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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.

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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
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EDITORIAL

Computational pharmacokinetics and *in vitro-in vivo* correlation of anti-diabetic synergistic phyto-composite blend

Baishakhi De, Koushik Bhandari, Nishant Chakravorty, Ranjan Mukherjee, Rohit Gundamaraju, Rajeev K Singla, Prakash Katakam, Shanta K Adiki, Biswajoy Ghosh, Analava Mitra

Baishakhi De, Koushik Bhandari, Nishant Chakravorty, Ranjan Mukherjee, Biswajoy Ghosh, Analava Mitra, School of Medical Science and Technology, IIT Kharagpur 721302, India

Rohit Gundamaraju, Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia

Rajeev K Singla, Division of Biotechnology, Netaji Subhas Institute of Technology, Sector-3, Dwarka, New Delhi 110078, India

Prakash Katakam, Faculty of Pharmacy, University of Zawia, Az-Zawiya 13, Libya

Shanta K Adiki, Nirmala College of Pharmacy, Guntur 522503, Andhra Pradesh, India

Author contributions: De B developed the central theme of the manuscript under the guidance of Chakravorty N, Katakam P and Mitra A; Bhandari K, Mukherjee R, Gundamaraju R and Singla RK contributed in literature review, and provided their valuable suggestions; De B developed the manuscript under the guidances of Chakravorty N, Shanta KA and Mitra A; Ghosh B prepared the pictorial presentations of receptor pathways and gave his suggestions.

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Correspondence to: Analava Mitra, MBBS, PhD, School of Medical Science and Technology, B-145, IIT Campus, IIT Kharagpur 721302, India. analavamitra@gmail.com
Telephone: +91-94-75258298
Fax: +91-32-22279970

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Abstract

Despite tremendous strides in modern medicine stringent control over insulin resistance or restoration of normoglycemia has not yet been achieved. With the growth of molecular biology, omics technologies, docking studies, and *in silico* pharmacology, modulators of enzymes and receptors affecting the molecular pathogenesis of the disease are being considered as the latest targets for anti-diabetic therapy. Therapeutic molecular targets are now being developed basing on the up or down regulation of different signaling pathways affecting the disease. Phytosynergistic anti-diabetic therapy is in vogue both with classical and non-classical medicinal systems. However its chemo-profiling, structural and pharmacokinetic validation awaits providing recognition to such formulations for international acceptance. Translational health research with its focus on benchside product development and its sequential transition to patient bedside puts the pharma RDs to a challenge to develop bio-waiver protocols. Pharmacokinetic simulation models and establishment of *in vitro-in vivo* correlation can help to replace *in vivo* bioavailability studies and provide means of quality control for scale up and post approval modification. This

review attempts to bring different shades highlighting phyto-synergy, molecular targeting of antidiabetic agents *via* different signaling pathways and bio-waiver studies under a single umbrella.

Key words: *In silico* pharmacology; Phytosynergistic; Anti-diabetic; Simulation models; Translational health research; Bio-waiver; Signaling pathways

Core tip: The current research scenario on anti-diabetic drug development pipeline focuses on pharmacological targets influencing the molecular pathogenesis of the disease. It encompasses receptors and enzymes that will increase insulin sensitivity, intracellular insulin signaling, enhance peripheral glucose utilizations, suppress hepatic glucose production and reduce circulating triglycerides levels. Combination therapy has gained significance either with herbal or synthetic drugs, though "phytosynergy" awaits proper validation to give rise to new generations of "phytopharmaceuticals". Pharmacokinetic simulation models and established *in vitro-in vivo* correlation that may be extrapolated to humans can serve the purpose of bio-waiver in product transition from lab bench to patient bedside.

De B, Bhandari K, Chakravorty N, Mukherjee R, Gundamaraju R, Singla RK, Katakam P, Adiki SK, Ghosh R, Mitra A. Computational pharmacokinetics and *in vitro-in vivo* correlation of anti-diabetic synergistic phyto-composite blend. *World J Diabetes* 2015; 6(11): 1179-1185 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i11/1179.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i11.1179

INTRODUCTION

The constant escalations in the number of diabetics worldwide has given an alarming signal and fueled intensified research for the development of new therapeutic entities and latest effective therapeutic regimen. The statistics of the global diabetic population is expected to show a steady growth to 366 million by 2030 of which 90% will be type 2 diabetics. The international diabetes federation has estimated the number of diabetics in India to be 40.9 million, which is expected to grow to 60.9 million by 2025[1-3]. Diabetes is a common metabolic disorder with abnormal elevations in the blood-gluco-lipid profile, leading to major complications like diabetic neuropathy, nephropathy leading to end stage renal disease, retinopathy leading to blindness and diabetic foot ulcers necessitating limb amputations[1,2]. Type 2 diabetes is characterized by the hallmark of insulin resistance and β-cell dysfunction and ultimate destruction of pancreatic insulin secreting cells. Combating insulin resistance with the existing pharmacological approaches are unsatisfactory primarily because although they may compensate for the defects in insulin secretion and action, but are ineffective in counteracting β-cell dysfunction and handling the secondary complications of type 2 diabetes[1-3]. While developing a novel anti-diabetic chemical entity, latest drug design approaches focuses on activation-inhibition of enzymes in insulin-sensitive cells, minimization of associated side effects like obesity, substitution or antagonizing of physiological hormones and their pathways. With the advancements in high throughput screening, proteomics, genomics, molecular docking, and combinatorial chemistry, new therapeutic entities are being developed that influence enzyme activities, signaling receptors and pathophysiological pathways[1,2]. Modern day quantitative structural activity relationship and docking studies are enabling development of bio-active molecules that can achieve structural modifications and thereby alter their pharmacological actions and pharmacokinetic profile so as to maximize bioavailability and minimize the side effects[4-10].

Latest anti-diabetic drug development pipeline focuses on pharmacological targets which include receptors and enzymes that will increase insulin sensitivity, intracellular insulin signaling, enhance peripheral utilizations of glucose, suppress hepatic glucose production and reduce the levels of circulating tri glycerides[4-10].

Medicine in recent times, whether western classical or phyto-therapy, advocates for combination therapy, instead of single approach. Synergy research in phyto-therapy, with the aid of "omics technologies" needs a rationale for establishing its pharmacological and therapeutic superiority to treat diseases which have hitherto been treated using synthetic drugs alone[11-15].

Along with the paradigm of translational health research with the perspectives of bench to bedside approach; all pharmaceutical RDs target to develop robust, cost effective, enhanced throughput *in vitro* assays which may be extrapolated to humans and serve the purpose of bio-waiver. The development of increased number of new chemical entities obviates the need of enhanced pharmacokinetic studies. Though human pharmacokinetic *in vivo* studies are often considered as the "gold standard" for assessment of bioequivalence but it is expensive, time consuming and difficult to handle enormous amount of pharmacokinetic data. Development of pharmacokinetic simulation models which are computational or mathematical tools help to interpret drug kinetics in living environment under specified conditions and can waive off bioequivalence requirements called bio-waiver studies. Establishment of *in vitro-in vivo* correlation (IVIVC) provides a justified explanation for bio-waiver during scale up or post approval changes[16-25].

Thus the editorial encompasses the broad areas highlighting phyto-synergy, targeting of different signaling pathways of type 2 diabetes and how computational pharmacokinetics and development of IVIVC serves the purpose of bio-waiver.

MOLECULAR PATHOGENESIS OF TYPE 2 DIABETES

Treatment regimen of type 2 diabetes advocates two different approaches, one recommending the sequential use of anti-diabetics and another is a pathophysiologic approach which aims to control the disease conditions basing on pathogenesis with a comparative preference on combination therapy.

American Diabetes Association guidelines incorporated an individualized ABCDE anti-diabetic therapy approach where each alphabet refers to A-age, B-body weight, C-complications (micro and macro vascular), D-disease duration and E-life expectancy and expense. Progressive β-cell destruction coupled with the development of insulin resistance in liver, muscles and adipocytes, subsequent elevation in glycated hemoglobin level being the common pathogenic hallmark in all type 2 diabetes mellitus, though variations are reported amongst different ethnic groups[4-6].

Apart from insulin resistance, a host of cardiovascular co-morbidities like dyslipidemia, hypertension, and central visceral adiposity occur in type 2 diabetes. Evidence based contemporary research paradigms have shown that intra abdominal or visceral fat depots synergize defective insulin action and secretion. Moreover leptins, adiponectins, tumor necrosis factor-α, resistin which are secreted from the adipose tissues interfere with glucose metabolism and insulin sensitivity giving rise to the concept of lipotoxicity in type 2 diabetes. These adipokines greatly modify insulin signaling pathways and promote development of insulin resistance. A triadic relation is found to exist amongst β-cell destruction, insulin resistance and adiposity[4-6].

Sedentary lifestyle, westernized dietary pattern, stress, anxiety, depression, smoking and alcohol consumption are other contributing risk factors of type 2 diabetes. Obesity is also found to be associated with endothelial dysfunction, impairs muscle microcirculation, retards entry of insulin and blood glucose into skeletal muscle and decreases their availability to muscle cells. Lack of physical activity is an important risk factor in type 2 diabetes. Daily physical activities decreases visceral and body fat, increase glycogen synthase (GS) content of the muscle, promotes non-oxidative disposal of glucose as glycogen and activates glucose transporter subtype 4 (GLUT4) to enhance peripheral glucose utilization. Physical activity up regulates expression and activity of proteins involved in insulin signal transduction, improves oxidative capacity of the skeletal muscles, decreases free fatty acid concentrations and enhances the increased expression of downstream signaling components of insulin. Regular exercises also trigger the release of anti-inflammatory cytokines, a protective role against insulin resistance[1,4-6].

An insight into the genetics of type 2 diabetes showed that genes encoding proteins are involved in insulin signaling, glycogen synthesis and glucose transportation, fatty acid uptake and synthesis, adipocyte differentiation and thus suggests associations with diabetes. A clear understanding of human genome sequence is necessary which will help in rapid identification of the genes associated with diabetes. Mutations of five genes viz. glucose metabolizing enzyme glucokinase, transcription factors hepatocyte nuclear factor (HNF) 1α and β, HNF4α and insulin promoter factor 1 (IPF1) affect moderate to significant reductions in insulin secretions. Latest research reporting does have mentioned that genetic variation of newly encoded gene Calpain, called as *CAPN10* gene can cause diabetes[5-9].

THERAPEUTIC MOLECULAR TARGETS BASED ON RECEPTOR SIGNALING PATHWAYS IN TYPE 2 DIABETES

Amongst the Oral Hypoglycemic Agents (OHAs) mostly recommended for type 2 anti-diabetic therapy, sulfonylureas (*e.g.*, tolbutamide, glibenclamide, acetohexamide) and biguanides (*e.g.*, phenformin, metformin) are in wide use followed by thiazolidinediones (also known as glitazones, *e.g.*, Rosiglitazone, Pioglitazone) and alpha glucosidase inhibitors (acarbose, miglitol, voglibose). Sulphonyl ureas work primarily by stimulating pancreatic insulin secretion and reduce the hepatic glucose output and enhance peripheral glucose utilizations. Biguanides are anti-hyperglycemic agents rather than hypoglycemics, suppress excessive hepatic glucose production, increases peripheral glucose utilizations to a lesser extent, reduce intestinal glucose absorption by reducing food intake. Alpha glucosidase inhibitors delay the breakdown of disaccharides and polysaccharides and hence glucose absorption is delayed. Thiazolidinediones enhance insulin sensitivity in peripheral tissues.

However, the available pharmacological approaches for anti-diabetic therapy are not successful enough to put a stringent control on insulin resistance. Instead of mono therapy now combination therapy and muti-drug formulations are in vogue. With the development of proteomics, genomics and a thorough understanding of the molecular pathways, the development of new molecular targets with anti-diabetic potentials focuses in modulating pharmacokinetics, cellular location, overall distribution etc. Modulators of enzymes and receptors are now becoming the molecular targets for any disease therapy[8-10].

The three targeted tissues of insulin action include skeletal muscle, adipose tissue and liver. Insulin binds with the target cell surface receptor and activates the tyrosine kinase which is a constituent of the receptor molecule. Tyrosine residues of the insulin receptors undergo autophosphorylation and the serine/threonine residues become phosphorylated[7-10]. In type 2 diabetes elevated levels of insulin stimulates serine kinases *via* IGF-1 receptor (Insulin like growth factor 1) leading to insulin resistance[7-10]. Protein kinase C (PKC) is known to play a significant role in developing

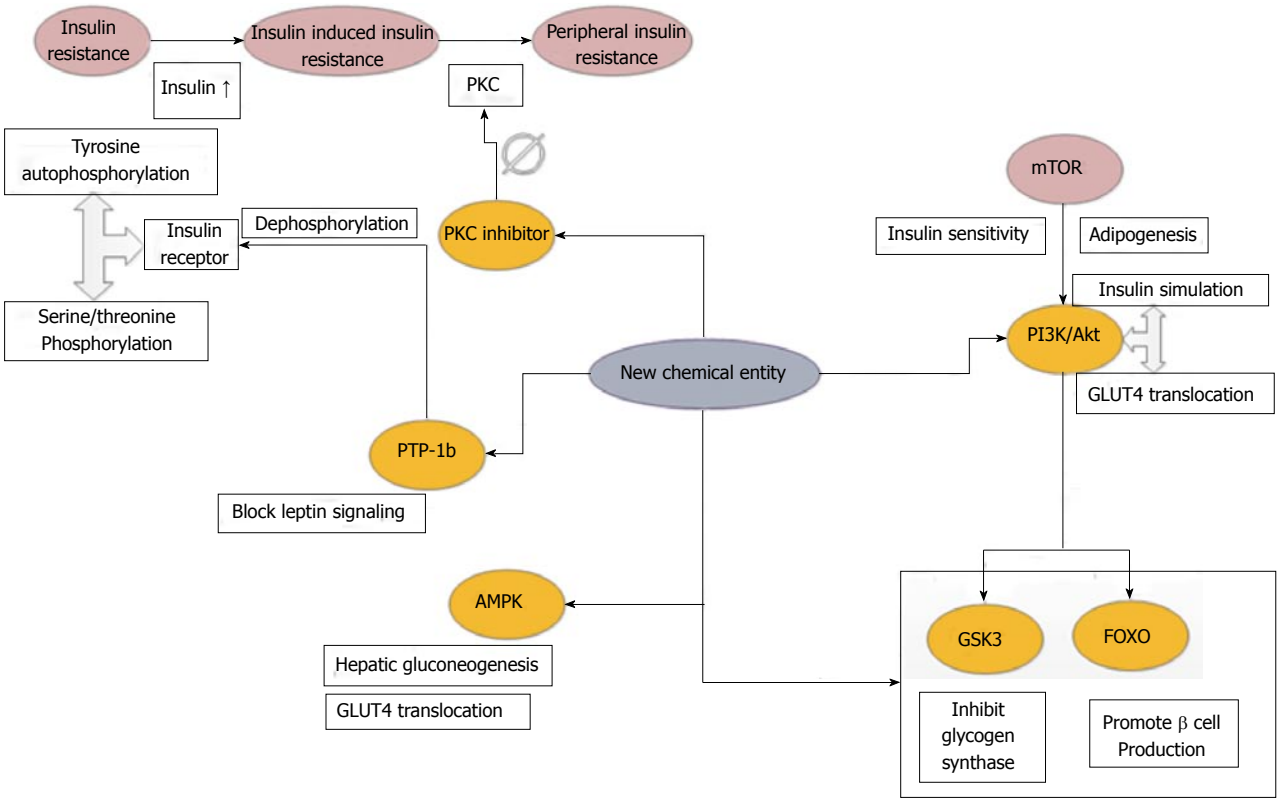

Figure 1 Schematic representation of different receptor signaling pathways in type 2 diabetes mellitus which can be targeted by new chemical entity. PKC: Protein kinase C; Akt: Also known as protein kinase B (PKB); GSK3: Glycogen synthase kinase 3; mTOR: Mammalian target of rapamycin; FOXO: Forkhead box subgroup O; PTP-1b: Protein tyrosine phosphatase-1b; GLUT4: Glucose transporter Subtype 4; PI3K: Phosphoinositide 3 kinase; AMPK: AMP activated protein kinase.

peripheral insulin resistance. Thus inhibition of PKC or its reduced expression may enhance insulin sensitivity and insulin receptor tyrosine kinase activity which can be an effective therapeutic strategy against type 2 diabetes. Protein tyrosine phosphatase-1b (PTP-1b) causes dephosphorylation of insulin receptor and is a negative regulator of the insulin signaling. It enhances insulin activity and is resistant to the development of obesity. PTP-1b down regulates or blocks leptin signaling by dephosphorylating Janus kinase (JAK). Thus PTP-1b serves as an essential therapeutic target. Phosphoinositide 3 kinase (PI3K) plays a significant role in the glucose uptake *via* insulin stimulation and GLUT4 translocation. PI3K is down regulated by two classes of serine/threonine kinases, Akt, also known as protein kinase B (PKB) and the isoforms of PKC[7-10]. Akt and isoforms of PKC are known to facilitate GLUT4 translocation. P70s6k directly phosphorylates IRS (insulin receptor substrate) which inhibits its activity and hinders Akt actively. Mammalian target of rapamycin (mTOR) has a significant role in obesity and IR and activates both Akt and p70s6k. The essential targets for Akt include the transcription factors, glycogen synthase kinase 3 (GSK3) and the forkhead box subgroup O (FOXO). GSK3 can phosphorylate and inhibit GS. Now phosphorylation of Akt inactivates GSK3 and leads to an increase in glycogen synthesis[7-10]. Akt phosphorylation also targets FOXO mediated transcription of target genes that promote the production of β-cells. To counteract IR and restore insulin sensitivity therapeutic agents should target to increase PI3K/Akt activity. Lipid phosphatase PTEN (phosphatase and tensin homolog) dephosphorylates phosphatidylinositol (3,4,5) trisphosphate (PIP3) making it less available to recruit Akt. Also downstream regulation of mTOR can regulate adipogenesis and insulin sensitivity[7-10].

AMPK (AMP activated protein kinase) regulates hepatic gluconeogenesis and increase muscle glucose uptake by translocation of GLUT4 which also serves the purpose of an essential therapeutic target[10]. A comprehensive scheme of the different receptor signaling pathways have been presented below in Figure 1.

Some of the novel molecular targets for anti-diabetic therapy have been mentioned in Table 1.

PHYTOSYNERGY IN TYPE 2 DIABETES

Synergy refers to the increased effectiveness that results when two or more elements work together, though here we will refer to phytochemical constituents. Synergism is the total outcome of a cumulative effect which is greater than the sum of individual effects. From the dimensions of pharmacology, molecular biology or clinical research, synergism can be either in the form of multi target effect where different phyto-constituents of a single extract or a composite extract will affect more than one targets agonistically and exhibit

Table 1 Possible therapeutic molecular targets for type 2 anti-diabetic therapy

Type	Target for action	Nature of action	Effect produced
Protein kinases	Protein kinase C	Inhibitory	Block receptor desensitization
	AMP activated kinase	Activator	Enhance glucose transport
	GSK-3	Inhibitory	Activate glycogen synthase
	MAP kinase	Inhibitory	Block receptor desensitization
Protein phosphatases	PTP-1b	Inhibitory	Block receptor dephosphorylation
	PP1	Activator	Activate glycogen synthase
	LAR	Inhibitory	Block receptor dephosphorylation
Lipid phosphatases	PTEN	Inhibitor	Increase PIP3-stimulated glucose transport
Cell surface receptors	Insulin receptor	Agonist	Insulin mimetic
	Glucagon receptor	Antagonist	Low fasting glucose
	GLP receptor	Agonist	Increase insulin secretion
	β-3 adrenergic receptor	Agonist	Increase lipolysis
Ion channels	Sulphonyl urea receptor	Inhibit K channel	Increase insulin secretion
Transcription factors	PPAR-γ	Selective modulator	Insulin sensitizer
	HNF4	Selective modulator	Increase insulin secretion

AMP: Adenosine monophosphate activated kinase; GSK-3: Glycogen synthase kinase 3; MAP: Mitogen activated protein; PTP-1b: Protein tyrosine phosphatase-1b; PP1: Protein phosphatase 1; LAR: Leukocyte antigen related; PTEN: Phosphatase and tensin homolog; PPAR-γ: Peroxisome proliferator-activated receptor; GLP: Glucagon-like peptide.

synergism[11,12]. Synergy can give better outcomes in terms of pharmacokinetic profile or physicochemical effects based on enhanced solubility profile, improved absorption and ultimately better bioavailability. Use of synergistic combinations also helps to restrict the development of resistance due to single prolonged drug use. While synthesizing or processing a single entity, unwanted adverse effects may develop due to either the extraction procedure or synthetic scheme being followed, or development of any by products; such adverse effects can be minimized or eliminated by use of combo formulations. Moreover stability issues of one to several bio-actives on long storage are more protected in combined form than in isolated form[11,12].

Combination therapy has made its way in the treatment of type 2 diabetes whether it is western classical medicine or herbal formulations. Resveratrol, a phytoalexin found in grapes which acts on various molecular targets in adipocytes and osteoblasts decreases the number of adipocytes and acts synergistically with quercetin and genistein to reduce adipogenesis[12]. Evidence based clinical research results have shown that miglitol in combination with metformin provides a better glycemic control than metformin monotherapy which is an example of synergism in anti-diabetic therapy with western medicine. Oleanolic acid, a pentacyclic triterpene, a natural component of many medicinal herbs in combination with metformin, first line antidiabetic drug showed synergistic anti-diabetic potentials in animal studies[13]. Experimental results showed that the combination reduced hepatic gluconeogenesis by decreasing mRNA expressions of PGC-1α, G-6-Pase and PEPCK (Phosphoenol pyruvate carboxykinase 1). The combination is also found to stimulate the PI3K pathway that phosphorylates Akt and down regulates mTOR to improve insulin resistance. Sesame oil, an edible oil rich in mono and polyunsaturated fatty acids is found to show synergistic anti-diabetic potentials with sulphonyl ureas *viz.* glibenclamide[14]. In case of allopathy, results of clinical trials have shown that combination therapy with miglitol and metformin was found to be more effective than the use of single drug alone[15].

Establishment of standard quality control profile in global context to confirm the validity and reproducibility of phytochemical constituents in the form of processed extract rather than single isolated compound; proper analytical and spectroscopic method development for structural characterizations in combined forms; rigorous validation of safety profile and pharmacokinetic parameters is essential to find a scientific basic of phytosynergy which may give rise to a new generation of medicinal products - phyto-pharmaceuticals[11].

BIOWAIVER-COMPUTATIONAL PHARMACOKINETICS AND IVIVC

Drug development procedure is very tedious and expensive and in many cases due to lack of adequate pharmacokinetic data of the candidate drug, completion of further research becomes questionable. With the vast expansions in the research arenas undertaking the development of new chemical entities, bioequivalence studies are of vital concern in drug development especially when there are absolute new entities or having narrow therapeutic index. Though *in vivo* animal experimentation for establishing the pharmacokinetic profile is still the surrogate, yet it's very tedious, expensive, and time consuming to handle enormous amount of data. Along with the development of *in silico* pharmacology, computational modeling now finds applications in pharmacokinetics and dynamics, as well as toxicokinetics and dynamics. Many multinational pharma R&Ds are now focusing on bio-waiver where in many cases *in vitro* results were considered more acceptable in different dosage formulations especially immediate release solid dosage forms[16-18]. In that condition to proceed with a bio-waiver study there's a need to establish dissolution profile and is to be characterized with both model dependent and independent approaches. *In vivo* performance of a dosage formulation or new chemical entity can be

Table 2 Types of *in vitro-in vivo* correlation and the parameters used

Level	*In vitro* parameters	*In vivo* parameters	Utility
Level A: direct relationship with *in vivo* data based on *in vitro* measurement alone	Dissolution curves	Absorption curves	Highest level of correlation depicting point to point relation between *in vitro* dissolution rate and *in vivo* input rate of drug from dosage form. Marks *in vitro* dissolution as the surrogate of *in vivo* performance
Level B: relation based on statistical moments analysis	MDT	MAT; MRT	Mean *in vitro* dissolution time of the product compared to mean *in vivo* residence time or mean *in vivo* dissolution time
Level C: relates one dissolution time point ($t_{50\%}$, $t_{90\%}$, *etc.*) to one mean pharmacokinetic parameter (AUC, Cmax, tmax)	Disintegration time, time to have 10%, 50%, 90% dissolved, dissolution rate, dissolution efficiency	Cmax, Tmax, Ka, time to have 10%, 50%, 90% absorbed, AUC (total or cumulative)	Single point weak correlation showing a partial relation between absorption and dissolution. Used in early stages of formulation development before pilot production

MDT: *In vivo* measurement of the dissolution rate in the digestive tract; MRT: The mean time that the drug resides in the body, MRT may also be the mean transit time; MAT: The mean time required for drug to reach systemic circulation from the time of drug administration. It is actually the mean time involved in the *in vivo* release and absorption processes as they occur in the input compartment and is estimated as MAT = MRT - MRToral/*i.v.*; AUC: In pharmacokinetics, AUC is the area under the curve (mathematically known as definite integral) in plot of concentration of drug in blood plasma against time, it reflects the actual body exposure to drug after administration of a dose of the drug and expressed in mg × h/L; Ka: It is the absorption rate constant which is a proportionality constant that relates the rate of drug absorbed in the body; Cmax: It refers to peak serum concentration that a drug achieves in a specified compartment or test area of the body after the drug has been administered and prior to the administration of a second dose; Tmax: It is the time after administration of a drug when the maximum plasma concentration, Cmax is reached and during which rate of absorption is equal to the rate of elimination. MDT: Mean dissolution time; MAT: Mean absorption time; MRT: Mean residence time.

simulated from the *in vitro* dissolution data after establishing a definitive IVIVC[19-23].

The biopharmaceutics classification system (BCS) proceeds with a predictive approach for developing correlation between physicochemical criteria of drug formulations and its *in vivo* bioavailability. BCS is not the direct IVIVC; IVIVC develops a mathematical relation between *in vitro* and *in vivo* data by either linear or non- linear correlation[19-25]. As per FDA guidelines IVIV correlation ranges from A-D with multiple level C correlation, the details of which have been presented in Table 2.

Apart from these three types of correlation, level D correlation is a rank order and qualitative method which may be applicable in some steps of formulation development but not recommended for regulatory purposes. A multiple point level C correlation is really a justified bio-waiver where correlation is established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. This correlation is based on three dissolution points (early, middle and end stages) and on achievement of this correlation level, the level A correlation is also likely to develop[19-21].

Even after the attainment of high level of correlation, till date no *in vitro* method can exactly simulate physiological conditions *in vivo* especially when it comes to replicate the exact gastro-intestinal (GI) conditions *in vitro viz*. appropriate amount, pH and exact physiological amounts of enzymes needed for digestion, physiological transits during digestion process, exact replication of peristalsis, food - drug interactions and its impact on dosage formulations. An artificial digestive system known as TIM1 have been developed by TNO Nutrition and Food Research mimicking the human stomach and three segments of small intestine where pH is monitored and computer controlled, constant generation of water pressure ensures mixing of enzymes by alternate compression and relaxation of flexible walls and removal of water and small molecules from lumen compartment by pumping dialysis fluid mimics the GI motility. Though such artificial models find applications in nutrition research but to be an effective quality control tool in drug development studies warrants further research[21].

CONCLUSION

Translational health research is the latest buzzword in the field of biomedicine which aims to bridge basic research with medical innovation with the perspectives of sequential development of products from lab bench to patient bedside. The landscape of drug discovery which is just the initiation of creating new chemical entities has undergone a drastic change after the emergence of computational biology, combinatorial chemistry and *in silico* docking studies. Now drug molecules are tailored as per requirements for maximizing bioavailability and stringent control over pharmacokinetics. Combination therapies with synergistic potentials are finding more prominence than monotherapy and even documentations are available in some anti-diabetic medications where combination of natural and synthetic medicine showed better results. However to capture the international pharma market and speed up the pilot scale production, there is an urgent need to boost bio-waivers which necessitates to develop robust and reproducible *in vitro* models simulating *in vivo* conditions.

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MINIREVIEWS

Incretins and selective renal sodium-glucose co-transporter 2 inhibitors in hypertension and coronary heart disease

Ramiro A Sanchez, Hugo Sanabria, Cecilia de los Santos, Agustin J Ramirez

Ramiro A Sanchez, Agustin J Ramirez, Arterial Hypertension and Metabolic Unit, University Hospital, Fundación Favaloro, Buenos Aires 1093, Argentina

Hugo Sanabria, Diabetes Unit, Instituto Cardiovascular de Buenos Aires, Buenos Aires 1428, Argentina

Cecilia de los Santos, Medical Affairs Department, Boehringer Ingelheim, Munro 1605, Argentina

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Correspondence to: Ramiro A Sanchez, MD, PhD, Professor, Head, Arterial Hypertension and Metabolic Unit, University Hospital, Fundación Favaloro, Av. Belgrano 1782, 4.º, Buenos Aires 1093, Argentina. rsanchez@ffavaloro.org
Telephone: +54-11-4371337

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Abstract

Hyperglycemia is associated with an increased risk of cardiovascular disease, and the consequences of intensive therapy may depend on the mechanism of the anti-diabetic agent(s) used to achieve a tight control. In animal models, stable analogues of glucagon-like peptide-1 (GLP-1) were able to reduce body weight and blood pressure and also had favorable effects on ischemia following coronary reperfusion. In a similar way, dipeptidyl peptidase IV (DPP-IV) showed to have favorable effects in animal models of ischemia/reperfusion. This could be due to the fact that DPP-IV inhibitors were able to prevent the breakdown of GLP-1 and glucose-dependent insulinotropic polypeptide, but they also decreased the degradation of several vasoactive peptides. Preclinical data for GLP-1, its derivatives and inhibitors of the DPP-IV enzyme degradation suggests that these agents may be able to, besides controlling glycaemia, induce cardio-protective and vasodilator effects. Notwithstanding the many favorable cardiovascular effects of GLP-1/incretins reported in different studies, many questions remain unanswered due the limited number of studies in human beings that aim to examine the effects of GLP-1 on cardiovascular endpoints. For this reason, long-term trials searching for positive cardiovascular effects are now in process, such as the CAROLINA and CARMELINA trials, which are supported by small pilot studies performed in humans (and many more animal studies) with incretin-based therapies. On the other hand, selective renal sodium-glucose co-transporter 2 inhibitors were also evaluated in the prevention of cardiovascular outcomes in type 2 diabetes. However, it is quite early to draw conclusions, since data on cardiovascular outcomes and cardiovascular death are limited and long-term studies are still ongoing. In this review, we will analyze the mechanisms underlying the cardiovascular effects of incretins and, at the same time, we will present a critical position about the real value of these compounds in the cardiovascular system and its protection.

Key words: Incretins; Hypertension; Cardiovascular effects; Dipeptidyl peptidase 4 inhibitors; Sodium-glucose co-transporter 2

Core tip: The dipeptidyl peptidase IV inhibitors prevent the breakdown of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide, but also decrease the degradation of several vasoactive peptides. Dipeptidyl peptidase IV inhibitors have shown to have favorable effects in animal models of ischemia/reperfusion and in hypertension. Clinical studies are most under way and final results could give reliable information on cardiovascular protection. Selective inhibitors of renal sodium glucose transport 2 have been also evaluated in the prevention of cardiovascular outcomes in type 2 diabetes. However, data on cardiovascular outcomes and cardiovascular death are limited and long term studies are on-going, therefore it is premature to draw conclusions.

Sanchez RA, Sanabria H, de los Santos C, Ramirez AJ. Incretins and selective renal sodium-glucose co-transporter 2 inhibitors in hypertension and coronary heart disease. *World J Diabetes* 2015; 6(11): 1186-1197 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i11/1186.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i11.1186

INCRETINES IN THE CARDIOVASCULAR SYSTEM

As a cardiovascular risk factor, hypertension together with disglycemia, hyperlipidemia and overweight, is one of the components of the so-called metabolic syndrome. From these four, hypertension takes the first position in mortality, particularly in middle- and low-income countries. Regarding disabilities, hypertension ranks in the third position after malnutrition and risky sex behavior[1-11]. As it is well known, diabetes mellitus is closely linked to cardiovascular diseases, and hypoglycemic agents may have either positive or negative effects on cardiovascular outcomes. Consequently, there is a growing interest in the evaluation of new compounds as therapeutic tools or with relation to side effects and interactions.

Incretins, either glucagon-like peptide-1 (GLP-1) analogues or dipeptidyl peptidase IV (DPP-IV) inhibitors, are just a new group of hypoglycemic drugs and their cardiovascular effects are being evaluated in different trials.

At the gastrointestinal level, incretins are able to increase insulin release after food intake in a glucose-dependent manner[12]. From these hormones, the most widely known ones are GLP-1 and the gastric inhibitory polypeptide.

The role of endogenous GLP-1 in the metabolic and cardiovascular systems has been intensively studied[13] with specific receptor antagonists (GLP-1R antagonists), with special attention to the cardiac effects of GLP-1 in different animal models. In conscious dogs with induced cardiomyopathy[14], GLP-1 infusion improved left ventricular contractility in 90%, stroke volume in 100% and cardiac output in 50%. Furthermore, an enhanced oxidative phosphorylation effect as a consequence of an increase in myocardial glucose uptake and oxygen consumption was also reported. Some authors suggested that the beneficial cardiovascular effects of GLP-1R stimulation are primarily due to the modulation of myocardial metabolism rather than direct mechanisms[14].

Other studies suggest that GLP-1 may induce vasodilation, possibly through the activation of specific endothelial and cardiovascular myocyte receptors[15].

In recent studies that used a mouse isolated heart preparation, both GLP-1 and its analog exenatide improved cardiac function following ischemia/reperfusion[16]. Moreover, data reported that GLP-1 cardioprotective effects result from additional mechanisms over the GLP receptor activation, affecting the GLP-1 degradation pathway[16-18]. Thus, the improvement of ischemic injury by coronary vasodilation induced by the metabolite GLP-1 seems to be mediated by a nitric oxide GLP-1 receptor-independent mechanism.

Studies in human beings seemed to have similar effects than those found in animal models. As an example of this, a significant improvement of left ventricular ejection fraction and wall motion scores were reported in a pilot study[19] in which 10 patients with acute myocardial infarction and coronary arterial graft surgery were perfused for three days with recombinant human GLP-1. These effects were independent from the infarction location or the diabetes history and, in some patients; they were detectable even months after cessation of the infusion. Similarly, the GLP-1 infusion improved left ventricular ejection fraction and exercise capacity in both diabetic and non-diabetic patients with congestive heart failure[15]. Finally, in diabetic patients with coronary heart disease that were pretreated with GLP-1 before cardiac surgery, an improvement of glycemic control and hemodynamic recovery indexes were reported[20].

In type 2 diabetes, endothelial dysfunction is an early alteration of the consecutive vascular disease that is responsible for an increase in cardiovascular (CV) morbidity and mortality. Furthermore, endothelial dysfunction, as a cluster of the metabolic syndrome, together with postprandial hyperglycemia and postprandial hypertriglyceridemia are commonly associated with oxidative stress, decreased fibrinolysis, sympathetic activation, and increased atherosclerotic coronary plaque burden[21]. It is interesting that incretins play a role in reducing endothelial dysfunction in experimental studies. In accordance with this information, Basu *et al*[22] reported that administration of GLP-1 enhanced forearm vasodilator response to intra-arterial acetylcholine but not to nitroprusside, which was consistent with a nitric oxide synthase-dependent effect. However, whether the role of GLP-1 or the products of its degradation mediated these effects was not

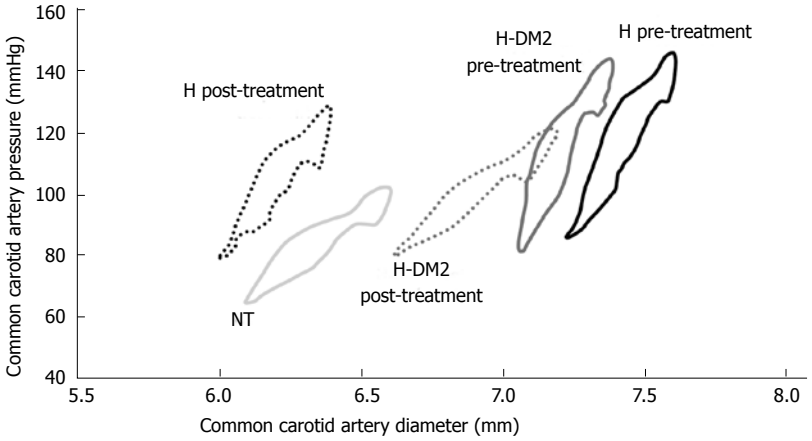

Figure 1 Pressure and diameter plot. The instantaneous pressure-diameter loops are shown, which were obtained from: H pre-treatment: Hypertensive patients without diabetes mellitus (DM) before administration of ramipril; H post-treatment: Hypertensive patients without DM after administration of ramipril; H-DM2 pre-treatment: Hypertensive patients with type 2 DM before ramipril administration; H-DM2 post-treatment: Hypertensive patients with type 2 Diabetes after ramipril administration; NT: Normotensive subjects. Adapt from Christen *et al*[23].

evaluated. In a review published by our group[23] that included patients with type 2 diabetes, we examined the endothelial function and the effects of treatment (Figure 1). The endothelial function was improved with ramipril, an angiotensin-converting enzyme inhibitor (ACEI), suggesting that GLP-1 may have endothelial effects that are similar to the ones of ACEI. In another study of Japanese diabetic patients with coronary artery disease, changes in endothelial function[24] were studied when patients were treated for 6 mo either with sitagliptin or conventional therapy. Patients receiving sitagliptin experienced a greater reduction in the C-reactive protein and systolic blood pressure (-7 mmHg), whereas hemoglobin A1c did not present any changes after treatment when compared to the control group. The authors concluded that sitagliptin, beyond its hypoglycemic action and blood pressure reduction, significantly improved the endothelial function and inflammatory state.

In conclusion, incretins as a family of anti-diabetic drugs may have additional protective effects on the cardiovascular system not only by improvement of glycemic control. In this regard, the mechanisms involved could be: the optimization of the endothelial function and the reduction of the inflammatory process with a subsequent improvement of the arterial and cardiac dynamics.

INCRETINS ON BLOOD PRESSURE

In addition to the well-demonstrated metabolic actions, incretins can reduce blood pressure as shown in different animal models of arterial hypertension. In Dahl salt-sensitive (DSS) rats, infusion of recombinant GLP-1 induces a reduction in blood pressure with concomitant attenuation of the development of hypertension[25]. This effect was related to higher levels of urine flow and sodium excretion, known as the natriuretic effect. In addition, a decrease in LV hypertrophy was observed. Similarly, in another study with DSS[26], a blunting effect of development of hypertension and cardiac left ventricular hypertrophy was described when the animals were pretreated with an exenatide-related GLP-1 receptor agonist. This was further confirmed during the pre-hypertensive period in spontaneously hypertensive rats[27] in which the administration of sitagliptin increased the levels of biologically active intact GLP-1 and significantly reduced the increase of blood pressure. These effects do not seem to be the only mechanisms involved in blood pressure reduction since, by using a mouse transgenic model, cardiac GLP-1R activation was able to induce the plasma levels of atrial natriuretic peptide (ANP) together with a decrease in blood pressure. Conversely, in GLP-1r-deficient mice, the GLP-1R agonist liraglutide failed to induce ANP secretion, vasodilation and blood pressure reduction. This supports the idea that different mechanisms of action like a gut-heart GLP-1R and an ANP-dependent axis are involved in blood pressure regulation with these compounds.

Studies of the stable GLP analogues on blood pressure were also performed in human beings[28]. Data obtained in six studies involving type 2 diabetic patients[29] showed that 6 mo of treatment with exenatide significantly reduces systolic blood pressure. Similarly, liraglutide in combination with other anti-diabetic drugs like metformin[30] also demonstrated the ability to reduce systolic blood pressure in diabetic hypertensive patients. In the LEAD-3 Mono trial[31], treatment with liraglutide *vs* glimepiride significantly decreased blood pressure. In a different study, Okerson *et al*[29] reported that six-month treatment with exenatide reduced systolic blood pressure when patients are pretreated with either insulin or placebo. The authors of these studies postulated that the exenatide antihypertensive effect seems to be partly independent from its metabolic activity. However, the weight loss effect cannot be ruled out[29] (Figure 2), raising one

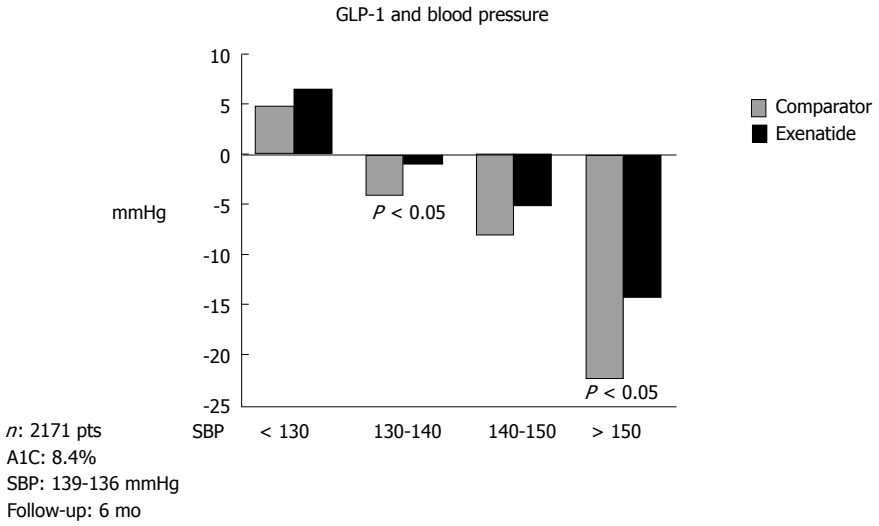

Figure 2 Glucagon-like peptide-1 and blood pressure. Summary of changes in systolic blood pressure (SBP) after the 6-mo study end point in subjects with type 2 diabetes treated with exenatide *vs* placebo. Data are presented as differences between baseline-to-end point in the least squares (mean ± SE). Adapt from Okerson *et al*[29]. GLP-1: Glucagon-like peptide-1.

important point of discussion: How weight loss may contribute to lowering blood pressure and whether this reduction is linked to the antihypertensive effect. In fact, in the Okerson study[29] the decrease observed in systolic blood pressure was significantly related to weight loss. Likewise, in the LEAD-3 trial[32], liraglutide treatment significantly reduced weight, whereas glimepiride did not. However, in another study[33], a decrease in blood pressure was observed prior to a decrease in body weight. Thus, the real association between weight reduction and blood pressure reduction is not yet clear.

Different studies re-analyzed the effects of the pressure-natriuretic mechanism in lowering of blood pressure by both GLP-1 analogues[34] and DPP-IV inhibitors[35]. In addition, Crajoinas *et al*[35] recently suggested that the activation of the cAMP/PKA signaling pathway by incretins interferes with the normal Na^+ transport in the proximal tubule that decreases sodium and water reabsorption, thus giving further support to the role of the natriuretic effect to the lowering of blood pressure through incretins.

ANTI-HYPERTENSIVE EFFECT OF DPP-IV INHIBITORS IN METABOLIC SYNDROME IN DIABETIC PATIENTS

Although a blood pressure decrease was reported in clinical studies with DPP-IV inhibitors in diabetes, these studies were not designed to evaluate the blood pressure effects and the conclusions were weak and failed to give support to the effect[36]. In this regard, patients with metabolic syndrome either under placebo or incomplete ACE inhibition were evaluated in one study carried out by Marney *et al*[37], who examined the interactive effect on blood pressure of the acute inhibition of both ACE and DPP-IV. The administration of sitagliptin was effective in lowering blood pressure. Yet, during maximal ACE inhibition sitagliptin had the opposite effect: It increased blood pressure with a concomitant increase in heart rate and circulating norepinephrine concentrations. These findings were similar to data previously reported in rats[38], where a dose-dependent decrease in blood pressure was observed with DPP-IV inhibition but later, when animals were pretreated with the ACE inhibitor captopril, the DPP-IV inhibition caused an increase in blood pressure. This effect was prevented with the blockade of the Neuropeptide Y (NPY1) receptors, thus suggesting that the combined inhibition of ACE and DPP-IV could raise blood pressure through their synergistic effects on substance P degradation. Moreover, Shah *et al*[39] showed that the inhibition of DPP-IV, similarly to GLP-1, is able to induce vasodilation (nitric oxide effect) with a consequent decrease in peripheral vascular resistance. Despite these controversial results, many investigators still favor the use of GLP-1 analogues and DPP-IV inhibitors for a better control of blood pressure in patients with diabetes and arterial hypertension[40,41]. In different studies performed in non-diabetic patients, sitagliptin[42] was associated with a 2-3 mmHg reduction in mean systolic blood pressure, assessed by 24-h ambulatory blood pressure monitoring and, in diabetic patients with inadequate glycemic control[43] that were receiving metformin, the addition of vildagliptin induced a dose-dependent decrease in both systolic and diastolic blood pressure.

Despite the data presented above, the ability of incretins to reduce blood pressure is still limited. Further studies must be performed in order to elucidate the real efficacy of GLP-1 analogues and DPP-IV inhibition on hypertension. Consequently, randomized trials in patients with either hypertension or diabetes and also with both hypertension and diabetes must be performed

in order to elucidate this important question.

ANTI-INFLAMMATORY EFFECTS OF INCRETINS IN THE CARDIOVASCULAR SYSTEM

Clinical studies of DPP-Ⅳ inhibitors on cardiovascular outcomes

Although the CV protective effects of DPP-Ⅳ inhibitors seem to be a result of an improvement of type 2 diabetes, the accumulating evidence that was mentioned earlier also suggests a possible direct myocardial effect of GLP-1 on the improvement of the endothelial function, lowering blood pressure and preventing myocardial injury[44,45].

Another important mechanism of cardiovascular protection is associated with the immune modulatory role of DPP-Ⅳ on cardiovascular inflammation. Even though this concept has been minimally investigated, this seems to be an area of emerging importance to evaluate the role of DPP-Ⅳ inhibitors in the modulation of innate and adaptive immunity[46-50]. In this regard, the decreased accumulation of specific inflammatory macrophages present in adipose tissue or atherosclerotic lesions related to the DPP-Ⅳ inhibitor treatment was studied[51,52]. The data provided raises the possibility of a DPP-Ⅳ facilitatory interaction with inflammatory related macrophages, resulting in an impairment of inflammation. On the other hand, since DPP-Ⅳ activity in serum and tissues is markedly increased in obesity in both animal models and human beings[53-55], the inhibition of DPP-Ⅳ might offer a novel strategy for suppression of low-grade inflammation present in diabetes and associated tissue insulin resistance with favorable effects that improve heart and coronary artery function. Thus, it is possible that the common effects of DPP-Ⅳ inhibition/GLP-1 signaling, in opposition to angiotensin Ⅱ/aldosterone effects, contribute to the beneficial modulation of immune responses in the cardio-renal system[56-58].

On the other hand, the vasodilator effect of both GLP-1 and DPP-Ⅳ inhibitors correlate with an increase in cGMP release, which is attenuated by the pre-incubation with nitric oxide synthase inhibitors, suggesting that at least part of their vasodilator mechanism is nitric oxide/cGMP-dependent. In addition, it seems that the anti-inflammatory effect precedes the blood pressure effect and mediates early improvements in endothelial function and atherosclerosis. Important *in vitro* studies with linagliptin performed in a mouse model of diabetic nephropathy[59] showed anti-inflammatory[49,60] and antioxidant[61] properties, improved re-epithelialization and healing of diabetes-related wounds[60] and, in a chronic renal failure rat model[62], renoprotective effects that were not linked to the worsening of glomerular and tubular pathological markers. In addition, in a uremic cardiomyopathy rat model, linagliptin significantly reduced the RNA messenger (mRNA) levels of several cardiac fibrosis markers and of a marker of left ventricular dysfunction. These results would demonstrate an important anti-fibrotic property of linagliptin[62].

In clinical studies, incretins seemed to reduce cardiovascular outcomes when compared to other hypoglycemic drugs as shown in a meta-analysis[63] in which the treatment with DPP-Ⅳ was associated with reduced CV events. The overall use of DPP-Ⅳ inhibitors compared to placebo or other oral hypoglycemic agents, apart from decreasing adverse CV effects, it was also able to reduce the risk of non-fatal myocardial infarction (MI) and acute coronary syndrome (ACS). Moreover, with DPP-Ⅳ inhibitor therapy the risk of adverse CV events was not significantly different compared to placebo, but was significantly lower compared to metformin and other oral hypoglycemic agents, including sulfonylureas and thiazolidinediones. In another small study[64] comparing sitagliptin *vs* placebo in patients with coronary artery disease and preserved left ventricular function awaiting revascularization, increased ejection fraction from 64.0% ± 8.0% to 73.0% ± 7.0% and increased plasma GLP-1 levels at peak stress (from 10.0 ± 9.0 pg/mL to 17.0 ± 11.0 pg/mL; $P \leqslant 0.003$) and at rest (from 9.0 ± 6.0 pg/mL to 12.0 ± 6.0 pg/mL) were reported.

In a large meta-analysis[65] of 25 phase Ⅲ studies, vildagliptin was administered either as monotherapy or in combination therapy for a period of 12 wk to 2 years and the drug safety was compared to a pool of placebo and active comparators. Relative to all comparators, the RRs for the composite endpoint were < 1 for both vildagliptin 50 mg *qd* and vildagliptin 50 mg *bid*, and the results were consistent across subgroups defined by age, gender and CV risk status, including the higher CV risk subgroups of elderly patients, males, or patients with a high CV risk status. The exposure-adjusted incidences of each component of the composite endpoint for vildagliptin 50 mg *bid* were also lower than or similar to those of all comparators. Based in these results, it was concluded that vildagliptin is a safe drug in the broad population of type 2 diabetes mellitus (T2DM), including patients at a higher risk of CCV events.

The incidence of major side effects (MACEs) was also evaluated in different studies with DPP-Ⅳ inhibitors. A meta-analysis[66] conducted to assess the effect of DPP-Ⅳ inhibitors on the incidence of MACE, cancer and pancreatitis compared to placebo or other treatment, determined that they were associated with a similar risk of cancer and pancreatitis and with a reduced risk of MACE. Frederich *et al*[67] analyzed eight randomized double-blind, phase Ⅱ and Ⅲ trials of patients with T2DM treated with saxagliptin, placebo, metformin, or glyburide. Cox proportional regression hazard model showed a 41% RR reduction of CV events with saxagliptin *vs* the comparators. The composite endpoint of CV death, MI or stroke was confirmed in 40 patients from whom 0.7% received saxagliptin and 1.4% received other comparator. The Cox RR

	Trials	Trials with events	Events (DPP-IVi)	Events (Comparator)	MH-OR (95%CI)	*P*	Kendall's tau	*P*
MACE	70	63	263	232	0.71 (0.59, 0.86)	< 0.001	0.04	0.64
Sitagliptin	27	24	77	67	0.86 (0.60, 1.24)	0.430	0.04	0.80
Vildagliptin	16	15	75	74	0.61 (0.43, 0.86)	0.005	0.03	0.89
Saxagliptin	13	12	62	46	0.67 (0.45, 0.99)	0.047	0.36	0.10
Linagliptin	9	8	37	41	0.72 (0.45, 1.16)	0.18	0.00	1.00
Alogliptin	5	4	12	4	0.86 (0.25, 2.93)	0.81	0.30	0.15
AMI	62	41	61	59	0.64 (0.44, 0.94)	0.023	-0.13	0.27
Stroke	63	29	41	33	0.77 (0.48, 1.24)	0.290	-0.24	0.14
Mortality	53	30	50	51	0.60 (0.41, 0.88)	0.008	0.13	0.28
CV Mortality	48	20	26	26	0.67 (0.39, 1.14)	0.140	0.05	0.76

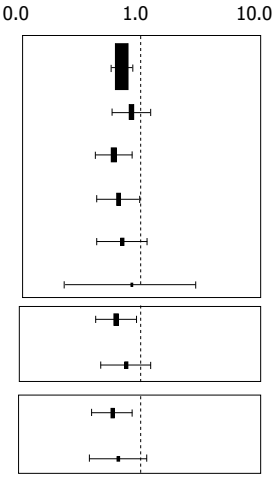

Figure 3 Mantel-Haenzel odds ratio for major cardiovascular events, acute myocardial infarction, stroke, mortality and cardiovascular mortality with 95%CI. Adapt from Monarini *et al*[90]. DPP-IVi: Dipeptidyl peptidase-IV inhibitors; MH-OR: Mantel-Haenzel odds ratio; CV: Cardiovascular; MACE: Major adverse CV events; AMI: Acute myocardial infarction.

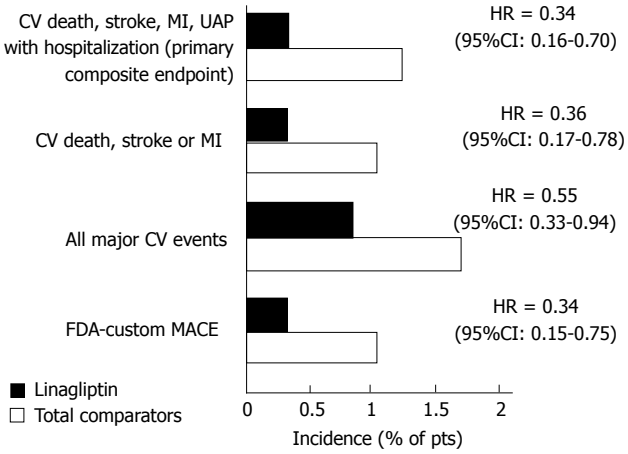

Figure 4 Cardiovascular tolerability profile of linagliptin in adults with type 2 diabetes mellitus. Results of a pre-specified meta-analysis of eight randomized, double-blind trials in which patients treated with linagliptin 5 or 10 mg/d (*n* = 3159 and 160), glimepiride 1-4 mg/d (*n* = 781), voglibose 0.6 mg/d (*n* = 162) or placebo (*n* = 977) as monotherapy or in combination with other oral anti-hyperglycemia drugs for 18-52 wk[84]. It shows the incidence of primary and secondary composite endpoints in the linagliptin and total comparators group (primary analysis), together with corresponding hazard ratios and 95%CI. Adapted from Deeks[74]. CV: Cardiovascular; FDA: Food and Drug Administration; MACE: Major adverse CV events; MI: Myocardial infarction; pts: Patients; UAP: Unstable angina pectoris.

estimate was 0.43 translating to a 57% risk reduction in patients assigned to saxagliptin. Thus, no CV harm and a potential for an actual reduction in CV events with saxagliptin was suggested[67].

Pooled information of MACEs[68-70] from different DPP-IV inhibitors is shown in Figure 3.

More recently, in a pre-specified meta-analysis assessing cardiovascular safety[71], cardiovascular risk did not increase with linagliptin 5 or 10 mg once daily (as monotherapy). Additional data suggested that linagliptin was not associated with a significantly greater risk of the primary composite endpoints, regardless of age, gender, and race, use of rescue therapy, hypoglycemia or cardiovascular risk. In an extension of one clinical trial[72], after receiving linagliptin monotherapy, the rate of patients reporting cardiovascular/cerebrovascular events was 4.1% and the rate of those with ischemic events amounted up to only 1.9%.

Finally, in a study[73] of 52 wk of follow-up in which 2.9% of the patients had severe renal impairment (a population with high cardiovascular risk), linagliptin was added to their hypoglycemic therapy, and the rate of death from cardiovascular causes was significantly lower and did not differ from the one observed with placebo.

Figure 4 shows safety indicators in other studies with linagliptin compared to other hypoglycemic drugs[74].

Trials specifically designed to evaluate the cardiovascular impact of DPP-IV inhibitors

In the SAVOR-TIMI 53 study[75], 16492 patients with type 2 diabetes and established atherosclerotic disease or high cardiovascular risk were randomized to receive saxagliptin or placebo. The primary endpoint was a composite of cardiovascular death, myocardial infarction or ischemic stroke, with a follow up of 2.1 years. No difference was observed for the primary endpoint when comparing saxagliptin to placebo (7.3% *vs* 7.2%, HR = 1.00, 95%CI: 0.89-1.12, $P = 0.99$ for superiority, $P < 0.001$ for non-inferiority). Surprisingly, a higher amount of hospitalizations due to heart failure were reported under saxagliptin compared to placebo (3.5% *vs* 2.8%; HR = 1.27, 95%CI: 1.07-1.51, $P = 0.007$). However, mortality secondary to heart failure did not increase (Figure 5).

The EXAMINE study[76] evaluated cardiovascular endpoints using alogliptin in patients with diabetes at very high cardiovascular risk. It randomized 5380 patients with diabetes and history of acute coronary syndrome. At a mean follow-up of 18 mo and compared to placebo, there was no difference in a composite of death from cardiovascular causes, non-fatal myocardial infarction, non-fatal stroke (11.3% *vs* 11.8%, HR =

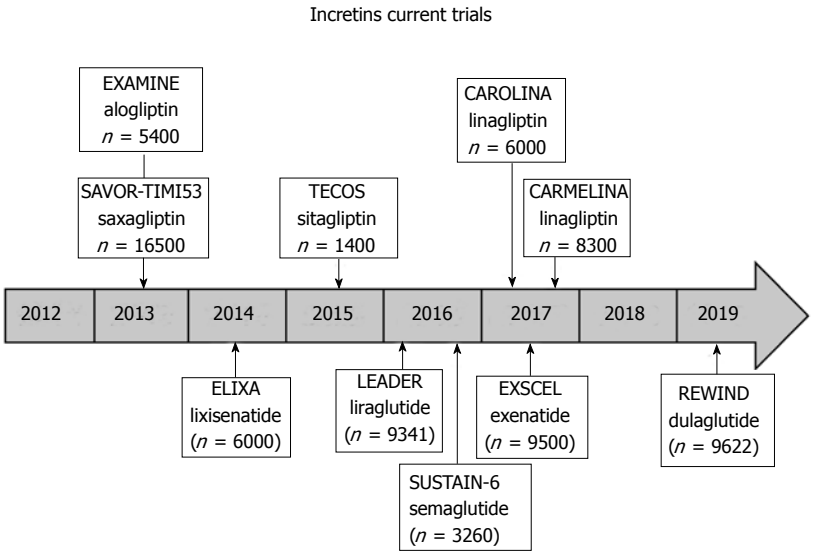

Figure 5 Flowchart of the clinical investigational trials which are completed or ongoing.

0.96; $P < 0.001$ for non-inferiority), in the different components of the primary endpoint nor in the incidence of heart failure.

TECOS: In this randomized, double-blind study recent published, 14671 patients were assigned to add either sitagliptin or placebo to their existing therapy. The primary cardiovascular outcome was a composite of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for unstable angina. Sitagliptin was noninferior to placebo for the primary composite cardiovascular outcome (HR = 0.98; 95%CI: 0.88-1.09; $P < 0.001$). Rates of hospitalization for heart failure did not differ between the two groups (HR = 1.00; 95%CI: 0.83-1.20; $P = 0.98$)[77].

The interim analysis of results of SITAGRAMI: Safety and Efficacy of Sitagliptin plus Granulocyte Colony-Stimulating Factor in Patients Suffering from Acute Myocardial Infarction[78]. It is a phase Ⅲ multicenter trial testing the myocardial regenerating effects of Sitagliptin combined with G-CSF after an acute MI. The results are encouraging, but they still need to be confirmed once the long-term study has been analyzed.

Others ongoing multicenter clinical trials

CAROLINA: Cardiovascular Outcome Study of Linagliptin *vs* Glimepiride in Patients with T2DM[79]. It is a long-term multicenter study planning to enroll 6000 patients with an expected completion date in September 2018.

CARMELINA: Cardiovascular safety and Renal Microvascular outcome with linagliptin patients with T2DM at high vascular risk. It is a long-term study investigating the efficacy and safety of linagliptin *vs* placebo on cardiovascular and renal micro-vascular outcomes in patients with type 2 diabetes and risk of cardiovascular events. The study will randomize patients with type 2 diabetes and previous CV complications and albuminuria [urinary albumin creatinine ratio (UACR) ⩾ 30 mg/g] with or without evidence of micro-vascular related end-organ damage and an estimated glomerular filtration rate (eGFR) between 15 and 45 mL/min and an UACR > 200 mg/g or eGFR ⩾ 45-75. The study will include more than 8000 adults with type 2 diabetes. The primary endpoint will be the time to the first occurrence of either CV death (including fatal stroke and fatal MI); non-fatal MI; non-fatal stroke; or hospitalization for unstable angina pectoris. The renal outcome is measured as a composite of renal death, sustained end-stage renal disease and sustained decrease of ⩾ 50% eGFR. The study will be completed in 2018. This kind of study could provide us with answers regarding the CV and renal outcomes for this type of drugs.

CARDIAC AND BLOOD PRESSURE EFFECTS OF RENAL GLUCOSE TRANSPORT INHIBITORS

The glucose reabsorption regulation is mainly performed in the kidneys where more than 99% of the plasma glucose that filters through the kidneys is reabsorbed. There are two transporters of glucose across cell membranes, the GLUTs, facilitative glucose transporters and an active sodium-dependent transport process mediated by the sodium/glucose co-transporters (SGLTs). These are a large family of intestinal epithelium and of the proximal renal tubules membrane proteins involved in the transportation of glucose, amino acids, vitamins, osmolytes, and some ions[80].

The high-capacity, low-affinity transporter sodium-glucose co-transporter 2 (SGLT2) is expressed primarily in the kidney, while SGLT1 plays an important function in the absorption of glucose in the intestine. The issue of gene expression and the possibility of SGLT

adaptation to chronic hyperglycemia is an area for further investigation. A small amount of adaptation and a near two-fold increase in the SGLT2 mRNA expression in diabetes animal models was shown. The induction of diabetes in rats increased mRNA expression of both SGLT2 and hepatocyte nuclear factor-1a in the renal cortex. Glycemic control was improved after 6 d of treatment with insulin or phlorizin accompanied by a reduced expression of SGLT2 and hepatocyte nuclear factor-1a to near-normal levels[81].

SGLT2 inhibitors are a new class of anti-diabetic drugs that reduce renal glucose reabsorption selectively in the proximal convoluted tubule leading to an increased urinary glucose excretion without potential gastrointestinal side effects. The SGLT2 inhibitors that are currently under investigation are dapagliflozin, a C-Aryl glucoside, empagliflozin and sergliflozin, an O-glucoside and canagliflozin[82,83] and represent an interesting and important tool to be added for the treatment of hyperglycemia. Additionally, SGLT2 inhibitors were associated with a reduction in systolic blood pressure compared to placebo (mean difference: -3.77 mmHg) and active comparators (mean difference: -4.45 mmHg). Diastolic blood pressure was also reduced with SGLT2 inhibitors compared to placebo (mean difference: -1.75 mmHg) and other anti-diabetic agents (mean difference: -2.01 mmHg). Risk of bias was high for both systolic and diastolic blood pressure analyses[84,85].

To be taken into account is the fact that SGLT2 inhibitors, like metformin, are associated with weight loss and also act as osmotic diuretics, resulting in a lowering of BP. While not approved for BP lowering, they may potentially aid BP goal achievement in people with a target reduction within 7-10 mmHg[86,87]. However, more studies are needed in order to determine a positive antihypertensive action of these compounds.

Regarding potential cardiovascular effects of SLGT2, different meta-analysis were performed: for dapagliflozin, the meta-analysis was based on 14 trials including 6300 patients. An OR of 0.73 (95%CI: 0.46-1.16) compared with the control group was reported, supporting the idea of an absence of cardiovascular risk. In a pooled analysis of two dapagliflozin trials[87] involving patients with established cardiovascular disease, the hazard ratio (HR) for the composite cardiovascular endpoint (cardiovascular death, myocardial infarction, stroke, and hospitalization for unstable angina) was 1.07 (95%CI: 0.64-1.72) compared to placebo. In another study that included data from 10 trials (10474 patients, OR = 0.95), of canagliflozin compared with placebo, no association of an increased risk for the composite cardiovascular outcome compared to placebo or an active comparator was found. Similarly, in the United States Food and Drug Administration report[87], the HR for non-fatal stroke was higher in patients receiving canagliflozin (6876 patient-years) than in the control groups (3470 patient-years; HR = 1.46; 95%CI: 0.83-2.58). On the other hand, an imbalance in the incidence of cardiovascular events was observed during the first 30 d[88] for canagliflozin (13 of 2886 patients) or placebo (1 of 1441 patients), which resulted in an HR = 6.50 (95%CI: 0.85-49.66). It was explained that this high risk of events resulted from volume depletion after the initiation of canagliflozin treatment, which failed to be observed after 30 d of treatment. In another recent study, systolic and diastolic blood pressure analyses were performed in response to empagliflozin during the euglycemic clamp in hypertensive patients. A reduction in systolic blood pressure was reported, as well as a decreased augmentation index at the radial, carotid and aortic arteries. Similar effects on arterial stiffness were observed, without changes in blood pressure. Carotid-radial pulse wave velocity decreased significantly under both glycemic conditions ($P \leqslant 0.0001$), whereas declines in carotid-femoral pulse wave velocity were only significant during clamped hyperglycemia. Finally, HRV, plasma noradrenalin and adrenaline remained unchanged under both euglycemic and hyperglycemic clamp conditions[89].

CONCLUSION

These new anti-diabetic compounds have shown additive CV protective effects in T2DM. Additional benefits include lowering of blood pressure, improvement of lipid profile and endothelial dysfunction, decrease in the macrophage-mediated inflammatory response, and reduction of myocardial injury. All these effects were mainly evaluated in animal models, since human clinical studies that include a high number of participants are still missing.

On the other hand, there are ongoing studies that aim to evaluate the CV effect and the safety of DPP-Ⅳ inhibitors. From the last studies that were published in which DPP-Ⅳ inhibitors were used, SAVOR TIMI, TECOS and EXAMINE, it seems that a neutral cardiovascular effect rather than a benefit is expected for these compounds. There are other studies with DPP-Ⅳ, which are still being developed, such as CAROLINA and CARMELINA, so additional effects could still be assessed.

As it was previously mentioned, further investigations in large cohorts of diabetic patients are needed in order to assess the exact mechanisms of CV protective effects held by renal glucose transport inhibitors. The reason supporting this need is based on the fact that these compounds have shown interesting natriuretic effect resulting in blood pressure decrease and loss of weight. Further trials may endorse these clinical features.

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Initial validation of the Yin-Yang Assessment Questionnaire for persons with diabetes mellitus

Yee Chi Peggy Wong, Mei Che Samantha Pang

Yee Chi Peggy Wong, Dietetic Department, Tuen Mun Hospital, Tuen Mun, New Territories, Hong Kong, China

Mei Che Samantha Pang, School of Nursing, Hong Kong Polytechnic University, Hong Kong, China

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Correspondence to: Yee Chi Peggy Wong, Dr, Dietetic Department, Tuen Mun Hospital, No. 1, Tsing Chung Koon Road, Tuen Mun, New Territories, Hong Kong, China. wongp@ha.org.hk
Telephone: +852-24685108
Fax: +852-24631314

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Abstract

AIM: To initially test for the content validity, comprehensibility, test-retest reliability and internal consistency reliability of the Yin-Yang Assessment Questionnaire (YY-AQ).

METHODS: The process of initial validity and reliability test covered: (1) content validation from the findings of 18 multiple-case studies, validated Yin- and Yang-deficiency assessment questionnaires, relevant literatures and registered Chinese medicine practitioners; (2) comprehension with the levels of comprehensibility for each item categorized on a 3-point scale (not comprehensible; moderately comprehensible; highly comprehensible). A minimum of three respondents selecting for each item of moderately or highly comprehensible were regarded as comprehensive; (3) test-retest reliability conducted with a 2-wk interval. The intraclass correlation coefficients (ICCs) and their 95%CIs were calculated using a two-way random effects model. Wilcoxon Signed Rank test for related samples was adopted to compare the medians of test-retest scores. An ICC value of 0.85 or higher together with $P > 0.05$, was considered acceptable; and (4) internal consistency of the total items was measured and evaluated by Cronbach's coefficient alpha (α). A Cronbach's α of 0.7 or higher was considered to represent good internal consistency.

RESULTS: Eighteen Yin-deficiency and 14 Yang-deficiency presentation items were finalized from content validation. Five participants with type 2 diabetes mellitus (T2DM) performed the comprehensibility and test-retest reliability tests. Comprehensibility score level of each presentation item was found to be moderate or high in three out of the five participants. Test-retest reliability showed that the single measure ICC of the total Yin-deficiency presentation items was 0.99 (95%CI: 0.89-0.99) and the median scores on the first and 14th

days were 17 (IQR 6.5-27) and 21 (IQR 6-29) (P = 0.144) respectively. The single measure ICC of the total Yang-deficiency presentation items was 0.88 (95%CI: 0.79-0.99) and the median scores on the first and 14th days were 10 (IQR 6-18) and 14 (IQR 7-23) (P = 0.144) respectively. The results of a descriptive correlation study on 140 survey participants with T2DM using the YY-AQ showed that internal consistency of the total Yin-deficiency and Yang-deficiency presentation items was satisfactory, with Cronbach's α of 0.79 and 0.78 respectively.

CONCLUSION: The YY-AQ will be tested further for comprehensibility, test-retest and internal consistency reliabilities, scoring system validity, construct validity, convergent and discriminant validities, responsiveness and predictive validity.

Key words: Body constitution; Traditional Chinese medicine; Diabetes mellitus; Yin-deficiency; Yang-deficiency; Yin-Yang-deficiency; Yin-Yang assessment questionnaire; Initial validity and reliability

Core tip: Unregulated "unhealthy" body constitution (BC) with an imbalanced Yin and Yang can induce chronic diseases. Past research findings support that food has natures that can regulate the "unhealthy" BC by balancing Yin and Yang. Yin-, Yang- and Yin-Yang-deficiency are the common "unhealthy" BC types in diabetes mellitus (DM). In order to identify the "unhealthy" BC presentations, it was necessary for dieticians to develop the Yin-Yang Assessment Questionnaire for DM. It has passed the initial validation and will be tested further for construct validity, convergent and discriminant validities, responsiveness and predictive validity; scoring system validity, comprehensibility, test-retest and internal consistency reliabilities.

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INTRODUCTION

From a perspective of traditional Chinese medicine (TCM), body constitution (BC) represents the health of an individual or a population in terms of the physical structure, physiological function, psychological reaction and metabolism[1,2]. BC can be classified as "healthy" and "unhealthy". "Healthy" BC occurs in the person with a balance of Yin (cold) and Yang (hot) while an unbalanced Yin and Yang leads to "unhealthy" BC, such as Yin-deficiency and Yang-deficiency[3]. In accordance with the theory of Yin-Yang interaction in TCM, the weaker the Yin, the weaker the Yang it will be and *vice versa*. That is, unregulated Yin-deficiency will weaken the Yang to further change to Yin-Yang-deficiency or *vice versa*. Different "unhealthy" BC types, such as Yin-deficiency and Yang-deficiency can be found in association with a single disease. Likewise, the same "unhealthy" BC type can be found to be associated with different diseases[4]. Without a prompt and appropriate treatment, "unhealthy" BC will induce diseases. Studies showed that both Yin-deficiency and Yang-deficiency types of BC have a negative influence on nervous system[5], long-term memory[6], blood pressure[7], heart health[8], carcinoma[9] and sleep quality[10]. In addition, persons with Yang-deficiency are also found to have hormone abnormality[11], organ dysfunction and decreasing metabolic rate[12], accumulation of free radicals (destructive substances inside the body), declining immunity and sterility[13].

Yin-deficiency, Yang-deficiency and Yin-Yang-deficiency are commonly recognized BC types in the population of diabetes mellitus (DM)[14-17]. Empirical study found that persons with type 2 diabetes mellitus (T2DM) have the presentations of these "unhealthy" BC types (Table 1)[18]. A validated Yin/Yang-deficiency assessment questionnaire will help provide prompt assessment of "unhealthy" BC presentations so that earlier and appropriate dietary therapy can be provided to regulate the "unhealthy" BC presentations by a balance of Yin and Yang. The ultimate goal of this treatment strategy is to prevent the development of other chronic diseases.

There are a few validated Yin-deficiency and Yang-deficiency assessment questionnaires available in the field of TCM, such as the Traditional Chinese Medical Yang-Xu Constitutional Questionnaire (TCMYCQ)[19], the Yin-Deficiency Questionnaire 1 (Yin-DQ1)[20] and the Cold-Heat Pattern Questionnaire[21]. However, these questionnaires do not target those with DM. The TCMYCQ is applied for pregnant women while the Yin-DQ1 was developed for general patients and the Cold-Heat Pattern Questionnaire is mainly adopted in clinical trials only. In view of the unavailability of a Yin- and Yang-deficiency assessment questionnaire for persons with DM, it was necessary to develop one such instrument for three reasons. First, DM prevalence has been increasing worldwide[22]. Second, research findings showed that Yin-deficiency, Yang-deficiency and Yin-Yang-deficiency are the common types of "unhealthy" BC in DM population[14-17]. Third, if BC assessment is to be integrated into the current dietary practice in future, this specific assessment instrument would be helpful to healthcare professionals, such as dieticians, in the assessment of Yin-deficiency, Yang-deficiency or Yin-Yang-deficiency presentations for persons with DM before a Yin/Yang enhancing dietary therapy would be provided for regulating the "unhealthy" BC

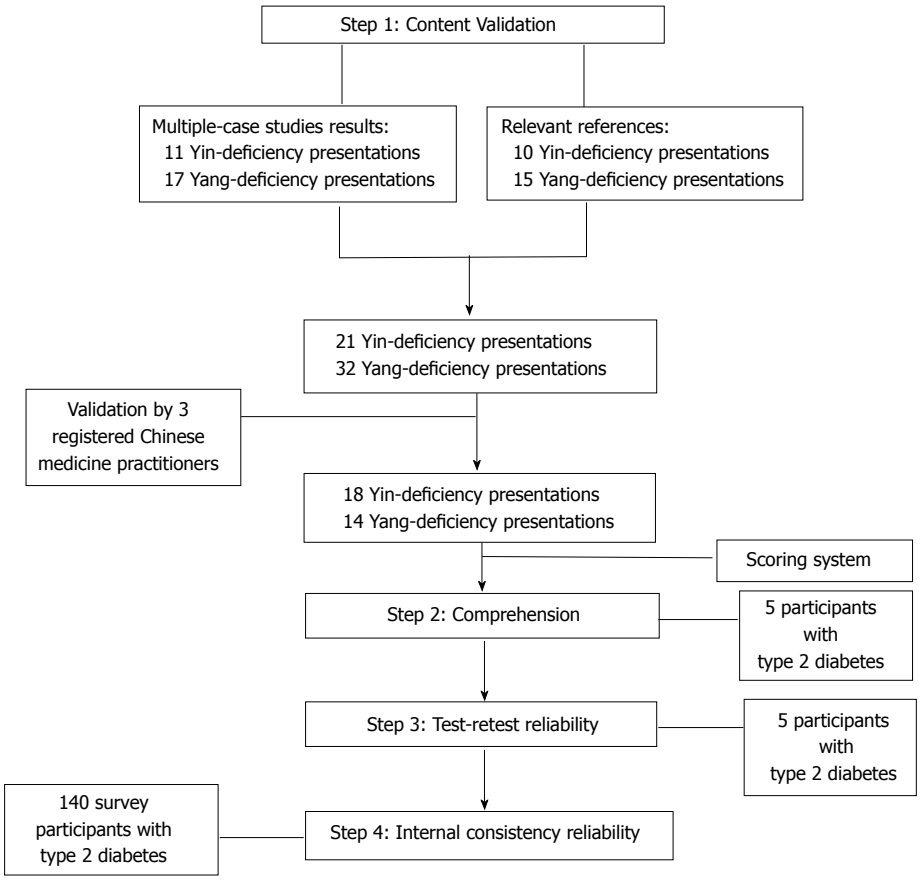

Figure 1 The process of developing the Yin-Yang Assessment Questionnaire.

presentations.

MATERIALS AND METHODS

The initial validation of the Yin-Yang Assessment Questionnaire (YY-AQ) for DM is summarized in four steps (Figure 1). They are content validation, comprehension, test-retest reliability and internal consistency reliability. Scoring system was set up in between the steps of content validation and comprehension.

Content validation

Content validation focuses on whether the full content of a conceptual definition is represented in the measure[23]. The YY-AQ was performed using the findings from the multiple-case studies conducted by Wong *et al*[18], validated Yin-deficiency and Yang-deficiency assessment questionnaires and relevant literatures.

The multiple-case studies

Between May 2013 and June 2013, 18 persons with T2DM were recruited in the multiple-case studies[18]. They were members of a non-profit organization for persons with DM. A specific characteristic of this participant group was that they all showed interests and faith in Chinese dietary therapy when asked why they liked to participate in this study. Apart from DM, all of them did not have other chronic diseases, non-diabetic medications and other medical treatments, such as kidney dialysis, chemotherapy, radiotherapy or Chinese medicine tonification. About three quarters of the 18 cases was female. The majority was aged less than 60 years. Most of the participants had more than three years of known diagnosis and were overweight. Nearly all of them were taking oral diabetic medications and their blood glucose levels were within normal range.

Each participant was assessed for BC type and presentations by a registered Chinese medicine practitioner (RCMP), who has 25 years of clinical experience in TCM, performed the TCM diagnostic technique using "Four Examinations" to collect the data with an application of the "Eight Principles of Syndrome Differentiation" to diagnose the BC type. Inter-rater reliability test was performed by two other RCMPs on five cases with a consistency rate of 80%. Eleven Yin-deficiency presentations and 17 Yang-deficiency presentations were identified from the multiple-case studies.

Procedure of content validation

First, Yin-deficiency presentation items were compiled from the multiple-case studies findings, validated Yin-DQ1[20] and two related studies[10,24]. Twenty Yin-deficiency presentation items were compiled, of which 11 items were made reference to the findings of the 18 multiple-case studies[18]. Apart from the Yin-deficiency

Table 1 Presentations of Yin-deficiency and Yang-deficiency in persons with type 2 diabetes mellitus

18 Yin-deficiency presentations	14 Yang-deficiency presentations
I have felt excessively warm in all seasons[1]	I have had an aversion to cold in all seasons[1]
I have worn thin clothes due to feeling excessively warm	I have worn thick clothes due to my aversion to cold
I have intermittent hot and cold spells	I have had an aversion to strong wind
My face has been flushed with crimson red	I have experienced heavy sweating[1]
I have been thin and slim[1]	My body looks puffy[1]
My palms or soles have felt hot	I have had pains on my knee, loin, shoulder, and back but feeling better with heat application[1]
I have had tinnitus[1]	I have running nose or and sneezing[1]
I have dry cough[1]	I have had clear sputum[1]
I have experienced hot flush especially in the afternoon	I have felt comfortable with hot drink[1]
I have experienced sweating at night[1]	I needed to wake up because of my diarrhea
My stools have been dry and hard[1]	My stools have been loose or watery[1]
I have felt hungry even after big meals	I have had diarrhea, itchy throat or cough after intake of cold food
I have passed minimal volumes of urine that were yellow colored[1]	I have passed large volumes of colorless urine[1]
I have always drunk water to quench my thirst	I have experienced bland taste in my mouth[1]
My thirst could not be relieved by frequent water intake[1]	
My skin has been very dry[1]	
My eyes have felt very dry	
My lips have felt very dry	

[1]Presentations found in multiple-case studies[18].

presentations, Yang-deficiency presentations were compiled from the multiple-case studies findings, validated TCMYCQ[19] and Wang[10]'s study. Thirty-three Yang-deficiency presentation items were compiled, of which 17 items were found from the multiple-case studies findings[18]. A total of 53 presentation items were finally compiled, of which 28 (52.8%) belonged to the findings of the multiple-case studies[18]. Second, three RCMPs validated the contents of the 53 Yin-deficiency and Yang-deficiency presentation items. One of them has clinical experience for more than 25 years. The other two RCMPs, who are at PhD level in Chinese medicine, have been practising TCM for eight and 18 years respectively. The 53 presentation items were validated further into 32 items, of which 18 were Yin-deficiency type and 14 were Yang-deficiency type (Table 1). Of these 32 presentation items, 19 (53%) belonged to the findings of the multiple-case studies[18].

Scoring system

With reference to the validated TCMYCQ[19], the presentation items of Yin-deficiency and Yang-deficiency were categorized into frequency scoring system including "never", "rare", "occasional", "often" and "always". Each score was set for the respondent on a scale from 0 score (never) to 1 score (rare), 2 scores (occasional), 3 scores (often) and 4 (always) (Table 2). The higher the presentation score level, the more severe the "unhealthy" BC presentations the person has experienced. For statistical purpose, the score levels of the Yin-deficiency, Yang-deficiency and Yin-Yang-deficiency presentations were defined into low, moderate, high and very high levels using median as the cut-off point.

Comprehension

Comprehension is an important procedure in the development of an instrument. It requires respondent to be able to understand the contents presented and to have the opportunity to read, evaluate and consider the content presented[25]. Five participants with T2DM were recruited and asked whether they understood the 32 presentation items from the YY-AQ. The levels of comprehensibility for each item were categorized on a 3-point scale (not comprehensible; moderately comprehensible; highly comprehensible). A minimum of three respondents selecting for each item of moderately or highly comprehensible was regarded as comprehensive.

Test-retest reliability

In order to ensure the reliability of the YY-AQ, it was necessary to ensure same score would be obtained when it would be given to the same person, under the same circumferences, but at a different time[23]. The test-retest reliability requires two administrations of the measuring instrument so as to assure stability of measurement over time. The YY-AQ was administered by five participants with T2DM with a 2-wk interval. This interval was used because the participants considered this length of time to be reasonable for time convenience. The participants completed the first YY-AQ at the recruitment. The second YY-AQ was sent to them by mail. They were requested to complete this second questionnaire on the 14th day since the completion of the first YY-AQ.

Internal consistency reliability

A measurement with a high degree of internal consistency is able to ensure all items are consistent with each other, or all working in the same direction[23]. Internal consistency estimation requires only one administration of the instrument. The YY-AQ was evaluated by a value of Cronbach's α[26] from a descriptive correlation

Table 2 The Yin-Yang Assessment Questionnaire

Your feelings (presentations)	Put a "tick" if applicable				
	No	Rare	Occasional	Often	Always
	0	(1 score)	(2 scores)	(3 scores)	(4 scores)
I have felt excessively warm in all seasons[1]					
I have worn thin clothes due to feeling excessively warm[1]					
I have had an aversion to cold in all seasons[2]					
I have worn thick clothes due to my aversion to cold[2]					
I have intermittent hot and cold spells[1]					
I have had an aversion to strong wind[2]					
My face has been flushed with crimson red[1]					
I have experienced hot flush especially in the afternoon[1]					
I have experienced sweating at night[1]					
My skin has been very dry[1]					
My eyes have felt very dry[1]					
My lips have felt very dry[1]					
I have been thin and slim[1]					
My body looks puffy[2]					
My palms or soles have felt hot[1]					
I have had pains on my knee, loin, shoulder, and back but feeling better with heat application[1]					
I have running nose or and sneezing[1]					
I have had tinnitus[2]					
I have always drunk water to quench my thirst[1]					
I have experienced heavy sweating[2]					
My thirst could not be relieved by frequent water intake[1]					
I have felt comfortable with hot drink[2]					
I have dry cough[1]					
I have had clear sputum[2]					
My stools have been dry and hard[1]					
My stools have been loose or watery[2]					
I needed to wake up because of my diarrhea[2]					
I have experienced bland taste in my mouth[2]					
I have felt hungry even after big meals[1]					
I have had diarrhea, itchy throat or cough after intake of cold food[2]					
I have passed minimal volumes of urine that were yellow colored[1]					
I have passed large volumes of colorless urine[2]					

[1]Yin-deficiency presentation; [2]Yang-deficiency presentation.

study on BC presentations in a sample of persons with T2DM[18].

The descriptive correlation study

One hundred and forty participants with T2DM were recruited to take part in the structured questionnaire survey between October 2013 and December 2013 after they had met the inclusion criteria: aged over 18 years; not on insulin injection and other medical treatments, such as kidney dialysis, chemotherapy or radiotherapy. Of the 140 survey participants, majority was the female. The mean age was about 65 years. Nearly half of the survey participants had more than 10 years of known diagnosis, taking either oral diabetic medications or non-diabetic medications. More than half of them had other chronic diseases, such as hypertension, heart disease, gout, cancer or liver disease. More than three quarters of the survey participants reported satisfactory blood glucose control. At the survey, each of them self-administered the YY-AQ. Completed questionnaires were then collected for data analysis of internal consistency reliability after data check by the researcher or trained helpers.

Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 20, SPSS Inc., United States) was used to analyze the questionnaire data. Intraclass correlation coefficient (ICC) was used to analyze the test-retest reliability of the YY-AQ. Wilcoxon Signed Rank test for related samples was adopted to compare the median of test-retest scores. If the ICC value of 0.85 or higher together with $P > 0.05$ for the related samples test, the test-retest reliability of the YY-AQ was considered acceptable[27]. A Cronbach's α of 0.7 or higher was considered to represent good internal consistency[23].

RESULTS

A total of 32 presentation items (18 Yin-deficiency items and 14 Yang-deficiency items) were validated for the YY-AQ (Table 2). Based on the cut-off point (median = 18), the score levels of the Yin-deficiency presentations questionnaire are categorized into low score (0-11 scores), moderate score (12-18 scores), high score (19-27 scores) and very high score (> 27 scores). The Yang-deficiency presentation score levels (median = 15)

Table 3 Categorization of the Yin-Yang Assessment Questionnaire score levels in research setting

Presentation	Presentation score levels			
	Low score	Moderate score	High score	Very high score
Yin-deficiency (median = 18)	0-11	12-18	19-27	> 27
Yang-deficiency (median = 15)	0-10	11-15	16-20	> 20
Yin-Yang-deficiency (median = 35)	0-23	24-35	36-48	> 48

Table 4 Comprehensibility of Yin-Yang Assessment Questionnaire (*n* = 5)

Your feelings (presentations)	Frequency of comprehensibility		
	Not	Moderately	Highly
I have felt excessively warm in all seasons	0	4	1
I have worn thin clothes due to feeling excessively warm	0	4	1
I have had an aversion to cold in all seasons	0	3	2
I have worn thick clothes due to my aversion to cold	0	3	2
I have intermittent hot and cold spells	1	4	0
I have had an aversion to strong wind	1	4	0
My face has been flushed with crimson red	0	5	0
I have experienced hot flush especially in the afternoon	1	4	0
I have experienced sweating at night	0	5	0
My skin has been very dry	0	5	0
My eyes have felt very dry	0	5	0
My lips have felt very dry	1	4	1
I have been thin and slim	0	0	5
My body looks puffy	1	4	0
My palms or soles have felt hot	0	5	0
I have had pains on my knee, loin, shoulder, and back but feeling better with heat application	0	4	1
I have running nose or and sneezing	0	5	0
I have had tinnitus	0	0	5
I have always drunk water to quench my thirst	0	0	5
I have experienced heavy sweating	0	5	0
My thirst could not be relieved by frequent water intake	0	3	2
I have felt comfortable with hot drink	0	0	5
I have dry cough	1	4	0
I have had clear sputum	0	5	0
My stools have been dry and hard	0	0	5
My stools have been loose or watery	0	0	5
I needed to wake up because of my diarrhea	1	4	0
I have experienced bland taste in my mouth	1	4	0
I have felt hungry even after big meals	0	0	5
I have had diarrhea, itchy throat or cough after intake of cold food	0	0	5
I have passed minimal volumes of urine that were yellow colored	0	4	1
I have passed large volumes of colorless urine	0	5	0

were set as: low score (0-10 scores), moderate score (11-15 scores), high score (16-20 scores) and very high score (> 20 scores). The Yin-Yang-deficiency scores were obtained by adding up the totals from the Yin- and Yang-deficiency scores. The Yin-Yang-deficiency score levels (median = 35) were classified as low score (0-23 scores), moderate score (24-35 scores), high score (36-48 scores) and very high score (> 48 scores) (Table 3). The results showed that more than three out of the five participants had selected each items of the YY-AQ as moderately or highly comprehensible (Table 4). Test-retest reliability showed that the single measure ICC of the total Yin-deficiency presentation items was 0.99 (95%CI: 0.89-0.99) and the median scores on the first and 14th days were 17 (IQR 6.5-27) and 21 (IQR 6-29) (P = 0.144) respectively. The single measure ICC of the total Yang-deficiency presentation items was 0.88 (95%CI: 0.79-0.99) and the median scores on the first and 14th days were 10 (IQR 6-18) and 14 (IQR 7-23) (P = 0.144) respectively. Internal consistency of the total Yin-deficiency and Yang-deficiency presentation items showed Cronbach's α of 0.79 and 0.78 respectively.

DISCUSSION

Although the initial validation of the YY-AQ was performed in a sample of persons with T2DM, this questionnaire can also be applied to those with type 1 diabetes mellitus (T1DM) or impaired glucose tolerance (IGT). It is because DM in TCM is not treated like those in the Western medicine. Similar to other diseases, treatment variation for persons with DM is based on the syndrome differentiation and then treated accordingly with the consideration of BC[28]. As such, the YY-AQ is not an instrument for diagnosing DM but serves to assess the presentations of Yin-deficiency, Yang-deficiency and

Table 5 Categorization of the Yin-Yang Assessment Questionnaire score levels in clinical setting

Presentation	Presentation score levels		
	Low score	Moderate score	High score
Yin-deficiency (median = 18)	0-11	12-18	> 18
Yang-deficiency (median = 15)	0-10	11-15	> 15
Yin-Yang-deficiency (median = 35)	0-23	24-35	> 35

Yin-Yang-deficiency in persons with DM. It is rather an assessment instrument that can help dieticians to make early identification of "unhealthy" BC presentations in people with DM so that an appropriate Yin/Yang enhancing dietary therapy could be provided to prevent them from development of other diseases.

In applying the YY-AQ to dietetic settings including clinical and community dietetics, it may be necessary to categorize the presentation scores into low, moderate and high levels (Table 5) because it is a common practice for dieticians to set an assessment instrument into three levels. A moderate score is the basic requirement for referral to a clinical dietician. For those who get low scores, protocols for providing dietetic service can be set, such as issue of relevant information leaflet without referring to a clinical dietician. The YY-AQ is designed for self-administration or by in-person interview. This is because some respondents might possibly be unable to understand all of the question contents for two reasons. First, some of them have poor vision due to the disease. Second, older age is a key factor in understanding, so there might be difficulties for those aged above 70 years or even 60 years. Assessment of the Yin- and Yang-deficiency presentations can be incorporated into conventional dietary therapy for DM care as well as community dietetic program so that Yin-deficiency and Yang-deficiency presentations can be identified, and persons thus identified can benefit from a Yin/Yang enhancing dietary program as early as possible.

We have found one limitation in the initial validation study. Some presentation items are similar in the meaning. First, "I have felt excessively warm in all seasons" and "I have worn thin clothes due to feeling excessively warm" mean persistent feelings of "burning hot" even though the temperatures were generally low. It was found from crosstabs statistics and Spearman's correlation coefficient (rho) that 55% of the 140 survey participants responded to both of these items (rho = 0.62, P = 0.000). Second, "I have had an aversion to cold in all seasons" and "I have worn thick clothes due to my aversion to cold" suggest that the respondents had experienced persistent feelings of "chilling cold" although the temperatures were general high. Of the 140 survey participants, 62% reported having both of these complaints (rho = 0.63, P = 0.000). Third, "My face has been flushed with crimson red" and "I have experienced hot flush especially in the afternoon" also mean the same thing. The results showed that 72.9% of the survey sample responded to both of the two items (rho = 0.63, P = 0.000). Fourth, "My stools have been loose or watery" also has the similar meaning to "I needed to wake up because of my diarrhea". Of the 140 survey participants, 50% responded to both of the complaints (rho = 0.35, P = 0.008). Finally, the results showed that only 39% of the survey participants responded to both "I have always drunk water to quench my thirst" and "My thirst could not be relieved by frequent water intake" (rho = 0.4, P = 0.000). In view of the above statistical findings, the first three pairs of items could be tentatively considered for integration respectively due to being responded by over 50% of the survey participants. However, there is no "gold standard" of percentage requirement in considering the integration of items for measuring instruments. A larger sample size would be considered in the future validity and reliability study for the YY-AQ to find more substantial evidence.

In conclusion, the YY-AQ has initially passed the content validity, comprehensibility, test-retest and internal consistency reliabilities. Due to the small sample size in the tests for comprehensibility and test and retest reliability, need of a larger sample size for substantiating the need of items integration and other important validity and reliability for the YY-AQ, it is necessary to test it further for establishing its validity and reliability: comprehension if the contents are user friendly and easily understood by respondents; test-retest reliability for testing if the YY-AQ can give the same measurement to the same people with DM on different occasions; internal consistency reliability for testing the degree to which the items of the questionnaire are all measuring the same for DM; determination of the scoring system for its clinical significance; construct validity with factor analysis for testing if each item of the YY-AQ corresponds to one of the factors to be derived in the questionnaire; convergent and discriminant validities for testing the degree of the positive and negative correlation respectively between the total items and total factors to be derived in the YY-AQ; responsiveness and predictive validity; and the need of items integration.

This questionnaire is intended for use by dieticians in clinical or community settings. It will help them to early identify the Yin-deficiency or Yang-deficiency presentations in persons with DM so that a Yin/Yang enhancing dietary therapy or education program with a TCM approach can be provided for regulating the "unhealthy" BC presentations, apart from blood glucose stabilization using the nutrition approach in conventional dietary therapy. The goal of the integrated dietetic practice is to prevent the disease advancement by slowing down or preventing the development of other diseases in persons with DM, apart from stabilization

of blood glucose level. Ultimately, the disease burden, such as reduced DM-related quality of life, stress of healthcare professionals and the rising healthcare cost would probably be reduced.

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COMMENTS

Background

Conventional dietary therapy has its limitations in dealing with the "unhealthy" body constitution (BC) presentations by balancing one's Yin and Yang in persons with diabetes mellitus (DM). It is because it controls blood glucose level with nutrition component only. An integration of current dietary practice and Chinese dietary approach may enhance the effectiveness on DM control as well as prevention of other diseases occurrence. In order to identify the "unhealthy" BC presentations, a validated Yin-Yang assessment questionnaire is required.

Research frontiers

The development of the Yin-Yang Assessment Questionnaire (YY-AQ) has completed its initial validation. However, it requires further test for validity and reliability.

Innovations and breakthroughs

It is comprehended that conventional dietary therapy has its limitations of regulating BC from a perspective of traditional Chinese medicine (TCM) while Chinese dietary therapy does not deal with nutrition component in the control of blood glucose. An integration of conventional dietary therapy and Chinese dietary therapy for DM care might be a future direction. The development of the YY-AQ has started a new page to the conventional dietary therapy for persons with DM.

Applications

The YY-AQ can be applied to persons with different types of DM in either clinical or community dietetic settings. In the future, this instrument could be further developed to be applied in different chronic diseases, such as hypertension.

Terminology

Yin-deficiency and Yang-deficiency are two common types of "unhealthy" BC in TCM. People with Yin-deficiency type of BC have the feelings of a "heat-dryness" due to an abnormally low level of humidity but extremely high level of temperature inside the body. Yang-deficiency is an indication of a "cold-dampness" nature of BC as a result of body humidity being at an extremely high above normal level but the body temperature is, however, extremely subnormal.

Peer-review

This study aimed to develop of a Yin/Yang assessment questionnaire for persons with type 2 diabetes from a Traditional Chinese Medicine perspective. This questionnaire is intended for use by dieticians in clinical and community settings. It will help them to early make identification of the Yin- or Yang-deficiency presentations in persons with T2DM so that a Yin/Yang enhancing dietary therapy or program can be provided for regulating the unhealthy body constitution presentations.

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