	0.20[a]	0.05	0.27[c]	0.21[a]	0.21[a]
Bitter	0.34[f]	-	-	0.14	-0.09	-0.13	0.02	0.03
Salty	0.26[c]	0.23[b]	-	0.10	-0.08	-0.11	0.23[a]	0.19
Sweet	0.24[d]	0.33[f]	0.26[c]	0.15	-0.15	0.10	0.08	-0.01
GTAS	-	-	-	-0.13[a]	-0.15	-0.05	-0.08	-0.11

[1]For BF%, waist circumference, presence of metabolic MetS and the number of MetS criteria correlations refer to overweight/obese patients (n = 93). Values are standardized coefficients adjusted for age and sex, [a]P < 0.05; [b]P < 0.01; [c]P <0.002; [d]P < 0.005; [f]P < 0.001, between BMI and both sour taste and GTAS, with lower sensitivity with increasing BMI. BMI: Body mass index; BF%: Percentage of body fat mass; MetS: Metabolic syndrome; GTAS: Global Taste Acuity Score.

(two-group comparisons) or ANOVA analysis (multiple-group comparisons) followed by post-hoc comparison of means by Tukey's test.

A linear regression model adjusted for sex and age was built to test the independent relationship between: (1) taste sensitivity (dependent variable) and both BMI and MetS (independent variables); and (2) outcomes, namely dropout and successful weight loss (as dependent variables), and taste sensitivity (each taste as independent variable).

Statistical analyses were performed by the SPSS 20.0 statistical package (SPSS for Windows; SPSS Inc., Chicago). Level of significance was established in a two-sided P value < 0.05.

RESULTS

Taste sensitivity according to nutritional status and metabolic syndrome

The features of the population investigated are presented in Table 2. Normal-weight controls, OW and OB patients were matched for age, gender and smoking and hormonal status. A higher prevalence of MetS characterized obese patients when compared to those overweight despite similar BF%. At baseline, no significant difference was detected neither in any of the taste sensitivity nor in GTAS. However, sex and age-adjusted multiple regression models revealed (Table 3) a significant association between BMI and both sour taste and GTAS, with lower sensitivity with increasing BMI. This trend in sensitivity for sour taste was also confirmed by the model refitted on the OW/OB group while the association with GTAS was marginally significant (P = 0.06).

MetS+ subjects presented higher thresholds for salty when compared to MetS- patients while no significant difference was detected for the other tastes and GTAS (unpaired Student t-test; Table 4). As assessed by multiple regression model, the association between salty taste and MetS appeared to be independent of sex, age and BMI.

Interestingly, similar differences in thresholds where found between MetS+ subjects and lean controls (for salty taste, P < 0.05), while sensitivity among lean controls and MetS- patients was almost comparable (data not reported in tables).

Taste sensitivity and outcome

The features of OW/OB group according to outcomes are presented in Table 5. During the follow-up 29 patients (31.2%) did not attend the second visit. However, among the others (n = 64) continuing the program and reaching the end of the study follow-up, only 37 obtained a successful weight loss (≥ 5%). These three outcome groups appeared well matched for all demographic parameters, prevalence of MetS and nutritional features (P > 0.05) with exception of weight loss (P < 0.001). Patients continuing the program did not show any difference in baseline taste sensitivity and GTAS when compared to drop-outs. Similarly, no significant difference was detected between patients reporting and not reporting a weight loss ≥ 5% of the initial body weight. Then, we sought to evaluate whether an effect of BMI and MetS was present in regard with outcome. No difference (P > 0.05 for all multiple group comparisons) was detected between controls and outcome groups, even after dividing patients on the basis of nutritional (OW and OB) or metabolic status (MetS+ and MetS-). Finally, sex, age and BMI-adjusted linear regression models, including program discontinuation or successful weight loss as alternative dependent variables, confirmed that taste thresholds or global taste acuity (alternative independent variables) are not able to predict the outcome of a diet-induced weight loss program.

DISCUSSION

Taste sensitivity may be involved both in the pathogenesis of weight excess, through food choice and energy intake, and in the lack of compliance to a diet-induced weight loss program. These were the issues we investigated in

Table 4 Taste sensitivity in overweight and obese patients according to metabolic syndrome and gender

	Overall			Women			Men		
	MetS+ (n = 33)	MetS- (n = 60)	P^1	MetS+ (n = 21)	MetS- (n = 45)	P^1	MetS+ (n = 12)	MetS- (n = 15)	P^1
BMI (kg/m^2)	33.7 ± 5.2	30.7 ± 4.2	0.002	33.5 ± 4.8	30.0 ± 3.9	0.002	34.1 ± 6.1	32.7 ± 4.4	0.485
BF%	47.7 ± 6.0	46.4 ± 4.7	0.281	50.3 ± 4.4	47.2 ± 4.5	0.013	43.1 ± 5.8	43.8 ± 4.2	0.744
Waist (cm)	106.2 ± 14.2	96.1 ± 16.3	0.004	101.9 ± 11.2	94.1 ± 9.4	0.005	113.9 ± 16.0	102.2 ± 28.0	0.288
Sweet (log g/L)	0.87 ± 0.41	0.80 ± 0.47	0.396	0.81 ± 0.45	0.72 ± 0.43	0.458	0.97 ± 0.31	1.02 ± 0.51	0.730
Salty (log g/L)	0.43 ± 0.56	0.16 ± 0.52	0.029	0.31 ± 0.50	0.10 ± 0.45	0.121	0.65 ± 0.62	0.33 ± 0.67	0.244
Sour (log g/L)	-0.01 ± 0.69	-0.27 ± 0.49	0.069	-0.08 ± 0.59	-0.39 ± 0.39	0.022	0.11 ± 0.85	0.08 ± 0.61	0.859
Bitter (log g/L)	-0.22 ± 0.28	-0.23 ± 0.31	0.956	-0.24 ± 0.27	-0.23 ± 0.29	0.846	-0.18 ± 0.30	-0.23 ± 0.37	0.757
GTAS	7.4 ± 2.0	7.7 ± 1.9	0.440	7.8 ± 2.0	7.8 ± 1.7	0.936	6.8 ± 1.9	7.5 ± 2.4	0.414

P values according to unpaired Student *t*-test or Wilcoxon-Mann-Whitney test. [1]MetS+ *vs* MetS- within the same group (overall or women or men). BMI: Body mass index; BF%: Percentage of body fat mass; MetS: Metabolic syndrome (+, presence; -, absence); GTAS: Global Taste Acuity Score.

Table 5 Features of overweight and obese patients according to the outcome

	Drop-out	Continuing the program		
		Overall	WL < 5%	WL ⩾ 5%
	(n = 29)	(n = 64)	(n = 27)	(n = 37)
Sex (M:F)	8:21	19:45	6:21	13:24
Age (yr)	45.3 ± 11.4	46.7 ± 11.7	48.1 ± 12.1	45.7 ± 11.4
Current smoking (n)	15 (51.7)	40 (62.4)	14 (51.6)	26 (70.2)
Menopause (n)	9 (42.9)	22 (48.9)	11 (52.4)	11 (45.8)
BMI (kg/m^2)	31.0 ± 4.3	32.1 ± 5.0	32.8 ± 4.8	31.6 ± 5.2
Body fat mass (%)	46.6 ± 4.9	46.9 ± 5.3	47.8 ± 5.3	46.3 ± 5.4
Waist (cm)	99.3 ± 13.2	99.9 ± 17.5	112.7 ± 12.3	99.7 ± 20.5
Metabolic syndrome (n)	10 (34.5)	23 (35.9)	10 (37.0)	13 (35.1)
Weight loss (%)	-	-5.6 ± 3.5	-2.5 ± 1.7	-7.8 ± 2.5
Taste thresholds				
Sweet (log g/L)	0.87 ± 0.35	0.80 ± 0.48	0.79 ± 0.52	0.81 ± 0.46
Salty (log g/L)	0.27 ± 0.49	0.25 ± 0.57	0.23 ± 0.53	0.26 ± 0.61
Sour (log g/L)	-0.25 ± 0.48	-0.15 ± 0.62	-0.24 ± 0.52	-0.08 ± 0.68
Bitter (log g/L)	-0.27 ± 0.31	-0.21 ± 0.29	-0.26 ± 0.28	-0.16 ± 0.29
GTAS	7.6 ± 2.0	7.6 ± 1.9	7.8 ± 1.7	7.4 ± 2.1

Data are reported as mean ± SD or counts (%). No significant differences were detected in ANOVA comparison among drop out, WL < 5% and WL > 5%. GTAS: Global Taste Acuity Score; BMI: Body mass index; M:F: Male: Female; WL: Weight loss.

the present study.

In the present study, we observed that taste thresholds appear related to metabolic disturbances (*e.g.*, MetS) only in the case of salty taste, MetS+ patients having higher threshold values than MetS- patients. Nonetheless, this association appeared independent of overall BMI. This result seems in conflict with the recent findings by Pasquet *et al*[16] who found a female-specific but positive association between taste sensitivity for sweet and salty tastes and the number of obesity-related metabolic disorders in a group of adolescents. This inconsistency may be ascribed to the different approach used to measure taste thresholds and to the fact that, contrary to Pasquet *et al*[16] study, adolescents were not considered in the present experiment. The positive association found between higher threshold for salty taste and Mets probably is dependent, at least partially, on association between higher threshold for salty taste and hypertension as suggested by Rabin *et al*[29]. Indeed, hypertension is a major component of the metabolic syndrome[24]. It should be pointed out that the association between metabolic syndrome and taste acuity still needs to be clarified, especially in adults, as several changes in perception could occur throughout life for example in reason of hormonal and psychological factors.

Concerning the relationship between taste sensitivity and nutritional status (BMI), the present study evidenced an independent effect of BMI on taste sensitivity for sour and global taste acuity. Moreover, obese individuals showed in general a tendency to higher taste thresholds than lean subjects.

Although the association between BMI and taste has been largely investigated, very few data are available on the relation between taste thresholds and body mass index and our findings appear partially in contrast with those already provided. Pasquet *et al*[16] observed that massively obese adolescents have lower thresholds for taste recognition than normal-weight controls. Obrebowski *et al*[30] found that children and adolescents with simple obesity have lowered electrogustometric thresholds. The authors attributed this behavior to obesity-related metabolic disturbances rather than to body mass *per se*. Similarly to our study, Simchen *et al*[11] have recently investigated the association between taste qualities (sweet, sour, bitter and salty) and BMI in a group of adults. They observed an age dependent relationship with respectively lower and higher sensory capabilities in overweight subjects aged < 65 years and ⩾ 65 years for sour and bitter tastes. However, despite the investigation by Simchen *et al*[11] has been performed in a larger cohort, the authors have recognized not to have controlled for an important potential confounder such as restrained eating behaviour, a factor that has been considered by us during recruitment. Besides, body composition and fat distribution assessments were helpful to better characterize our subjects nutritional status, as the pathophysiology of metabolic complications is substantially related to overall and compartmental body fatness[24]. Indeed, a prospective study would be the best way to assess their relationship of taste acuity with future overweight/obesity.

It is also interesting to know if partial or total failure to comply with diet is related to sensory capabilities. We reported that, regardless of the presence of obesity-related metabolic derangements, namely MetS, no apparent

effect of taste sensitivity on the adherence to a diet-based weight loss program seems to exist. Accordingly, the assessment of taste sensitivity may not assist in predicting the outcome of dieting and may not be useful to the improvement of clinical practice. A possible explanation of our findings is that a 3-mo follow-up is probably a too short period of time to observe differences. One would argue that other factors (*e.g.*, portions size, psychosocial factors) may be involved in the short-term adherence to a weight loss program[31]. We recognize the lack of sensory capabilities reassessment at the end of the follow-up as a study limitation as we cannot exclude a modification of taste acuity during the program itself. Despite conflicting reports are available on this issue[32], it seems likely that acute fasting (14-16-h-long) results in lower sensory thresholds[18,19]. It should be noted that we performed our study postabsorptively (14-16 h after last meal), in physiologic state. Accordingly, it is reasonable to sustain a lack of involvement of taste perception in dietary compliance. However, motivation to comply is generally high in the initial phases and the long-term effect of diet-related restrained eating behaviour on gustatory sensitivity has never been explored. We know only a study by Tepper and Ullrich[33] in which it is reported that in non-dieting subjects the relationship between body weight and sensory capabilities may be masked by dietary restraint.

The relationship between putative changes in taste sensitivity and drop-out is more difficult to explain but we cannot exclude those patients not attending the second visit did so also for organizing reasons. Finally, we cannot exclude a "pathological" regulation of sensory capabilities in satiated state. It would be probably useful to assess taste sensitivities also in this condition.

With this background, it is clear that the relationship between nutritional status and taste sensitivity deserves further investigation also in view of the fact that present data generalizability is limited in view of the method used and the study sample size.

In conclusion, taste sensitivity (sour and global taste acuity) appears related to weight excess with lower sensitivity with increasing BMI and to metabolic syndrome only in the case of salty taste. However, no implication seems to exist in the compliance to a weight loss program. Further studies still needs to be done to clarify the cause-effect association between taste perception and BMI.

COMMENTS

Background

The prevalence of obesity has grown in parallel with the worldwide rise in metabolic syndrome and diabetes becoming a global public health problem that threatens the economies of all nations. Obesity is fuelled by individual factors, nutrition transition and increasingly sedentary lifestyles that lead to excess caloric intake. Among individual factors, taste sensitivity plays an important role in food preferences, choices, and thus consumption. The role of taste thresholds in the physiopathology and the management of overweight and obesity has been not completely clarified and data available are rather contradictory.

Research frontiers

Recently new findings have suggested that overweight and obese subjects may have a reduced or distorted sensory sensitivity that might increase the desire and ingestion of food, thus leading to excessive energy intake and weight gain. Moreover, some investigators have reported that taste sensitivity may be affected by short-term caloric deprivation in both overweight and lean subjects, with lower thresholds of perception in fasted state than in satiated state. However, evidence in regard to this issue is still in lack.

Innovations and breakthroughs

The authors investigated in overweight and obese patients the relationship between taste sensitivity, nutritional status and metabolic syndrome parameters and the possible implications of this relationship on the outcome of weight loss dietary program.

Applications

The authors shown a direct independent relationship between body mass index and metabolic syndrome and the threshold for sour taste. Successful weight-loss appeared unrelated to sensory capabilities.

Terminology

Taste sensitivity can be defined as the minimum concentration at which the subject is able to perceive a specific taste quality, such as sweet, sour, salty and bitter.

Peer review

The authors sought to determine a plausible relationship between taste sensitivity, nutritional status and metabolic syndrome. They evaluated implications for success in weight loss dietary intervention. The methodology is adequate and analysis well carried out. The work leads to the conclusion that taste sensitivity appears in some measure related to weight excess and metabolic derangements.

REFERENCES

1 **French SA**, Story M, Jeffery RW. Environmental influences on eating and physical activity. *Annu Rev Public Health* 2001; **22**: 309-335 [PMID: 11274524 DOI: 10.1146/annurev.publhealth.22.1.309]

2 **Sørensen LB**, Møller P, Flint A, Martens M, Raben A. Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. *Int J Obes Relat Metab Disord* 2003; **27**: 1152-1166 [PMID: 14513063 DOI: 10.1038/sj.ijo.0802391]

3 **Bufe B**, Breslin PA, Kuhn C, Reed DR, Tharp CD, Slack JP, Kim UK, Drayna D, Meyerhof W. The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. *Curr Biol* 2005; **15**: 322-327 [PMID: 15723792 DOI: 10.1016/j.cub.2005.01.047]

4 **Kim UK**, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science* 2003; **299**: 1221-1225 [PMID: 12595690 DOI: 10.1126/science.1080190]

5 **Dinehart ME**, Hayes JE, Bartoshuk LM, Lanier SL, Duffy VB. Bitter taste markers explain variability in vegetable sweetness, bitterness, and intake. *Physiol Behav* 2006; **87**: 304-313 [PMID: 16368118 DOI: 10.1016/j.physbeh.2005.10.018]

6 **Keller KL**, Steinmann L, Nurse RJ, Tepper BJ. Genetic taste sensitivity to 6-n-propylthiouracil influences food preference and reported intake in preschool children. *Appetite* 2002; **38**: 3-12 [PMID: 11883912 DOI: 10.1006/appe.2001.0441]

7 **Duffy VB**, Davidson AC, Kidd JR, Kidd KK, Speed WC, Pakstis AJ, Reed DR, Snyder DJ, Bartoshuk LM. Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake. *Alcohol Clin Exp Res* 2004; **28**: 1629-1637 [PMID: 15547448 DOI: 10.1097/01.ALC.0000145789.55183.D4]

8 **Donaldson LF**, Bennett L, Baic S, Melichar JK. Taste and weight: is there a link? *Am J Clin Nutr* 2009; **90**: 800S-803S [PMID: 19571216 DOI: 10.3945/ajcn.2009.27462Q]

9 **Malcolm R**, O'Neil PM, Hirsch AA, Currey HS, Moskowitz G. Taste hedonics and thresholds in obesity. *Int J Obes* 1980; **4**: 203-212 [PMID: 7419338]

10 **Frijters JE**, Rasmussen-Conrad EL. Sensory discrimination, intensity perception, and affective judgment of sucrose-

sweetness in the overweight. *J Gen Psychol* 1982; **107**: 233-247 [PMID: 7175511 DOI: 10.1080/00221309.1982.9709931]
11 **Simchen U**, Koebnick C, Hoyer S, Issanchou S, Zunft HJ. Odour and taste sensitivity is associated with body weight and extent of misreporting of body weight. *Eur J Clin Nutr* 2006; **60**: 698-705 [PMID: 16435003 DOI: 10.1038/sj.ejcn.1602371]
12 **Monneuse MO**, Rigal N, Frelut ML, Hladik CM, Simmen B, Pasquet P. Taste acuity of obese adolescents and changes in food neophobia and food preferences during a weight reduction session. *Appetite* 2008; **50**: 302-307 [PMID: 17904687 DOI: 10.1016/j.appet.2007.08.004]
13 **Sartor F**, Donaldson LF, Markland DA, Loveday H, Jackson MJ, Kubis HP. Taste perception and implicit attitude toward sweet related to body mass index and soft drink supplementation. *Appetite* 2011; **57**: 237-246 [PMID: 21600942 DOI: 10.1016/j.appet.2011.05.107]
14 **Tepper BJ**, Koelliker Y, Zhao L, Ullrich NV, Lanzara C, d' Adamo P, Ferrara A, Ulivi S, Esposito L, Gasparini P. Variation in the bitter-taste receptor gene TAS2R38, and adiposity in a genetically isolated population in Southern Italy. *Obesity* (Silver Spring) 2008; **16**: 2289-2295 [PMID: 18719631 DOI: 10.1038/oby.2008.357]
15 **Szalay C**, Aradi M, Schwarcz A, Orsi G, Perlaki G, Németh L, Hanna S, Takács G, Szabó I, Bajnok L, Vereczkei A, Dóczi T, Janszky J, Komoly S, Örs Horváth P, Lénárd L, Karadi Z. Gustatory perception alterations in obesity: an fMRI study. *Brain Res* 2012; **1473**: 131-140 [PMID: 22885291 DOI: 10.1016/j.brainres.2012.07.051]
16 **Pasquet P**, Frelut ML, Simmen B, Hladik CM, Monneuse MO. Taste perception in massively obese and in non-obese adolescents. *Int J Pediatr Obes* 2007; **2**: 242-248 [PMID: 17852551 DOI: 10.1080/17477160701440521]
17 **Turner-McGrievy G**, Tate DF, Moore D, Popkin B. Taking the bitter with the sweet: relationship of supertasting and sweet preference with metabolic syndrome and dietary intake. *J Food Sci* 2013; **78**: S336-S342 [PMID: 23323969 DOI: 10.1111/1750-3841.12008]
18 **Zverev YP**. Effects of caloric deprivation and satiety on sensitivity of the gustatory system. *BMC Neurosci* 2004; **5**: 5 [PMID: 15028115 DOI: 10.1186/1471-2202-5-5]
19 **Glöckner L**, Fikentscher R, Ulrich FE. [Taste perception in fasting overweight patients]. *Z Gesamte Inn Med* 1986; **41**: 244-246 [PMID: 3727647]
20 **American Psychiatric Association**. Diagnostic and statistical manual of mental disorders, (4th ed., text revision). American Psychiatric Association: Washington, DC, 2000
21 **Lohman TG**, Roche AF, Martorel R. Anthropometric standardization Reference manual. Champagne, IL: Human Kinetics Books, 1988
22 **Deurenberg P**, van der Kooy K, Leenen R, Weststrate JA, Seidell JC. Sex and age specific prediction formulas for estimating body composition from bioelectrical impedance: a cross-validation study. *Int J Obes* 1991; **15**: 17-25 [PMID: 2010255]
23 **WEIR JB**. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949; **109**: 1-9 [PMID: 15394301]
24 **Tong PC**, Kong AP, So WY, Yang X, Ho CS, Ma RC, Ozaki R, Chow CC, Lam CW, Chan JC, Cockram CS. The usefulness of the International Diabetes Federation and the National Cholesterol Education Program's Adult Treatment Panel III definitions of the metabolic syndrome in predicting coronary heart disease in subjects with type 2 diabetes. *Diabetes Care* 2007; **30**: 1206-1211 [PMID: 17259472 DOI: 10.2337/dc06-1484]
25 ISO 8589 Sensory analysis-General guidance for the design of test rooms. International Organization for Standardization, Geneva, Switzerland, 2007
26 ASTM E679-04: Standard practice for determination of odor and taste threshold by a forced-choice ascending concentration series method of limits. ASTM International, West Conshohocken, PA, 2004
27 **Mojet J**, Christ-Hazelhof E, Heidema J. Taste perception with age: generic or specific losses in threshold sensitivity to the five basic tastes? *Chem Senses* 2001; **26**: 845-860 [PMID: 11555480 DOI: 10.1093/chemse/26.7.845]
28 **Mojet J**, Heidema J, Christ-Hazelhof E. Taste perception with age: generic or specific losses in supra-threshold intensities of five taste qualities? *Chem Senses* 2003; **28**: 397-413 [PMID: 12826536 DOI: 10.1093/chemse/28.5.397]
29 **Rabin M**, Poli de Figueiredo CE, Wagner MB, Antonello IC. Salt taste sensitivity threshold and exercise-induced hypertension. *Appetite* 2009; **52**: 609-613 [PMID: 19501757 DOI: 10.1016/j.appet.2009.02.007]
30 **Obrebowski A**, Obrebowska-Karsznia Z, Gawliński M. Smell and taste in children with simple obesity. *Int J Pediatr Otorhinolaryngol* 2000; **55**: 191-196 [PMID: 11035176 DOI: 10.1016/S0165-5876(00)00397-9]
31 **Inelmen EM**, Toffanello ED, Enzi G, Gasparini G, Miotto F, Sergi G, Busetto L. Predictors of drop-out in overweight and obese outpatients. *Int J Obes* (Lond) 2005; **29**: 122-128 [PMID: 15545976 DOI: 10.1038/sj.ijo.0802846]
32 **Pasquet P**, Monneuse MO, Simmen B, Marez A, Hladik CM. Relationship between taste thresholds and hunger under debate. *Appetite* 2006; **46**: 63-66 [PMID: 16298017 DOI: 10.1016/j.appet.2005.09.004]
33 **Tepper BJ**, Ullrich NV. Influence of genetic taste sensitivity to 6-n-propylthiouracil (PROP), dietary restraint and disinhibition on body mass index in middle-aged women. *Physiol Behav* 2002; **75**: 305-312 [PMID: 11897256 DOI: 10.1016/S0031-9384(01)00664-3]

P- Reviewer: Gervois P, Niculescu M, Nishikawa T, Rasool A
S- Editor: Song XX **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v5.i5.724

World J Diabetes 2014 October 15; 5(5): 724-729
ISSN 1948-9358 (online)

PROSPECTIVE STUDY

Perfluorocarbon in vitreoretinal surgery and preoperative bevacizumab in diabetic tractional retinal detachment

J Fernando Arevalo, Martin A Serrano, Juan D Arias

J Fernando Arevalo, From the Retina Division, Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21287, United States
J Fernando Arevalo, the Vitreoretinal Division, King Khaled Eye Specialist Hospital, Riyadh 11462, Saudi Arabia
J Fernando Arevalo, Division of Ophthalmology, Faculty of Medicine and Health Sciences, University of Stellenbosch, Stellenbosch 7600, South Africa
Martin A Serrano, Juan D Arias, the Retina and Vitreous Service, Clinica Oftalmologica Centro Caracas, Caracas 1010, Venezuela
Author contributions: Arevalo JF performed all surgeries, designed the study and wrote the manuscript; and Serrano MA and Arias JD assisted during all surgeries and collected data; Serrano MA and Arias JD were also involved in editing the manuscript.
Supported by The Arevalo-Coutinho Foundation for Research in Ophthalmology, Caracas, Venezuela
Correspondence to: J Fernando Arevalo, MD, FACS, Chief of the Vitreoretinal Division, King Khaled Eye Specialist Hospital, Al-Oruba Street, PO Box 7191, Riyadh 11462, Saudi Arabia. arevalojf@jhmi.edu
Telephone: +966-11-48212343860 Fax: +966-1-48212343727
Received: November 28, 2013 Revised: June 26, 2014
Accepted: July 15, 2014
Published online: October 15, 2014

Abstract

AIM: To describe the en bloc perfluorodissection (EBPD) technique and to demonstrate the applicability of using preoperative intravitreal bevacizumab during small-gauge vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy) in eyes with advanced proliferative diabetic retinopathy (PDR) with tractional retinal detachment (TRD).

METHODS: This is a prospective, interventional case series. Participants included 114 (eyes) with advanced proliferative diabetic retinopathy and TRD. EBPD was performed in 114 eyes (consecutive patients) during 23-gauge vitrectomy with the utilization of preoperative bevacizumab (1.25 mg/0.05 mL). Patients mean age was 45 years (range, 21-85 years). Surgical time had a mean of 55 min (Range, 25-85 min). Mean follow up of this group of patients was 24 mo (range, 12-32 mo). Main outcome measures included best-corrected visual acuity (BCVA), retinal reattachment, and complications.

RESULTS: Anatomic success occurred in 100% (114/114) of eyes. Significant visual improvement [$\geqslant$ 2 Early Treatment Diabetic Retinopathy Study (ETDRS) lines] was obtained in 69.2% (79/114), in 26 eyes (22.8%) BCVA remained stable, and in 8 eyes (7%) BCVA decreased ($\geqslant$ 2 ETDRS lines). Final BCVA was 20/50 or better in 24% of eyes, between 20/60 and 20/400 in 46% of eyes, and worse than 20/400 in 30% of eyes. Complications included cataract in 32 (28%) eyes, iatrogenic retinal breaks in 9 (7.8%) eyes, vitreous hemorrhage requiring another procedure in 7 (6.1%) eyes, and phthisis bulbi in 1 (0.9%) eye.

CONCLUSION: This study demonstrates the usefulness of using preoperative intravitreal bevacizumab and EBPD during small-gauge vitreoretinal surgery in eyes with TRD in PDR.

Key words: Avastin; Intravitreal bevacizumab; Intravitreal injections; Proliferative diabetic retinopathy; Tractional retinal detachment; Perfluorodissection; Minimally invasive vitreoretinal surgery; Vitrectomy

Core tip: *En bloc* perfluorodissection and preoperative intravitreal bevacizumab use for small-gauge vitrectomy in patients with proliferative diabetic retinopathy and tractional retinal detachment are very useful, the combination reduces complications and operative time. *En bloc* perfluorodissection and preoperative intravitreal bevacizumab use seems to have many advantages including that the retina remains stable during vitrectomy, better visibility of the ocular structures in the vitreous cavity, immediate reattachment of the retina,

bleeding control, subretinal fluid reabsorbsion and drainage, bleeding sites' tamponade, and easier dissection of epiretinal tissues.

Arevalo JF, Serrano MA, Arias JD. Perfluorocarbon in vitreoretinal surgery and preoperative bevacizumab in diabetic tractional retinal detachment. *World J Diabetes* 2014; 5(5): 724-729 Available from: URL: http://www.wjgnet.com/1948-9358/full/v5/i5/724.htm DOI: http://dx.doi.org/10.4239/wjd.v5.i5.724

INTRODUCTION

Pars plana vitrectomy is a successful surgical technique for the complications of proliferative diabetic retinopathy (PDR)[1,2]. It is usually necessary within one year in up to 10% of patients presenting with PDR[3]. The commonest indication for surgery is non-clearing vitreous hemorrhage. Unfortunately[1,2], postoperative vitreous hemorrhage is a significant complication occurring in about 20% to 30% of cases[4-10].

Some advances in surgical techniques and instrumentation, such as; *en bloc* dissection, delamination, segmentation, and bimanual surgical techniques, have allowed better results in the treatment of severe PDR[11-13]. Viscodissection, described by Stenkula and Tornquist[12], and the use of perfluorocarbon liquids (PFCL), introduced as a surgical adjuvant in vitrectomy in 1987 by Chang *et al*[14], facilitate removal of epiretinal membranes, the management of proliferative vitreoretinopathy (PVR) with retinal detachment, tractional retinal detachments in diabetics, and control of intraoperative hemorrhage.

Quiroz-Mercado *et al*[15,16] published a technique called perfluorocarbon-perfused vitrectomy (PCPV). In their technique, PFCL is used in the infusion in a continuous way during vitrectomy. In selected cases PFCL may offer several advantages over saline solution, because of their properties including gravitational forces, immiscibility with fluids, and ability to transport oxygen[15,16]. Regardless of PFCL's advantages, the use of PCPV has not extended worldwide. In addition, PCPV utilizes a considerable amount of PFCL, and membranes may be pushed against the retina during PCPV.

We have previously described "*En bloc* perfluorodissection" (EBPD), which combines the advantages of viscodissection and PCPV. EBPD helps the surgeon during removal of membranes over the retina and to create a posterior vitreous detachment by injecting PFCL between the retina and the posterior hyaloid separating tissues over the retina[17,18]. In addition, identification and removal of all posterior vitreoretinal traction is very important. Furthermore, vitreoschisis can also occur in patients with PDR, it is important to identify this feature and to perform dissection in the true vitreoretinal plane, to avoid recurrent traction and postoperative bleeding from retinal neovascularization[19].

Postoperative vitreous cavity hemorrhage is a significant complication following vitrectomy for the treatment of PDR. It has two main forms, "early" when hemorrhage (bleeding) is present in the first few postoperative days and "late", when hemorrhage occurs a number of months after surgery. The presence of postoperative vitreous hemorrhage delays visual recovery can lead to elevated pressure within the eye and can make further treatment for diabetic retinopathy difficult. Revision surgery is required in 10% of patients, which has significant implications for resources, time and cost. The use of anti-vascular endothelial growth factor (anti-VEGF) before surgery (preoperatively) has been proposed as an intervention to reduce the incidence of postoperative vitreous hemorrhage[20].

Recently, it has been reported that intravitreal bevacizumab in patients with vitreous hemorrhage and PDR resulted in regression of retinal neovascularization and resolution of vitreous hemorrhage[21]. Chen *et al*[22] and Avery *et al*[23], have reported that preoperative intravitreal bevacizumab (Avastin®, Genentech Inc., San Francisco, CA) reduce the risk of bleeding during vitrectomy facilitating the removal of fibrovascular tissues.

The aim of this article is to describe the surgical technique and demonstrate the usefulness of combining *en bloc* perfluorodissection and preoperative intravitreal bevacizumab use for membrane peeling in tractional retinal detachment in advanced diabetic retinopathy with small-gauge vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy).

MATERIALS AND METHODS

This is a prospective, interventional case series. One hundred fourteen (eyes) with tractional retinal detachment (TRD) in PDR participated. The authors performed EBPD in 114 eyes (consecutive patients) during 23-gauge transconjunctival sutureless vitrectomy for tractional retinal detachment in severe PDR with the utilization of preoperative bevacizumab (1.25 mg/0.05 mL). Main outcome measures were best-corrected visual acuity (BCVA), retinal status, and complications. This study has been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki and it was approved by the Institution's Ethics Committee.

An aliquot of commercially available bevacizumab was prepared for each patient and placed in a tuberculin syringe using aseptic techniques. Four days before vitrectomy, after preparation of the eye using 5% povidone/iodine, an eyelid speculum was used to open the eyelids, and the injection of 1.25 mg (0.05 mL) of bevacizumab was performed 4 mm posterior to the limbus, through the superotemporal or inferotemporal pars plana with a 30-gauge needle under topical anesthesia. After the injection, retinal artery perfusion was checked with the indirect ophthalmoscope. In none of our cases an anterior chamber paracenthesis was necessary. No topical antibiotics were administered preoperatively.

A 23-gauge transconjunctival sutureless vitrectomy was performed in all cases. A core vitrectomy is done first to clear any vitreous hemorrhage present. A hole is then

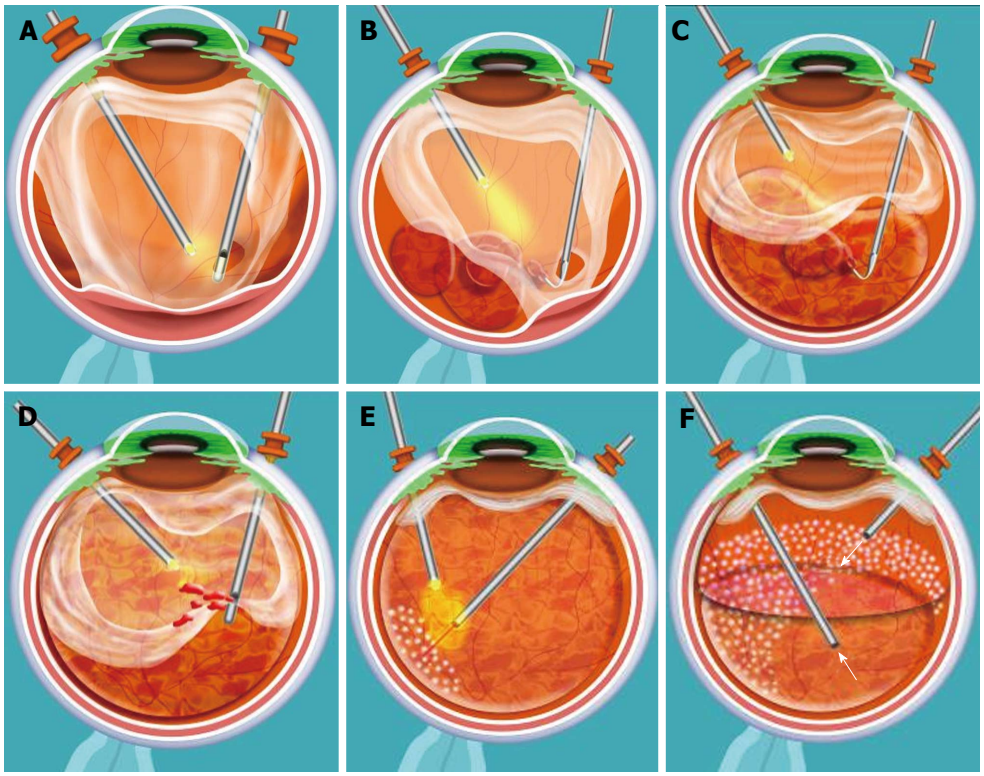

Figure 1 Artist's representation of surgical technique. A: An opening is made with the vitrector in the mid-periphery of the posterior hyaloid; B and C: Perfluorocarbon liquid (PFCL) is injected to separate the posterior hyaloid from the retina. A dual bore cannula (for 23-gauge cases) attached to a 5 cc syringe filled with PFCL is used to separate membranes and posterior hyaloid from the underlying retina; D: Once all the tissues have been separated from the retina, vitrectomy can be continued up to the periphery; E: Endolaser is applied under PFCL; F: An air-fluid and an air-gas (C3F8) exchange exchange are performed to end the case.

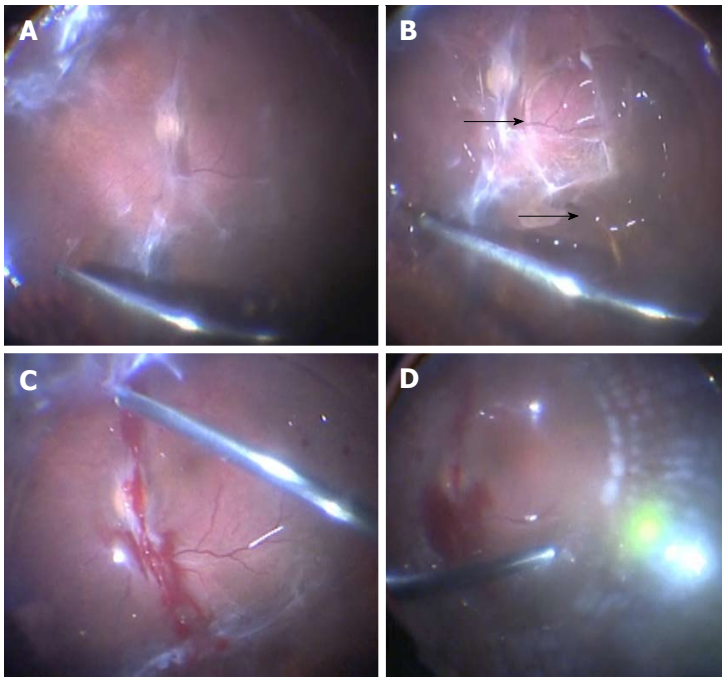

Figure 2 En bloc perfluorodissection performed in a case of tractional retinal detachment in proliferative diabetic retinopathy. A: An opening is made with the vitrector in the mid-periphery of the posterior hyaloid; B: Perfluorocarbon liquid (PFCL) is injected to separate the posterior hyaloid from the retina (arrows). A dual bore cannula (for 23-gauge cases) attached to a 5 cc syringe filled with PFCL is used to separate membranes and posterior hyaloid from the underlying retina; C: Once all the tissues have been separated from the retina, vitrectomy can be continued up to the periphery; D: Endolaser is applied under PFCL (shown). An air-fluid and an air-gas (C3F8) exchange are performed to end the case (not shown).

made in the mid-peripheral posterior hyaloid (Figures 1A and 2A) to inject the perfluorocarbon liquid (PFCL) [Perfluorooctane (C_8F_{18})] and mechanically detach the posterior hyaloid from the retina (Figures 1B, 1C and 2B). We

use a 23-gauge Dual Bore cannula (Dual Bore cannula 0.6 mm, MedOne, Sarasota, FL) attached to a 5 cc syringe filled with PFCL to separate the posterior hyaloid and membranes from the retina. After all the membranes and posterior hyaloid have been separated from the retina, vitrectomy is completed up to the periphery (Figures 1D and 2C), endolaser is applied (Figures 1E and 2D), an air-fluid and air-gas [Perfluoropropane (C_3F_8), Escalon Medical Corporation, New Berlin, WI] exchange is performed to finish the case (Figure 1F).

Non-illuminated instrumentation was usually used in our cases[7] combined with a non-contact wide-angle viewing system (BIOM, Oculus, Wetzlar, Germany). An illuminated cannula was utilized (25ga, Awh chandelier, Synergetics Inc., O'Fallon, MO) in some cases for bimanual surgery.

RESULTS

Patients were prospectively enrolled from January 2006 to January 2010 at Clinica Oftalmologica Centro Caracas in Caracas, Venezuela. Inclusion criteria included patients with TRD in advanced PDR and macular involvement or impending macular involvement with or without vitreous hemorrhage. EBPD was performed in 114 consecutive eyes (patients) during small-gauge vitrectomy for severe PDR with TRD. The mean age of the patients was 45 years (range, 21-85 years). Surgical time had a mean of 55 min (Range, 25-85 min). Mean follow up of our patients was 24 mo (range: 12-32 mo).

Each patient underwent BCVA measurement with ETDRS. Patients were followed postoperatively on day 1, at one week, at three weeks, at 7 wk, and every 3 mo with complete eye examination at each visit, including BCVA, anterior segment examination, IOP determination, and fundus biomicroscopy. Patients were included only with a minimum 12 mo of follow-up. An increase or decrease in BCVA was considered to have occurred if there was a change of two or more Early Treatment Diabetic Retinopathy Study (ETDRS) lines. Main outcome measures were changes in BCVA, and retinal reattachment.

En bloc perfluorodissection was performed using a mean volume of PFCL of 4 mL (range: 3 to 8 mL). No patients in our series have shown ocular hypertension or inflammation. Anatomic success occurred in 100% (114/114) of eyes. Significant visual improvement ($\geq$ 2 ETDRS lines) was seen in 69.2% (79/114), in 26 eyes (22.8%) BCVA remained stable, and in 8 eyes (7%) BCVA decreased ($\geq$ 2 ETDRS lines). Final BCVA was 20/50 or better in 24%, between 20/60 and 20/400 in 46%, and worse than 20/400 in 30%. Complications included cataract in 32 (28%) eyes, iatrogenic retinal breaks in 9 (7.8%) eyes, vitreous hemorrhage requiring another procedure in 7 (6.1%) eyes, and phthisis bulbi in 1 (0.9%) eye.

DISCUSSION

In selected cases *en bloc* perfluorodissection during vitrectomy in eyes with TRD in PDR and preoperative use of intravitreal bevacizumab, we can obtained an anatomic (100%) and functional success (69.2%). Other benefits of this technique include that the retina remains stable during vitrectomy, less blood in the vitreous cavity, rapid retinal reattachment, better visualization of vitreous and intraocular structures, blood confinement, and easier dissection of epiretinal membranes.

In our study, the authors have not seen any difficulties with the technique. However, in one case PFCL was injected within a vitreous schisis. After a short amount of instillation (1 mL) that situation was apparent, and PFCL was aspirated and a new hole in the posterior hyaloid was made at another location making sure that the proper plane was found between the posterior hyaloid and the retina this time. No complications rose from this event. In addition, there were 2 eyes (1.7%) with subretinal PCL that were solved with a peripheral retinotomy, aspiration with an extrusion cannulae, and the injection of additional PCL in the posterior pole. In our study the prevalence of postoperative vitreous hemorrhage was lower (6.1%) than that reported in other studies (20% to 30%)[4-10] which can be explained by the use of intravitreal bevacizumab 4 d preoperatively.

Surgeons with extensive experience can manage complex retinal detachments in patients with TRD using either viscodissection or conventional techniques with pick and scissors. Thus, surgeons should deal with these cases selectively according to their level of experience. An ideal case for EBPD might be one in which there is a TRD with no tears, with limited posterior vitreous detachment, and relatively loose attachment of the posterior hyaloid to the retina. We use a combination of several techniques in our cases including EBPFD, and the use of picks and forceps with bimanual surgery. Currently, the use of small-gauge vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy) and preoperative intravitreal bevacizumab for TRD in diabetics have improved our surgical time and results.

In the future, MIVS with 23-gauge transconjunctival sutureless vitrectomy techniques will be increasingly performed in diabetic patients due to the increased incidence of diabetes and its complications. In the coming years we will use techniques that are less invasive in vitreoretinal surgery such as 25+, and 27-gauge. We will have available other anti-VEGF antibodies capable of blocking all types of VEGF isoforms before and after surgery, reducing intraoperative bleeding, and postoperative inflammation. It is likely that the use of preoperative agents that promote the detachment of the posterior hyaloid and facilitate the removal of membranes will become routine. They will facilitate surgery of complex cases such as PDR cases. Optical coherence tomography equipment will be available in the operating room and that will facilitate intraoperative tissue differentiation, and help us get better functional results. The advent of new lasers will permit us faster retinal photocoagulation, and will minimize collateral damage of the retina.

In summary, EBPD and preoperative intravitreal bevacizumab use for vitrectomy in eyes with TRD in PDR it is very useful. *En bloc* perfluorodissection and preoperative intravitreal bevacizumab use seems to have many advantages including that the retina remains stable during vitrectomy, better visibility of intraocular structures, immediate reattachment of the retina, bleeding control, reabsorbsion and drainage of subretinal fluid, bleeding sites' tamponade, and easier dissection of epiretinal tissues.

ACKNOWLEDGMENTS

Dr. Arevalo is a PhD student at Faculty of Health Sciences, Stellenbosch University, Stellenbosch, South Africa. This article is part of his PhD thesis on "Intravitreal Bevacizumab as Anti-Vascular Endothelial Growth Factor in the Management of Complications of Diabetic Retinopathy".

COMMENTS

Background

Authors have previously described a new surgical dissection technique, namely "*En bloc* perfluorodissection" (EBPD), which combines the advantages of viscodissection and perfluorocarbon-perfused vitrectomy. EBPD helps the surgeon during removal of epiretinal membranes and to detach the posterior hyaloid by injecting perfluorocarbon liquid between the retina and the posterior hyaloid to separate the epiretinal tissues from the retina.

Research frontiers

The objective of this article is to describe the surgical technique and demonstrate the usefulness of combining *en bloc* perfluorodissection and preoperative intravitreal bevacizumab use for membrane peeling in tractional retinal detachment in advanced diabetic retinopathy with small-gauge vitreoretinal surgery (23-gauge transconjunctival sutureless vitrectomy).

Innovations and breakthroughs

En bloc perfluorodissection and preoperative intravitreal bevacizumab use seems to have many advantages including that the retina remains stable during vitrectomy, better visibility of vitreous and intraocular structures, immediate retinal reattachment, bleeding control in the vitreous cavity, subretinal fluid reabsorbsion and drainage, bleeding sites' tamponade, and easier dissection of epiretinal tissues.

Applications

En bloc perfluorodissection and preoperative intravitreal bevacizumab use for vitrectomy in eyes with tractional retinal detachment in advanced proliferative diabetic retinopathy it is very useful technique, reduces complication and operative time.

Peer review

The report is interesting, well documented, and the paper should be published.

REFERENCES

1 **Ho T**, Smiddy WE, Flynn HW. Vitrectomy in the management of diabetic eye disease. *Surv Ophthalmol* 1992: 190-202 [PMID: 1475753 DOI: 10.1016/0039-6257(92)90137-I]
2 **McLeod D**. Microsurgical management of neovascularisation secondary to posterior segment ischaemia. *Eye* (Lond) 1991; **5** (Pt 2): 252-259 [PMID: 1712738 DOI: 10.1038/eye.1991.40]
3 **Kaiser RS**, Maguire MG, Grunwald JE, Lieb D, Jani B, Brucker AJ, Maguire AM, Ho AC, Fine SL. One-year outcomes of panretinal photocoagulation in proliferative diabetic retinopathy. *Am J Ophthalmol* 2000; **129**: 178-185 [PMID: 10682970 DOI: 10.1016/S0002-9394(99)00322-0]
4 **Benson WE**, Brown GC, Tasman W, McNamara JA. Complications of vitrectomy for non-clearing vitreous hemorrhage in diabetic patients. *Ophthalmic Surg* 1988; **19**: 862-864 [PMID: 3231410]
5 **Blankenship GW**. Management of vitreous cavity hemorrhage following pars plana vitrectomy for diabetic retinopathy. *Ophthalmology* 1986; **93**: 39-44 [PMID: 2419815 DOI: 10.1016/S0161-6420(86)33791-6]
6 **Liggett PE**, Lean JS, Barlow WE, Ryan SJ. Intraoperative argon endophotocoagulation for recurrent vitreous hemorrhage after vitrectomy for diabetic retinopathy. *Am J Ophthalmol* 1987; **103**: 146-149 [PMID: 3812616]
7 **Novak MA**, Rice TA, Michels RG, Auer C. Vitreous hemorrhage after vitrectomy for diabetic retinopathy. *Ophthalmology* 1984; **91**: 1485-1489 [PMID: 6521989 DOI: 10.1016/S0161-6420(84)34099-4]
8 **Tolentino FI**, Cajita VN, Gancayco T, Skates S. Vitreous hemorrhage after closed vitrectomy for proliferative diabetic retinopathy. *Ophthalmology* 1989; **96**: 1495-1500 [PMID: 2587044 DOI: 10.1016/S0161-6420(89)32700-X]
9 **Virata SR**, Kylstra JA. Postoperative complications following vitrectomy for proliferative diabetic retinopathy with sew-on and noncontact wide-angle viewing lenses. *Ophthalmic Surg Lasers* 2001: 193-197 [PMID: 11371085]
10 **Yorston D**, Wickham L, Benson S, Bunce C, Sheard R, Charteris D. Predictive clinical features and outcomes of vitrectomy for proliferative diabetic retinopathy. *Br J Ophthalmol* 2008; **92**: 365-368 [PMID: 18303158 DOI: 10.1136/bjo.2007.124495]
11 **Abrams GW**, Williams GA. "En bloc" excision of diabetic membranes. *Am J Ophthalmol* 1987; **103**: 302-308 [PMID: 3826236]
12 **Stenkula S**, Tornquist R. Healon: a guide to its use in ophthalmic surgery. In: Miller D, Stegman R. Use of healon in vitrectomy and difficult retinal detachments. New York: Wiley, 1983: 207-221
13 **Grigorian RA**, Castellarin A, Bhagat N, Del Priore L, Von Hagen S, Zarbin MA. Use of viscodissection and silicone oil in vitrectomy for severe diabetic retinopathy. *Semin Ophthalmol* 2003; **18**: 121-126 [PMID: 15513472 DOI: 10.1076/soph.18.3.121.29811]
14 **Chang S**, Zimmerman NJ, Iwamoto T, Ortiz R, Faris D. Experimental vitreous replacement with perfluorotributylamine. *Am J Ophthalmol* 1987; **103**: 29-37 [PMID: 3799787]
15 **Quiroz-Mercado H**, Guerrero-Naranjo J, Agurto-Rivera R, Leizaola-Fernández C, Suárez-Tatá L, Murillo-López S, Reategui-Escalante G, García-Aguirre G, Fromow-Guerra J. Perfluorocarbon-perfused vitrectomy: a new method for vitrectomy--a safety and feasibility study. *Graefes Arch Clin Exp Ophthalmol* 2005; **243**: 551-562 [PMID: 15965676 DOI: 10.1007/s00417-004-1063-z]
16 **Quiroz-Mercado H**, Garcia-Aguirre G, Ustáriz-González O, Martín-Avià J, Martinez-Jardon S. Perfluorocarbon-perfused vitrectomy using a transconjunctival 25-gauge system. *Retina* 2007; **27**: 926-931 [PMID: 17891018 DOI: 10.1097/IAE.0b013e31804b3cf9]
17 **Arevalo JF**. En bloc perfluorodissection for tractional retinal detachment in proliferative diabetic retinopathy. *Ophthalmology* 2008; **115**: e21-e25 [PMID: 18423870 DOI: 10.1016/j.ophtha.2008.02.008]
18 **Arevalo JF**. En bloc perfluorodissection in vitreoretinal surgery: a new surgical technique. *Retina* 2008; **28**: 653-656 [PMID: 18398370 DOI: 10.1097/IAE.0b013e3181619bd5]
19 **Schwatz SD**, Alexander R, Hiscott P, Gregor ZJ. Recognition of vitreoschisis in proliferative diabetic retinopathy. A useful landmark in vitrectomy for diabetic traction retinal detachment. *Ophthalmology* 1996; **103**: 323-328 [PMID: 8594521 DOI: 10.1016/S0161-6420(96)30697-0]
20 **Yeoh J**, Williams C, Allen P, Buttery R, Chiu D, Clark B, Essex R, McCombe M, Qureshi S, Campbell WG. Avastin as an adjunct to vitrectomy in the management of severe proliferative diabetic retinopathy: a prospective case series. *Clin*

Experiment Ophthalmol 2008; **36**: 449-454 [PMID: 18942219]
21 **Spaide RF**, Fisher YL. Intravitreal bevacizumab (Avastin) treatment of proliferative diabetic retinopathy complicated by vitreous hemorrhage. *Retina* 2006; **26**: 275-278 [PMID: 16508426 DOI: 10.1097/00006982-200603000-00004]
22 **Chen E**, Park CH. Use of intravitreal bevacizumab as a preoperative adjunct for tractional retinal detachment repair in severe proliferative diabetic retinopathy. *Retina* 2006; **26**: 699-700 [PMID: 16829817 DOI: 10.1097/01.iae.0000225351.87205.69]
23 **Avery RL**, Pearlman J, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ, Wendel R, Patel A. Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. *Ophthalmology* 2006; **113**: 1695.e1-1695.15 [PMID: 17011951]

P- Reviewer: Koleva-Georgieva DN, Velez-Montoya R
S- Editor: Wen LL **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
www.wjgnet.com

World J Diabetes 2014 October 15; 5(5): I-V
ISSN 1948-9358 (online)

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

Aims and scope

WJD covers topics concerning α, β, δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

WJD is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 43 OA clinical medical journals, including 42 in English, has a total of 15471 editorial board members or peer reviewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJD* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cuttingedge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in diabetes; (12) Research Report: To briefly report the novel and innovative findings in diabetes; (13) Meta-Analysis: Covers the systematic review, mixedtreatment comparison, meta-regression, and overview of reviews, in order to summarize a given quantitative effect, *e.g.*, the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJD*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of diabetes; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal

World Journal of Diabetes

ISSN

ISSN 1948-9358 (online)

Frequency

Bimonthly

Editors-in-chief

MA 02115, United States

Editorial office
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Diabetes
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

Publisher
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

Instructions to authors
Full instructions are available online at http://www.wjgnet.com/1948-9358/g_info_20100107165233.htm.

Indexed and abstracted in
PubMed Central, PubMed, Digital Object Identifier, and Directory of Open Access Journals

SPECIAL STATEMENT

All articles published in journals owned by the BPG represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc.* The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJD* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the International Committee of Medical Journal Editors to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www. clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs

and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1948-9358/g_info_20100107165233.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to bpgofficw@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, *e.g.*, Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g., Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJD*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present P values where appropriate and must provide relevant data to illustrate how they were obtained, *e.g.*, 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$), and CONCLUSION (no more than 26 words).

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles, rapid communication and case reports, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over

shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of P values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, *e.g.*, PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001

Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 200201 03498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1948-9358/g_info_20100107145507.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*

Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*

Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of BPG. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1948-9358/g_info_20100107144846.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1948-9358/g_info_20100107170340.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

STATEMENT ABOUT ANONYMOUS PUBLICATION OF THE PEER REVIEWERS' COMMENTS

In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

PUBLICATION FEE

WJD is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 698 USD per article. All invited articles are published free of charge.

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com