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EDITORIAL

Assessing and treating insulin resistance in women with polycystic ovarian syndrome

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Abstract

Polycystic ovarian syndrome (PCOS) is a highly prevalent hormonal and metabolic disorder among reproductive aged women worldwide. Women with PCOS have widely varying phenotypes and seek medical care for differing reasons. In addition to concern for menstrual cycle function, ovulation, hirsutism and acne, many PCOS women have abnormal glucose metabolism. While diabetes mellitus and impaired glucose tolerance are easily diagnosed, the diagnosis of and concern for insulin resistance as a precursor disorder is underappreciated. Insulin resistance may be the first important marker of metabolic disease in PCOS women at risk for metabolic syndrome and coronary artery disease.

Key words: Polycystic ovarian syndrome; Insulin resistance; Impaired glucose tolerance; Diabetes mellitus; Infertility

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INTRODUCTION

Diagnosis of polycystic ovarian syndrome (PCOS) is relatively straightforward. Common criteria established by the Rotterdam Conference in 2003 include at least two of three characteristics (oligomenorrhea, clinical and/or biochemical hyperandrogenism and ultrasound criteria) in the absence of other disease. PCOS is the most common hormonal disorder in women worldwide with prevalence estimates between 4%-8% but as high as 25% in some populations[1]. Women often initiate medical care for a cluster of PCOS symptoms (infertility, hirsutism and irregular menstrual cycles) that ultimately are not the most concerning medical consequences of PCOS [diabetes mellitus (DM), coronary artery disease (CAD), endometrial hyperplasia/cancer]. Here exists an important paradigm in the recognition and treatment of PCOS.

Clinically speaking, the hyperandrogenism seen in PCOS is associated with hirsutism more than acne or alopecia and therefore hirsutism is an impetus for young women seeking care[2]. Many PCOS women are also overweight (BMI > 25kg/m^2) or obese (BMI > 30kg/m^2), although adiposity is not a defining criteria for PCOS. Obesity is highly prevalent in the general population and in PCOS women and is an independent risk factor for CAD[3]. Obesity in adolescents is correlated with insulin resistance (IR) and dyslipidemia[4]. PCOS related ovulatory dysfunction in adolescents often correlates to adolescent obesity[5]. Genetic predisposition to PCOS has been sus-

pected for many years[6] and data link obesity and metabolic disturbances in PCOS with genetic polymorphisms[7,8]. Even male first degree relatives of women with PCOS have a higher incidence of metabolic syndrome (MS), the closest corollary to PCOS in men[9].

Once a diagnosis of PCOS is confirmed, it is imperative to assess women for CAD risk factors. Despite the many reasons women seek medical care for PCOS, the greatest long term risk for these women is CAD. This is generally not viewed or even recognized as a concern by women seeking care in the first place. The link between PCOS and CAD is multi-faceted. C-reactive protein (CRP) is higher in age matched PCOS women and is linked to BMI[10] with some ethnic variation in this risk[11]. The prevalence of MS in PCOS women is as high as 40% with increased prevalence of hypertension, dyslipidemia and abnormal glucose metabolism, all before age 30[12]. PCOS women aged 20-40 already demonstrate poor vascular function measured by brachial artery vascular flow[13]. No single blood test can predict or quantify this CAD risk. Although no standard recommendation for assessment of CAD risk factors exists, measurement of glucose metabolism, blood pressure screening, lipid screening and carotid intimal media thickness measurements have been suggested[14].

The routine use of OGTT is advocated by some in all PCOS women[15]. In teenagers, abnormalities in glucose metabolism manifest prior to dyslipidemia, suggesting that assessment of glucose metabolism is even more important in younger women[16]. DM is diagnosed by an 8 h fasting plasma glucose $\geqslant$ 126 mg/dL, 2 h glucose value $\geqslant$ 200 mg/dL after oral glucose tolerance test (OGTT) or random glucose $\geqslant$ 200 mg/dL with symptoms of DM confirmed by either fasting plasma glucose or OGTT. Hemoglobin AIC > 6.5% may also be issued to diagnose DM[17]. Impaired glucose tolerance (IGT) is defined by a 2 h cutoff of 140-200 mg/dL on OGTT[18].The prevalence of IGT in obese adolescents is surprisingly as high as 15%[19].

INSULIN RESISTANCE AND PCOS

As many as 70% of PCOS women are insulin resistant and 10% have DM[20-22]. In PCOS women with normal glucose metabolism initially, the rate of conversion to abnormal glucose metabolism can be 25% over just three years[23]. More alarming, insulin abnormalities are highly prevalent in adolescents with PCOS[24]. Almost 20% of young Thai women with PCOS actually have DM[25]. Overall, normal glucose levels on an OGTT do not predict IR and IR, despite normal glucose levels, is correlated with CRP, dyslipidemia and other CAD risk factors[26]. Therefore, glucose levels alone lack the sensitivity to predict metabolic risk in PCOS patients. Precursor states of insulin abnormalities likely predict long term CAD risk well before glucose abnormalities. IR can be just as severe in diabetics and non-diabetics[27], stressing the seriousness of this metabolic impairment as a precursor and not a separate disease. Animal models have shown that IR alone damages myocardial cells, providing direct evidence of end organ disease[28]. Human data link HOMA-IR to left ventricular dysfunction[29]. Abnormal glucose metabolism short of IGT and DM still deserves attention, identification and treatment[30].

PCOS women with different phenotypes have been found similarly insulin resistant in response to a 3 h 75 g OGTT[31]. Obese (compared to lean) PCOS women tend to have a higher degree of IR. Correlation between hyperandrogenism and IR is significant in many studies but not as significant as the link between insulin abnormalities and obesity[32]. PCOS women demonstrate greater variation in insulin parameters compared to controls, independent of weight[33]. Animal studies of prenatal testosterone exposure show downstream IR in early postnatal life[34]. Some human data shows a high degree of correlation between hyperandrogenism and IR[35,36] and the relationship between hyperandrogenism and IR seem to differ between PCOS and non-PCOS women[35].

Reproductive dysfunction in PCOS women may also be a manifestation of IR. Menstrual cycle irregularity has been correlated with HOMA-IR[37]. Molecular defects in insulin action may be responsible for reproductive difficulties in PCOS women. Although endometrial tissue appears morphologically similar in PCOS to controls and may have similar insulin receptor prevalence, insulin receptor action at the local endometrial level is impaired and may be reflected in lower pregnancy implantation rates[38]. HOMA-IR has been correlated with follicle count in PCOS during *in vitro* fertilization[39]. Follicular insulin levels correlate with pregnancy outcome after IVF[40]. These are areas of unresolved understanding with regard to PCOS. Proposed mechanisms for insulin reproductive abnormalities include abnormalities of ovarian steroidogenesis, excessive LH secretion and abnormalities in glucose uptake[41]. PCOS women have been found to have post-receptor insulin abnormalities as well as reduced peripheral insulin receptor binding[42].

ASSESSMENT OF INSULIN RESISTANCE

No universal definition of insulin resistance exists and therefore no standard clinical technique to measure insulin resistance exists. Insulin resistance can be thought of as a metabolic state where normal glucose homeostasis mechanisms fail to operate properly. Translating theory to clinical practice has been a source of frustration for many practitioners. The American Diabetes Association has characterized IR as a state of impaired metabolic response to insulin[43]. IR is characterized by an inability of normal amounts of insulin to achieve the normal predicted response, often in the clinical setting of central adiposity. To achieve euglycemia, the pancreas over secretes insulin[44]. Investigators define IR based on hyperinsulinemic-euglycemic clamp techniques as a state of impaired glucose disposal in response to insulin[22]. Despite no consensus, clamp techniques have become the refe-

rence for understanding IR.

Hyperinsulinemic-euglycemic clamp techniques rely on an intravenous insulin infusion to maintain steady serum glucose concentrations at fasting levels to measure glucose uptake. Lower glucose uptake signifies resistance to insulin action (i.e. IR). Since the technique requires intravenous infusions, frequent blood sampling, extensive time and significant financial resources, it is experimentally useful but clinically cumbersome[45]. Clamp studies in PCOS women show conflicting results; some studies show IR only in obese PCOS women[46] and others demonstrate IR in lean PCOS patients[47]. Of importance, the studies which failed to demonstrate IR in lean PCOS women did, however, demonstrate elevated basal insulin levels compared to weight matched, non PCOS controls[46]. Other sophisticated testing methods using intravenous infusions of insulin have been attempted (insulin sensitivity test and insulin tolerance test) but they do not alleviate the time, financial and testing burdens to make them relevant for widespread clinical practice and normal cutoffs are not widely disseminated[45]. Clamp techniques have been used as comparisons to validate other modes of assessment of IR.

Fasting methods to measure IR have been advocated for many years as an adjunct to DM screening. Elevated fasting insulin levels greater than 20 μU/mL may alone indicate IR. Fasting glucose/insulin ratio (G/I) has also gained some clinical traction. A ratio < 4.5 has in general been shown to be > 90% sensitive in some populations[45] but has never been validated with clamp studies[48]. Some ethnic variation in G/I cutoff ratios may exist[49]. There has been some suggestion that G/I < 7 in very young girls may predict IR[50,51].

The homeostatic model assessment (HOMA), a more complex fasting calculation, has been compared to clamp techniques with good results. HOMA is the product of fasting glucose (mg/dL) and insulin (μU/mL) divided by a constant[45]. One major limitation of HOMA rests on the previous reflection that many young PCOS women display stimulated but not fasting metabolic abnormalities. In fact, HOMA in young PCOS patients missed 50% of IR as compared to OGTT with insulin-AUC calculations[52]. G/I ratio correlated strongly with clamp-demonstrated IR in a small study of PCOS women - interestingly, both lean and obese PCOS women had evidence of IR. Sex hormone binding globulin (SHBG) did not correlate with IR in this study[47], as has been previously postulated[53].

Quantitative insulin sensitivity check index (QUICKI) was developed to improve the sensitivity of fasting measurements. QUICKI is calculated as: 1/[log(insulin fasting) + log(glucose fasting)] and has been well correlated to clamp measurements in obese and non-obese patients[15]. QUICKI also demonstrates correlation with HOMA-IR[53]. QUICKI research calculations in young PCOS women are often identical to age matched women with DM[54].

OGTT with 75-g glucose and hourly glucose and insulin measurements has been compared to clamp techniques. Insulin sensitivity calculated by mathematical transformation of measurements has shown good correlation with glucose disposal using clamp techniques[48]. Although the OGTT is easy to perform, these calculations are more complex and make this particular calculation less desirable for clinical use. However these data show that 1 and 2 h levels are often needed to diagnose IR and stress the potential for false negative results with fasting measurements alone. In patients undergoing clamp and OGTT no correlation was observed between fasting glucose/insulin ratios and IR on the clamp[48].

Some have tried to utilize ultrasound to detect IR. Of note, normoglycemic women often have the phenotypic criteria for polycystic ovaries on ultrasound[54], consistent with other data in young adolescents showing that polycystic ovaries by ultrasound appearance often does not correlate with either anovulatory menstrual cycles or metabolic abnormalities[55]. Therefore ultrasound is too nonspecific to use with any reliability in measuring IR.

Limitations of direct insulin testing and cumbersome calculations have led to research for indirect serum markers to provide evidence of IR. SHBG correlations to IR as previously mentioned have been inconsistent. Adiponectin is a protein found in adipose tissue associated with both inflammation and insulin action. Recent studies have linked plasma adiponectin level to IR (but not hyperandrogenism) measured by HOMA[56-58]. Serum soluble glycoprotein-130 levels (local cytokine) have been inversely correlated to IR[59]. Resistin plasma levels have been correlated with fasting glucose and HOMA-IR in PCOS women[60]. Inhibin A levels in PCOS women were not found to correlate with IR in PCOS women[61]. Most of these serum markers share common limitations and have been poorly studied. How they might vary with different PCOS phenotypes is unknown. None are adequately compared to IR measured by clamp studies. Their usefulness serially in clinical practice to monitor patients over time and undergoing treatment is also unknown. Some genetic work has recently shown promise. Although far from clinical use, microarray analysis of genes in muscle, adipose tissue and the liver shows alterations in the setting of IR[62]. Serum genetic markers may lead to future genetic techniques to detect and monitor IR.

TREATMENT

Why treat IR in PCOS women? For many years only PCOS women with DM were treated. As the link between IGT and CAD became more apparent, many PCOS women with IGT were treated. We now understand that IR is often the first step in a progression to DM and CAD. Those who now advocate treatment for IR do so for the following reasons: reduction of insulin and androgen levels, prevention of IGT and DM, potential for improved ovulation, symptomatic improvement, prevention of MS[63]. Ultimately, secondary prevention in young women with identifiable and treatment precursor conditions is far more desirable and easier than treatment of these same

women later in life with serious disease.

Metformin has been the mainstay of treatment for IR and IGT in PCOS women over the past decade. Metformin is a biguanide that acts principally on the liver to inhibit hepatic gluconeogenesis. It also inhibits acetyl-CoA carboxylase activity and suppresses fatty acid production. Metformin acts on skeletal muscle to inhibit lipid production and acts peripherally on adipose tissue to stimulate glucose transport and uptake. Metformin reduces insulin levels and promotes improved insulin receptor activity[64]. Metformin may also have direct and indirect effects on the ovary with respect to insulin action and steroidogenic enzymatic activity. In the endothelium, metformin seems to improve nitric oxide vasodilatory effects. Many other mechanisms of action have been studied in both animal and human models but consistent effects are not always demonstrated with local tissue concentrations that result from therapeutic doses[65].

Human data regarding metformin improvement in IR in PCOS women shows mixed results and is complicated by varying methods of assessing IR. Short term (3 mo) treatment with metformin (1500 mg per day) failed to affect IR as measured by AUC-Insulin after 75-g OGTT. Metformin (1600 mg per day) in obese PCOS women treated for 6 mo failed to reduce IR as measured by QUICKI[66]. This is in contrast to similar length studies on obese PCOS women who demonstrated decreased IR as measured by HOMA-IR, QUICKI and ISI, and correlated with alterations in phosphoproteins related to IR[67]. Longer term metformin therapy (2 years, 1600 mg per day) in young, obese PCOS women reduced fasting insulin, hyperandrogenism and produced borderline reductions in HOMA-IR ($P = 0.05$)[68]. Metformin was compared prospectively to naltrexone and prenisolone in combination with oral contraceptive pills (OCPS). IR was unchanged despite lowered androgen levels[69]. Metformin has been compared to orlistat and pioglitazone over a 4 mo treatment course and although each treatment reduced IR as measured by HOMA-IR, metformin (1500 mg per day) had the least reduction (< 20%)[70].

Studies have attempted for years to show an advantage to metformin for ovulation induction and as an adjunct to more advanced fertility treatments. In ovulatory PCOS women metformin was associated with improved serum and follicular fluid AMH levels as well as insulin values; these changes were not seen in anovulatory PCOS women[71]. Despite the demonstration of negative effects of IR on reproductive outcome, the vast majority of evidence does not show improvement in live birth rates when metformin is used strictly for fertility[72], although treatment does improve ovulatory status[72,73].

Metformin has been studied specifically in adolescent PCOS women. Metformin therapy for 10 mo decreased fasting serum insulin levels in obese girls with PCOS[74]. The positive effects of metformin in adolescents wore off within 3 mo of medication discontinuation[75]. Metformin in obese PCOS adolescents has shown improvements in IR by clamp studies, fasting measurements and OGTT after just 3 mo of therapy[76,77]. Other studies have found non-significant trends to improved IR by HOMA and OGTT-AUC in adolescent PCOS patients[78]. Metformin has also been shown to effectively contribute to BMI reduction in PCOS adolescents[79].

Metformin has been tested in combination with cholesterol lowering medications. Pretreatment of obese PCOS patients with atorvastatin (20 mg per day for 3 mo) followed by 3 mo of metformin (1500 mg per day) resulted in more effective lowering of HOMA-IR than metformin alone[80]. Other similar data show that combined treatment with metformin and atorvastatin compared to metformin alone produced similar but significant improvements in IR. Combination therapy only showed successful reduction of hyperandrogenism and not IR[81].

The ultimate goal is to prevent metabolic disease. Metformin (1500 mg per day) compared to placebo in a prospective 12 wk randomized control trial decreased arterial stiffness (by peripheral pressure waveforms in the brachial artery) and endothelial function (measured by augmentation index). Metformin did not reduce HOMA-IR[82]. The study population was obese but young (mean age 30 years), demonstrating the ability to reduce CAD risk even in very young women. Metformin has reduced both carotid intimal media thickness and endothelin levels in obese PCOS women[83]. In many studies metformin has reduced both total cholesterol and LDL cholesterol levels[84-86], triglyceride levels[84] and increased HDL levels[87,88]. Animal studies have shown that acarbose given to insulin resistant rats decreased carotid intimal hyperplasia and blood flow velocities[89]. Taken as a whole, the ability of metformin (and likely other insulin sensitizing agents) to elicit an overall reduction in the risk for CAD may be easier than the ability to produce consistent measureable improvements.

Other insulin sensitizing agents have been advocated and studied for the treatment of IR in PCOS, principally thiazolinediones. Thiazolinediones stimulate gene transcription that alters lipid and glucose metabolism, decreases lipolysis and decreases fat deposition[90]. Thiazolinediones decrease fatty acid release, suppress gluconeogenesis and reduce tumor necrosis factor α disruption of insulin activity[64]. Pioglitazone and rosiglitazone have decreased IR (measured by clamp studies) in PCOS women[90-93]. Glitazones have also decreased IR by OGTT AUC-Insulin in PCOS women[91,93,94]. In patients with DM, thiazolinediones reduce central adiposity[95], a trait commonly shared with PCOS women. Pioglitazone by way of IR and adiponectin levels also has improved menstrual regularity in PCOS women[96,97]. Adverse outcomes have been seen in pregnant animals with limited to no human data. Therefore, as a class, thiazolinediones are not considered first line therapy for PCOS women seeking pregnancy. Rosiglitazone has even been found to decrease pro-inflammatory markers in human granulosa cells cultured following *in vitro* fertilization oocyte retrieval, thus showing additional target tissue for therapy[98]. However, these effects have not been adequately studied and have no current practical application.

Other pharmacological treatments have attempted to lower IR. Vitamin D has been shown to decrease HO-

MA-IR despite a lack of change in hyperandrogenism in young, obese PCOS women[99]. Animal studies have demonstrated that treatment with glycyrrhizic acid affecting lipoprotein lipase activity decreases serum insulin and HOMA-IR[100]. Although oral contraceptive pills positively affect hyperandrogenism, they have little to no effect on glucose metabolism by OGTT[101]. Long term oral contraceptive pill use may have some limited benefit in IR but data are limited[102]. A 6 mo course of oral contraceptive pill treatment in adolescent obese PCOS women has demonstrated some improvement in IR[103].

Lifestyle interventions are usually required for long term sustainable results. PCOS women who smoke have higher free androgen levels and IR as measured by HO MA-IR, QUICKI and the insulin sensitivity index following 75 g OGTT[104]. Thus PCOS women who smoke have an additional reason to stop smoking. In more general population studies (non-PCOS) comprised mostly of middle-aged women, lifestyle intervention is more effective than metformin in preventing the progression to DM. Dietary and exercise intervention decreased the 4 year progression to DM in patients at risk (non-diabetic, elevated fasting and/or OGTT glucose) by almost 50%[105]. Realizing the limitations of applying this population sample to young PCOS women, it still highlights the benefit of non-pharmacological treatment. PCOS women randomized to both metformin and lifestyle interventions (compared to placebo) showed improvements in HOMA-IR after 4 mo[106]. In European adolescents with PCOS who failed to achieve improvements in HOMA-IR after 6 mo of lifestyle intervention, both metformin and placebo reduced IR over 6 mo, although metformin offered no benefit over placebo[107]. Lifestyle modification in adolescents has been successful in reducing hyperandrogenism[103]. Modest weight loss of about 5% bodyweight has also been shown to lower hyperandrogenism[108] which may ultimately improve IR.

Acupuncture has been studied as a means to reduce IR in PCOS phenotype animals. Acupuncture decreased IR by euglycemic-hyperinsulinemic clamp and altered glucose transporter expression (GLUT4) in a rat model of PCOS[109]. In humans, acupuncture has shown both metabolic and hormonal benefits in women with PCOS[110].

CONCLUSION

Regardless of what reasons women have for seeking diagnosis and treatment of PCOS, it is imperative for practitioners to assess a woman's risk for CAD. Assessment should probably be made in all PCOS patients regardless of BMI. Especially in young women or adolescents, IR may be the first identifiable risk factor. Practitioners must recognize that no universal test for IR exists and must use good clinical judgment to assess metabolic status in women. Stimulated testing with OGTT may be more sensitive than fasting measurements. Women who demonstrate IR should be counseled on lifestyle modifications. Physicians should discuss with their patients a target BMI that is realistically obtainable. It is often advisable for patients to seek nutritional assessment and counseling to help with this goal. In many individuals, consideration should be given to pharmacological treatment. Although the most commonly used medication is metformin, other medications may be appropriate first line therapy, especially in women not actively seeking pregnancy.

REFERENCES

1 **Teede H**, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med* 2010; **8**: 41

2 **Ozdemir S**, Ozdemir M, Görkemli H, Kiyici A, Bodur S. Specific dermatologic features of the polycystic ovary syndrome and its association with biochemical markers of the metabolic syndrome and hyperandrogenism. *Acta Obstet Gynecol Scand* 2010; **89**: 199-204

3 **Lee M**, Aronne LJ. Weight management for type 2 diabetes mellitus: global cardiovascular risk reduction. *Am J Cardiol* 2007; **99**: 68B-79B

4 **Bouglé D**, Zunquin G, Sesbouë B, Sabatier JP. Relationships of cardiorespiratory fitness with metabolic risk factors, inflammation, and liver transaminases in overweight youths. *Int J Pediatr* 2010; **2010**: 580897

5 **Kasa-Vubu JZ**, Jain V, Welch K. Impact of fatness, insulin, and gynecological age on luteinizing hormone secretory dynamics in adolescent females. *Fertil Steril* 2010; **94**: 221-229

6 **Deligeoroglou E**, Kouskouti C, Christopoulos P. The role of genes in the polycystic ovary syndrome: predisposition and mechanisms. *Gynecol Endocrinol* 2009; **25**: 603-609

7 **Hanzu FA**, Radian S, Attaoua R, Ait-El-Mkadem S, Fica S, Gheorghiu M, Coculescu M, Grigorescu F. Association of insulin receptor genetic variants with polycystic ovary syndrome in a population of women from Central Europe. *Fertil Steril* 2010; **94**: 2389-2392

8 **Tan S**, Scherag A, Janssen OE, Hahn S, Lahner H, Dietz T, Scherag S, Grallert H, Vogel CI, Kimmig R, Illig T, Mann K, Hebebrand J, Hinney A. Large effects on body mass index and insulin resistance of fat mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS). *BMC Med Genet* 2010; **11**: 12

9 **Coviello AD**, Sam S, Legro RS, Dunaif A. High prevalence of metabolic syndrome in first-degree male relatives of women with polycystic ovary syndrome is related to high rates of obesity. *J Clin Endocrinol Metab* 2009; **94**: 4361-4366

10 **Tosi F**, Dorizzi R, Castello R, Maffeis C, Spiazzi G, Zoppini G, Muggeo M, Moghetti P. Body fat and insulin resistance independently predict increased serum C-reactive protein in hyperandrogenic women with polycystic ovary syndrome. *Eur J Endocrinol* 2009; **161**: 737-745

11 **Alemzadeh R**, Kichler J, Calhoun M. Spectrum of metabolic dysfunction in relationship with hyperandrogenemia in obese adolescent girls with polycystic ovary syndrome. *Eur J Endocrinol* 2010; **162**: 1093-1099

12 **El-Mazny A**, Abou-Salem N, El-Sherbiny W, El-Mazny A. Insulin resistance, dyslipidemia, and metabolic syndrome in women with polycystic ovary syndrome. *Int J Gynaecol Obstet* 2010; **109**: 239-241

13 **Dokras A**, Jagasia DH, Maifeld M, Sinkey CA, VanVoorhis BJ, Haynes WG. Obesity and insulin resistance but not hyperandrogenism mediates vascular dysfunction in women with polycystic ovary syndrome. *Fertil Steril* 2006; **86**: 1702-1709

14 **Alexander CJ**, Tangchitnob EP, Lepor NE. Polycystic ovary syndrome: a major unrecognized cardiovascular risk factor in women. *Rev Obstet Gynecol* 2009; **2**: 232-239

15 **Katz A**, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; **85**: 2402-2410

16 **Fulghesu A**, Magnini R, Portoghese E, Angioni S, Minerba L, Melis GB. Obesity-related lipid profile and altered insulin incretion in adolescents with polycystic ovary syndrome. *J Adolesc Health* 2010; **46**: 474-481
17 **Gillett MJ**. International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes: Diabetes Care 2009; 32(7): 1327-1334. *Clin Biochem Rev* 2009; **30**: 197-200
18 Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2003; **26** Suppl 1: S5-S20
19 **Ghergherechi R**, Tabrizi A. Prevalence of impaired glucose tolerance and insulin resistance among obese children and adolescents. *Ther Clin Risk Manag* 2010; **6**: 345-349
20 **Freeman R**, Pollack R, Rosenbloom E. Assessing impaired glucose tolerance and insulin resistance in Polycystic Ovarian Syndrome with a muffin test: Alternative to glucose tolerance test. *Endocr Pract* 2010; 1-24
21 **Farrell K**, Antoni MH. Insulin resistance, obesity, inflammation, and depression in polycystic ovary syndrome: biobehavioral mechanisms and interventions. *Fertil Steril* 2010; **94**: 1565-1574
22 **Ovalle F**, Azziz R. Insulin resistance, polycystic ovary syndrome, and type 2 diabetes mellitus. *Fertil Steril* 2002; **77**: 1095-1105
23 **Pesant MH**, Baillargeon JP. Clinically useful predictors of conversion to abnormal glucose tolerance in women with polycystic ovary syndrome. *Fertil Steril* 2011; **95**: 210-215
24 **Bhattacharya SM**, Ghosh M. Insulin resistance and adolescent girls with polycystic ovary syndrome. *J Pediatr Adolesc Gynecol* 2010; **23**: 158-161
25 **Indhavivadhana S**, Wongwananuruk T, Rattanachaiyanont M, Techatraisak K, Leerasiri P, Tanmahasamut P, Popijan M. Prevalence of metabolic syndrome in reproductive-aged polycystic ovary syndrome Thai women. *J Med Assoc Thai* 2010; **93**: 653-660
26 **Karakas SE**, Kim K, Duleba AJ. Determinants of impaired fasting glucose versus glucose intolerance in polycystic ovary syndrome. *Diabetes Care* 2010; **33**: 887-893
27 **Ishihara M**, Inoue I, Kawagoe T, Shimatani Y, Kurisu S, Nakama Y, Kagawa E, Dai K, Ootani T, Ikenaga H, Morimoto Y, Ejiri K, Oda N. Glucometabolic responses during Glucose Tolerance Test: A comparison between known diabetes and newly detected diabetes after acute myocardial infarction. *Int J Cardiol* 2010; Epub ahead of print
28 **Yang ZH**, Peng XD. Insulin resistance and heart injury in rats with insulin resistance or type 2 diabetes mellitus. *Acta Cardiol* 2010; **65**: 329-335
29 **Başyiğit F**, Temizhan A, Malçok O, Kahraman E, Cakal E, Selçuk MT, Korkmaz S. [The relationship between insulin resistance and left ventricular systolic and diastolic functions and functional capacity in patients with chronic heart failure and metabolic syndrome]. *Turk Kardiyol Dern Ars* 2010; **38**: 173-181
30 **Hsueh WA**, Orloski L, Wyne K. Prediabetes: the importance of early identification and intervention. *Postgrad Med* 2010; **122**: 129-143
31 **Wang Y**, Qu J, Wu X, Hou L, Erkkola R, Wang Y. Different phenotypes of polycystic ovary syndrome by Rotterdam criteria are differently steroidogenic but similarly insulin resistant. *Fertil Steril* 2010; **93**: 1362-1365
32 **Acién P**, Quereda F, Matallín P, Villarroya E, López-Fernández JA, Acién M, Mauri M, Alfayate R. Insulin, androgens, and obesity in women with and without polycystic ovary syndrome: a heterogeneous group of disorders. *Fertil Steril* 1999; **72**: 32-40
33 **Jayagopal V**, Kilpatrick ES, Holding S, Jennings PE, Atkin SL. The biological variation of insulin resistance in polycystic ovarian syndrome. *J Clin Endocrinol Metab* 2002; **87**: 1560-1562
34 **Padmanabhan V**, Veiga-Lopez A, Abbott DH, Recabarren SE, Herkimer C. Developmental programming: impact of prenatal testosterone excess and postnatal weight gain on insulin sensitivity index and transfer of traits to offspring of overweight females. *Endocrinology* 2010; **151**: 595-605
35 **Luque-Ramírez M**, Alpañés M, Escobar-Morreale HF. The determinants of insulin sensitivity, β-cell function, and glucose tolerance are different in patients with polycystic ovary syndrome than in women who do not have hyperandrogenism. *Fertil Steril* 2010; **94**: 2214-2221
36 **Yildiz BO**, Gedik O. Insulin resistance in polycystic ovary syndrome: hyperandrogenemia versus normoandrogenemia. *Eur J Obstet Gynecol Reprod Biol* 2001; **100**: 62-66
37 **Strowitzki T**, Capp E, von Eye Corleta H. The degree of cycle irregularity correlates with the grade of endocrine and metabolic disorders in PCOS patients. *Eur J Obstet Gynecol Reprod Biol* 2010; **149**: 178-181
38 **Fornes R**, Ormazabal P, Rosas C, Gabler F, Vantman D, Romero C, Vega M. Changes in the expression of insulin signaling pathway molecules in endometria from polycystic ovary syndrome women with or without hyperinsulinemia. *Mol Med* 2010; **16**: 129-136
39 **Dickerson EH**, Cho LW, Maguiness SD, Killick SL, Robinson J, Atkin SL. Insulin resistance and free androgen index correlate with the outcome of controlled ovarian hyperstimulation in non-PCOS women undergoing IVF. *Hum Reprod* 2010; **25**: 504-509
40 **Takikawa S**, Iwase A, Goto M, Harata T, Umezu T, Nakahara T, Kobayashi H, Suzuki K, Manabe S, Kikkawa F. Assessment of the predictive value of follicular fluid insulin, leptin and adiponectin in assisted reproductive cycles. *Gynecol Endocrinol* 2010; **26**: 494-499
41 **Baptiste CG**, Battista MC, Trottier A, Baillargeon JP. Insulin and hyperandrogenism in women with polycystic ovary syndrome. *J Steroid Biochem Mol Biol* 2010; **122**: 42-52
42 **Marsden PJ**, Murdoch A, Taylor R. Severe impairment of insulin action in adipocytes from amenorrheic subjects with polycystic ovary syndrome. *Metabolism* 1994; **43**: 1536-1542
43 Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association. *Diabetes Care* 1998; **21**: 310-314
44 **Garruti G**, Depalo R, Vita MG, Lorusso F, Giampetruzzi F, Damato AB, Giorgino F. Adipose tissue, metabolic syndrome and polycystic ovary syndrome: from pathophysiology to treatment. *Reprod Biomed Online* 2009; **19**: 552-563
45 **Legro RS**, Castracane VD, Kauffman RP. Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls. *Obstet Gynecol Surv* 2004; **59**: 141-154
46 **Vrbíková J**, Cibula D, Dvoráková K, Stanická S, Sindelka G, Hill M, Fanta M, Vondra K, Skrha J. Insulin sensitivity in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2004; **89**: 2942-2945
47 **Ducluzeau PH**, Cousin P, Malvoisin E, Bornet H, Vidal H, Laville M, Pugeat M. Glucose-to-insulin ratio rather than sex hormone-binding globulin and adiponectin levels is the best predictor of insulin resistance in nonobese women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2003; **88**: 3626-3631
48 **Matsuda M**, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999; **22**: 1462-1470
49 **Kauffman RP**, Baker VM, Dimarino P, Gimpel T, Castracane VD. Polycystic ovarian syndrome and insulin resistance in white and Mexican American women: a comparison of two distinct populations. *Am J Obstet Gynecol* 2002; **187**: 1362-1369
50 **Silfen ME**, Manibo AM, McMahon DJ, Levine LS, Murphy AR, Oberfield SE. Comparison of simple measures of insulin sensitivity in young girls with premature adrenarche: the fasting glucose to insulin ratio may be a simple and useful measure. *J Clin Endocrinol Metab* 2001; **86**: 2863-2868
51 **Vuguin P**, Saenger P, Dimartino-Nardi J. Fasting glucose insulin ratio: a useful measure of insulin resistance in girls with premature adrenarche. *J Clin Endocrinol Metab* 2001; **86**:

4618-4621
52 **Fulghesu AM**, Angioni S, Portoghese E, Milano F, Batetta B, Paoletti AM, Melis GB. Failure of the homeostatic model assessment calculation score for detecting metabolic deterioration in young patients with polycystic ovary syndrome. *Fertil Steril* 2006; **86**: 398-404
53 **Kajaia N**, Binder H, Dittrich R, Oppelt PG, Flor B, Cupisti S, Beckmann MW, Mueller A. Low sex hormone-binding globulin as a predictive marker for insulin resistance in women with hyperandrogenic syndrome. *Eur J Endocrinol* 2007; **157**: 499-507
54 **Seneviratne HR**, Lankeshwara D, Wijeratne S, Somasunderam N, Athukorale D. Serum insulin patterns and the relationship between insulin sensitivity and glycaemic profile in women with polycystic ovary syndrome. *BJOG* 2009; **116**: 1722-1728
55 **Codner E**, Villarroel C, Eyzaguirre FC, López P, Merino PM, Pérez-Bravo F, Iñiguez G, Cassorla F. Polycystic ovarian morphology in postmenarchal adolescents. *Fertil Steril* 2011; **95**:702-706.e1-2.
56 **Trolle B**, Lauszus FF, Frystyk J, Flyvbjerg A. Adiponectin levels in women with polycystic ovary syndrome: impact of metformin treatment in a randomized controlled study. *Fertil Steril* 2010; **94**: 2234-2238
57 **Demirci H**, Yilmaz M, Ergun MA, Yurtcu E, Bukan N, Ayvaz G. Frequency of adiponectin gene polymorphisms in polycystic ovary syndrome and the association with serum adiponectin, androgen levels, insulin resistance and clinical parameters. *Gynecol Endocrinol* 2010; **26**: 348-355
58 **Liu X**, Zhang J, Li Y, Xu L, Wei D, Qiu D, Han D. [On the relationship between serum total adiponectin and insulin resistance in polycystic ovary syndrome]. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* 2010; **27**: 636-640
59 **Nikolajuk A**, Kowalska I, Karczewska-Kupczewska M, Adamska A, Otziomek E, Wolczynski S, Kinalska I, Gorska M, Straczkowski M. Serum soluble glycoprotein 130 concentration is inversely related to insulin sensitivity in women with polycystic ovary syndrome. *Diabetes* 2010; **59**: 1026-1029
60 **Chu Y**, Cui Q, Feng G, Song Z, Jiang X. The expression of resistin in adipose tissues of patients with polycystic ovary syndrome and insulin resistance. *J Huazhong Univ Sci Technolog Med Sci* 2009; **29**: 642-645
61 **Segal S**, Elmadjian M, Takeshige T, Karp S, Mercado R, Rivnay B. Serum inhibin A concentration in women with polycystic ovarian syndrome and the correlation to ethnicity, androgens and insulin resistance. *Reprod Biomed Online* 2010; **20**: 675-680
62 **Kim E**. Insulin resistance at the crossroads of metabolic syndrome: systemic analysis using microarrays. *Biotechnol J* 2010; **5**: 919-929
63 **Pasquali R**, Gambineri A. Targeting insulin sensitivity in the treatment of polycystic ovary syndrome. *Expert Opin Ther Targets* 2009; **13**: 1205-1226
64 **De Leo V**, Musacchio MC, Palermo V, Di Sabatino A, Morgante G, Petraglia F. Polycystic ovary syndrome and metabolic comorbidities: therapeutic options. *Drugs Today (Barc)* 2009; **45**: 763-775
65 **Diamanti-Kandarakis E**, Christakou CD, Kandaraki E, Economou FN. Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. *Eur J Endocrinol* 2010; **162**: 193-212
66 **Tang T**, Glanville J, Hayden CJ, White D, Barth JH, Balen AH. Combined lifestyle modification and metformin in obese patients with polycystic ovary syndrome. A randomized, placebo-controlled, double-blind multicentre study. *Hum Reprod* 2006; **21**: 80-89
67 **Savastano S**, Valentino R, Pizza G, De Rosa A, Orio F, Passaretti F, Formisano P, Lombardi G, Beguinot F, Colao A. Preliminary data on effects of metformin on PED/PEA-15 cellular levels in obese women with polycystic ovary syndrome. *J Endocrinol Invest* 2010; **33**: 446-450
68 **Oppelt PG**, Mueller A, Jentsch K, Kronawitter D, Reissmann C, Dittrich R, Beckmann MW, Cupisti S. The effect of metformin treatment for 2 years without caloric restriction on endocrine and metabolic parameters in women with polycystic ovary syndrome. *Exp Clin Endocrinol Diabetes* 2010; **118**: 633-637
69 **Hadžiomerović-Pekić D**, Wildt L, Weiss JM, Moeller K, Mattle V, Seeber BE. Metformin, naltrexone, or the combination of prednisolone and antiandrogenic oral contraceptives as first-line therapy in hyperinsulinemic women with polycystic ovary syndrome. *Fertil Steril* 2010; **94**: 2385-2388
70 **Cho LW**, Kilpatrick ES, Keevil BG, Coady AM, Atkin SL. Effect of metformin, orlistat and pioglitazone treatment on mean insulin resistance and its biological variability in polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2009; **70**: 233-237
71 **Falbo A**, Rocca M, Russo T, D'Ettore A, Tolino A, Zullo F, Orio F, Palomba S. Serum and follicular anti-Mullerian hormone levels in women with polycystic ovary syndrome (PCOS) under metformin. *J Ovarian Res* 2010; **3**: 16
72 **Tang T**, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev* 2009; CD003053
73 **Roy KK**, Baruah J, Sharma A, Sharma JB, Kumar S, Kachava G, Karmakar D. A prospective randomized trial comparing the clinical and endocrinological outcome with rosiglitazone versus laparoscopic ovarian drilling in patients with polycystic ovarian disease resistant to ovulation induction with clomiphene citrate. *Arch Gynecol Obstet* 2010; **281**: 939-944
74 **Glueck CJ**, Wang P, Fontaine R, Tracy T, Sieve-Smith L. Metformin to restore normal menses in oligo-amenorrheic teenage girls with polycystic ovary syndrome (PCOS). *J Adolesc Health* 2001; **29**: 160-169
75 **Ibáñez L**, Valls C, Potau N, Marcos MV, de Zegher F. Sensitization to insulin in adolescent girls to normalize hirsutism, hyperandrogenism, oligomenorrhea, dyslipidemia, and hyperinsulinism after precocious pubarche. *J Clin Endocrinol Metab* 2000; **85**: 3526-3530
76 **Arslanian SA**, Lewy V, Danadian K, Saad R. Metformin therapy in obese adolescents with polycystic ovary syndrome and impaired glucose tolerance: amelioration of exaggerated adrenal response to adrenocorticotropin with reduction of insulinemia/insulin resistance. *J Clin Endocrinol Metab* 2002; **87**: 1555-1559
77 **La Marca A**, Artensio AC, Stabile G, Volpe A. Metformin treatment of PCOS during adolescence and the reproductive period. *Eur J Obstet Gynecol Reprod Biol* 2005; **121**: 3-7
78 **Bridger T**, MacDonald S, Baltzer F, Rodd C. Randomized placebo-controlled trial of metformin for adolescents with polycystic ovary syndrome. *Arch Pediatr Adolesc Med* 2006; **160**: 241-246
79 **De Leo V**, Musacchio MC, Morgante G, Piomboni P, Petraglia F. Metformin treatment is effective in obese teenage girls with PCOS. *Hum Reprod* 2006; **21**: 2252-2256
80 **Sathyapalan T**, Kilpatrick ES, Coady AM, Atkin SL. Atorvastatin pretreatment augments the effect of metformin in patients with polycystic ovary syndrome (PCOS). *Clin Endocrinol (Oxf)* 2010; **72**: 566-568
81 **Kazerooni T**, Shojaei-Baghini A, Dehbashi S, Asadi N, Ghaffarpasand F, Kazerooni Y. Effects of metformin plus simvastatin on polycystic ovary syndrome: a prospective, randomized, double-blind, placebo-controlled study. *Fertil Steril* 2010; **94**: 2208-2213
82 **Agarwal N**, Rice SP, Bolusani H, Luzio SD, Dunseath G, Ludgate M, Rees DA. Metformin reduces arterial stiffness and improves endothelial function in young women with polycystic ovary syndrome: a randomized, placebo-controlled, crossover trial. *J Clin Endocrinol Metab* 2010; **95**: 722-730
83 **Palomba S**, Falbo A, Giallauria F, Russo T, Tolino A, Zullo

F, Colao A, Orio F. Effects of metformin with or without supplementation with folate on homocysteine levels and vascular endothelium of women with polycystic ovary syndrome. *Diabetes Care* 2010; **33**: 246-251
84 **Banaszewska B**, Duleba AJ, Spaczynski RZ, Pawelczyk L. Lipids in polycystic ovary syndrome: role of hyperinsulinemia and effects of metformin. *Am J Obstet Gynecol* 2006; **194**: 1266-1272
85 **Lord J**, Thomas R, Fox B, Acharya U, Wilkin T. The effect of metformin on fat distribution and the metabolic syndrome in women with polycystic ovary syndrome--a randomised, double-blind, placebo-controlled trial. *BJOG* 2006; **113**: 817-824
86 **Palomba S**, Falbo A, Russo T, Manguso F, Tolino A, Zullo F, De Feo P, Orio F Jr. Insulin sensitivity after metformin suspension in normal-weight women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2007; **92**: 3128-3135
87 **Fleming R**, Hopkinson ZE, Wallace AM, Greer IA, Sattar N. Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind placebo-controlled trial. *J Clin Endocrinol Metab* 2002; **87**: 569-574
88 **Moghetti P**, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. *J Clin Endocrinol Metab* 2000; **85**: 139-146
89 **Nyalala JO**, Luo S, Campbell DN, Brown AT, Moursi MM. The effects of acarbose treatment on intimal hyperplasia in a rat carotid endarterectomy model of diet-induced insulin resistance. *Vasc Endovascular Surg* 2010; **44**: 560-567
90 **Glintborg D**, Andersen M. Thiazolinedione treatment in PCOS--an update. *Gynecol Endocrinol* 2010; **26**: 791-803
91 **Aroda VR**, Ciaraldi TP, Burke P, Mudaliar S, Clopton P, Phillips S, Chang RJ, Henry RR. Metabolic and hormonal changes induced by pioglitazone in polycystic ovary syndrome: a randomized, placebo-controlled clinical trial. *J Clin Endocrinol Metab* 2009; **94**: 469-476
92 **Brettenthaler N**, De Geyter C, Huber PR, Keller U. Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2004; **89**: 3835-3840
93 **Rautio K**, Tapanainen JS, Ruokonen A, Morin-Papunen LC. Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: a randomized placebo-controlled study. *Hum Reprod* 2006; **21**: 1400-1407
94 **Aigner E**, Bachofner N, Klein K, De Geyter C, Hohla F, Patsch W, Datz C. Retinol-binding protein 4 in polycystic ovary syndrome--association with steroid hormones and response to pioglitazone treatment. *J Clin Endocrinol Metab* 2009; **94**: 1229-1235
95 **Mayerson AB**, Hundal RS, Dufour S, Lebon V, Befroy D, Cline GW, Enocksson S, Inzucchi SE, Shulman GI, Petersen KF. The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes* 2002; **51**: 797-802
96 **Glintborg D**, Frystyk J, Højlund K, Andersen KK, Henriksen JE, Hermann AP, Hagen C, Flyvbjerg A, Andersen M. Total and high molecular weight (HMW) adiponectin levels and measures of glucose and lipid metabolism following pioglitazone treatment in a randomized placebo-controlled study in polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2008; **68**: 165-174
97 **Glintborg D**, Hermann AP, Andersen M, Hagen C, Beck-Nielsen H, Veldhuis JD, Henriksen JE. Effect of pioglitazone on glucose metabolism and luteinizing hormone secretion in women with polycystic ovary syndrome. *Fertil Steril* 2006; **86**: 385-397
98 **Chen Q**, Sun X, Chen J, Cheng L, Wang J, Wang Y, Sun Z. Direct rosiglitazone action on steroidogenesis and proinflammatory factor production in human granulosa-lutein cells. *Reprod Biol Endocrinol* 2009; **7**: 147
99 **Selimoglu H**, Duran C, Kiyici S, Ersoy C, Guclu M, Ozkaya G, Tuncel E, Erturk E, Imamoglu S. The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. *J Endocrinol Invest* 2010; **33**: 234-238
100 **Eu CH**, Lim WY, Ton SH, bin Abdul Kadir K. Glycyrrhizic acid improved lipoprotein lipase expression, insulin sensitivity, serum lipid and lipid deposition in high-fat diet-induced obese rats. *Lipids Health Dis* 2010; **9**: 81
101 **Uras R**, Orrù M, Pani F, Marotto MF, Pilloni M, Guerriero S, Etzi R, Zedda P, Sorge R, Lello S, Melis GB, Paoletti AM. Endocrinological, metabolic and clinical features of treatment with oral contraceptive formulation containing ethinylestradiol plus chlormadinone acetate in nonobese women with polycystic ovary syndrome. *Contraception* 2010; **82**: 131-138
102 **Pasquali R**, Gambineri A, Anconetani B, Vicennati V, Colitta D, Caramelli E, Casimirri F, Morselli-Labate AM. The natural history of the metabolic syndrome in young women with the polycystic ovary syndrome and the effect of long-term oestrogen-progestagen treatment. *Clin Endocrinol (Oxf)* 1999; **50**: 517-527
103 **Hoeger K**, Davidson K, Kochman L, Cherry T, Kopin L, Guzick DS. The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. *J Clin Endocrinol Metab* 2008; **93**: 4299-4306
104 **Cupisti S**, Häberle L, Dittrich R, Oppelt PG, Reissmann C, Kronawitter D, Beckmann MW, Mueller A. Smoking is associated with increased free testosterone and fasting insulin levels in women with polycystic ovary syndrome, resulting in aggravated insulin resistance. *Fertil Steril* 2010; **94**: 673-677
105 **Knowler WC**, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403
106 **Fux Otta C**, Wior M, Iraci GS, Kaplan R, Torres D, Gaido MI, Wyse EP. Clinical, metabolic, and endocrine parameters in response to metformin and lifestyle intervention in women with polycystic ovary syndrome: A randomized, double-blind, and placebo control trial. *Gynecol Endocrinol* 2009; 1-6
107 **Wiegand S**, l'Allemand D, Hübel H, Krude H, Bürmann M, Martus P, Grüters A, Holl RW. Metformin and placebo therapy both improve weight management and fasting insulin in obese insulin-resistant adolescents: a prospective, placebo-controlled, randomized study. *Eur J Endocrinol* 2010; **163**: 585-592
108 **Hoeger KM**, Kochman L, Wixom N, Craig K, Miller RK, Guzick DS. A randomized, 48-week, placebo-controlled trial of intensive lifestyle modification and/or metformin therapy in overweight women with polycystic ovary syndrome: a pilot study. *Fertil Steril* 2004; **82**: 421-429
109 **Johansson J**, Feng Y, Shao R, Lönn M, Billig H, Stener-Victorin E. Intense electroacupuncture normalizes insulin sensitivity, increases muscle GLUT4 content, and improves lipid profile in a rat model of polycystic ovary syndrome. *Am J Physiol Endocrinol Metab* 2010; **299**: E551-E559
110 **Stener-Victorin E**, Jedel E, Mannerås L. Acupuncture in polycystic ovary syndrome: current experimental and clinical evidence. *J Neuroendocrinol* 2008; **20**: 290-298

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REVIEW

Osteoporosis in diabetes mellitus: Possible cellular and molecular mechanisms

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Abstract

Osteoporosis, a global age-related health problem in both male and female elderly, insidiously deteriorates the microstructure of bone, particularly at trabecular sites, such as vertebrae, ribs and hips, culminating in fragility fractures, pain and disability. Although osteoporosis is normally associated with senescence and estrogen deficiency, diabetes mellitus (DM), especially type 1 DM, also contributes to and/or aggravates bone loss in osteoporotic patients. This topic highlight article focuses on DM-induced osteoporosis and DM/osteoporosis comorbidity, covering alterations in bone metabolism as well as factors regulating bone growth under diabetic conditions including, insulin, insulin-like growth factor-1 and angiogenesis. Cellular and molecular mechanisms of DM-related bone loss are also discussed. This information provides a foundation for the better understanding of diabetic complications and for development of early screening and prevention of osteoporosis in diabetic patients.

NORMAL BONE REMODELING

Being a primary structural framework of the body, bone undergoes dynamic microstructural remodeling throughout life to accommodate mechanical stress and calcium demand[1]. Bone remodeling is a coupled process of bone resorption and formation, and requires coordination of all three types of bone cells, namely osteocytes, osteoblasts and osteoclasts[1,2]. Under mechanical stress, osteocytes act as mechanosensors to detect changes in the flow of bone fluid within bone canaliculi, and respond by transmitting signals to the osteoblasts via their syncytial processes. Osteoblasts later stimulate osteoclast differentiation and subsequent bone resorption. Normally, osteoblast-mediated bone formation takes place at the same site to fill up the resorption pit with new bone[1,2].

Osteoclastic bone resorption occurs in areas of structurally weak bone caused by mechanical stress or disuse. At the cellular and molecular level, osteoclast-mediated bone resorption commences by osteoblasts initiating

proliferation of osteoclast precursors and their differentiation into mature osteoclasts by secreting a cytokine called macrophage colony stimulating factor (MCSF)[2,3]. Osteoblasts also secrete the key mediator for osteoclastogenesis, receptor activator of nuclear factor-κB (RANK) ligand (RANKL), which binds to its receptor (RANK) on the plasma membrane of osteoclast precursors, thereby stimulating differentiation of pre-osteoclasts into mature osteoclasts. RANKL and MCSF are differentially upregulated by various osteoclastogenic factors, such as parathyroid hormone (PTH), PTH-related peptide and prolactin[2,4,5]. Moreover, to counterbalance RANKL action, osteoblasts synthesize and secrete osteoprotegerin (OPG), a soluble decoy receptor capable of inhibiting RANK-RANKL interaction and osteoclastogenesis[2,6]. In the presence of activated osteoclasts, bone resorption begins with dissolution of inorganic and organic components by hydrochloric acid, cathepsin K and lysosomal protease from osteoclasts[2,7].

Following bone resorption, osteoblast-mediated bone formation takes place to fill the resorption pits with newly mineralized bone. The type I collagen fibrils secreted by osteoblasts are arranged into the organic matrix osteoid, which is subsequently mineralized by calcium and phosphate in the presence of alkaline phosphatase, osteocalcin and osteopontin. Eventually, hydroxide ions are gradually added and mature hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$] are formed[1]. Humoral factors, such as insulin-like growth factor (IGF)-1, insulin, bone morphogenetic proteins and OPG, serve as anabolic signals to promote bone formation[5,8-10]. Among these anabolic mediators, liver-derived IGF-1 is of particular interest since profound growth retardation, small bone size, low bone mineral density (BMD) and osteoporosis were reported in IGF-1 and IGF-1 receptor deficiencies[5,10,11]. Furthermore, insulin was found to directly induce osteogenic action by increasing cell proliferation, differentiation, alkaline phosphatase activity and expression of type I collagen and osteocalcin in human osteoblast-like MG-63 cells[12]. Matrix mineralization was also found to be enhanced by IGF-1 and insulin[11,12].

OSTEOPOROSIS AND RISK FACTORS

Osteoporosis is a global health care problem characterized by a reduction in BMD with increased porosity and susceptibility to fractures[13]. It can be caused by acceleration of bone resorption and/or deceleration of bone formation. Clinically, osteoporosis most often results from a combination of postmenopausal estrogen deficiency and age-related bone loss[2,14]. Irreversible bone loss can result from an imbalance between osteoclast and osteoblast activities, i.e. enhanced bone resorption and/or suppressed bone formation, resulting in an uncoupling event that can prolong duration of the bone remodeling cycle[5,13]. Other risk factors for osteoporosis are abnormally high plasma PTH levels, advancing age, genetic background, cigarette smoking, alcohol consumption, physical inactivity and the chronic use of some medications, such as corticosteroids. Low physical activity as found in the sedentary lifestyle of elderly, paralyzed or immobilized patients is also associated with accelerated bone loss[13,15-17]. Furthermore, other medical conditions, particularly hyperparathyroidism and diabetes mellitus (DM) are also risk factors for osteoporotic bone loss[13,16,17].

Regardless of the etiology, osteoporosis is initiated by the uncoupling of bone resorption and bone formation[5,13,17]. At the molecular level, enhanced bone resorption and osteoporosis generally result, in part, from the overproduction of RANKL and other cytokines/mediators regulating osteoclast differentiation and function. These include cyclooxygenase (Cox)-2, prostaglandin (PG) E_2, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 or IL-11[5,18,19], all of which lead to recruitment and differentiation of pre-osteoclasts[5,18,19]. Thus, the greater the increase in the levels of these osteoclastogenic cytokines, the faster the progression of bone loss.

DM-INDUCED OSTEOPOROSIS

DM is a group of pandemic debilitating metabolic diseases featuring chronic hyperglycemia which results from defective insulin secretion and/or insulin actions[20]. Such chronic hyperglycemia typically elicits dysfunction and failure of various organs, particularly the eyes (diabetic retinopathy and cataract), kidneys (diabetic nephropathy), nerves (diabetic neuropathy), heart (diabetic cardiomyopathy) and blood vessels (microangiopathy)[20]. In addition, DM has been found to be associated with metabolic bone diseases, osteoporosis and low-impact fractures, as well as other bone-related events including falls in geriatric patients[15,21]. Indeed, DM not only aggravates osteopenia (T-scores between -1 and -2.5, as determined by dual energy X-ray absorptiometry; DXA) and osteoporosis (T-scores $\leqslant$ -2.5), but is also one of the "causes" of both conditions. Nevertheless, bone deteriorations differ markedly between type 1 and type 2 DM and possibly stem from different cellular and molecular mechanisms[22-27].

Type 1 DM, also known as insulin-dependent DM, results from insulin insufficiency which leads to hyperglycemia in the young[20]. Besides the usual neurovascular complications, both male and female patients with type 1 DM manifest low bone mass at the hip, femoral neck and spine (Table 1), which may eventually lead to an increased incidence of bone fractures[22-25,28,29]. In contrast, data on skeletal abnormalities in type 2 DM, or non-insulin-dependent DM, appear conflicting, and the exact explanation of this is still unknown[26,27,30]. For example, by using DXA Yamaguchi and colleagues demonstrated that, of 187 males with type 2 DM, there was an increase in BMD at the femoral neck with low prevalence of vertebral fracture in diabetic men with metabolic syndromes[26]. Similarly, Petit and colleagues reported a higher BMD in elderly patients with type 2 DM when compared to age-matched non-DM volunteers[27]. In contrast, several

Table 1 Bone changes in patients with type 1 diabetes mellitus

References	Sample size	Age	Gender (F/M)	Major findings
Hamilton *et al*, 2009	102	20-71	52/50	Adult males with type 1 DM had lower BMD at hip, femoral neck and spine compared with age-matched controls ($P \leqslant 0.048$). No significant difference in BMD between female type 1 DM *vs* age-matched controls.
Mastrandrea *et al*, 2008	63	2-37	63/0	Type 1 DM women ⩾ 20 years of age had a reduction in BMD at hip, femoral neck and whole body. No significant difference in BMD between type 1 DM women < 20 years of age *vs* age-matched controls.
Soto *et al*, 2010	45	15-39	45/0	Adolescent and adult women with type 1 DM had lower BMD at spine, femoral neck and whole body. No correlation between decreased BMD and sex steroid hormones.
Saha *et al*, 2009	48	12-18	26/22	Adolescent men and women with type 1 DM had lower BMC at the proximal femur. Men with type 1 DM had lower cortical bone mass and cross-sectional size than age-matched women with type 1 DM.
Lumachi *et al*, 2009	18	36-51	8/10	Type 1 DM patients had ~60% lower BMD compared with age-matched controls.
Heilman *et al*, 2009	30	5-19	11/19	Type 1 DM patients had lower total BMC and lumbar BMD. Type 1 DM men had less physical activity than age-matched male controls.

DM: diabetes mellitus; BMD: bone mineral density; BMC: bone mineral content; F: female; M: male.

other investigators reported a negative effect of type 2 DM on BMD. For instance, Yaturu and colleagues found a significantly low BMD of hip in type 2 DM patients when compared to age-matched normal subjects[30]. Moreover, an increased fracture risk at several sites, including spine and hip has been reported[31]. However, these fractures and falls could have resulted from visual impairment (from diabetic retinopathy and cataract), gait imbalance (from peripheral neuropathy) and overweight, all of which are common clinical features in type 2 DM. Peripheral neuropathy in type 2 DM may also lead to local destruction of bones around the weight-bearing joints (especially in the ankle and foot), known as Charcot osteoarthropathy, which can cause pain, fracture and joint deformity[21].

Type 1 DM featuring low circulating insulin and IGF-1 levels usually occurs in young children prior to peak bone mass attainment, whereas type 2 DM is common in adults who have already attained peak bone mass[32,33]. Thus, type 1 and 2 DM induce detrimental skeletal complications of different magnitudes. Specifically, in both genders, BMD of the proximal femur appears to be significantly lower in type 1 DM than in type 2 DM[34]. This difference in severity might be because type 1 DM patients lack insulin, which is an osteogenic factor capable of stimulating osteoblast proliferation and differentiation[12]. Alternatively, different the time course of type 1 and 2 DM might contribute to their different outcomes and prognosis. A recent population-based investigation on 1964 diabetic patients in Rochester, Minnesota, revealed that the incidence of hip fractures, one of the most common osteoporotic fractures, increased only over 10 years of follow-up, and was not correlated with obesity or prolonged DM treatments[35]. However, other factors, including advanced age, previous fracture and long-term corticosteroid use, might also predispose DM patients to osteoporosis and low-impact fracture, whereas physical activity/exercise and high body mass index are protective[35].

BONE LOSS IN DIABETIC MOTHERS

Pregnancy and lactation increase calcium demand for fetal skeletal development and milk production, respectively, and bone serves to supply calcium during these reproductive periods[36-38]. Although maternal BMD is not decreased during pregnancy in humans and rodents[36,37], our recent histomorphometric study in rats showed that osteoclastic bone resorption was indeed enhanced at trabecular sites from mid-pregnancy to late lactation[39]. Significant bone loss with a decrease in BMD was, therefore, observed in late lactation. Maternal BMD is usually restored within 12 mo post-weaning. However, some breastfeeding mothers manifest a long-term sequela known as pregnancy/lactation-induced osteoporosis, which features back pain, height loss and/or vertebral fracture[38,40].

Bone loss is, therefore, expected to be greater in mothers with previously diagnosed DM or even with gestational DM (GDM; which affects ~4% of all pregnant women without previous history of DM[41]). A recent densitometric study in GDM women revealed a reduction in vertebral BMD when compared with non-DM pregnant women[42]. Moreover, it has been reported that greater than normal bone loss is present in ~40% of GDM women within 3 mo postpartum[15]. Nevertheless, the effects of previously diagnosed DM on maternal bone resorption and the long-term sequelae remain to be elucidated.

POSSIBLE MECHANISMS OF DM-INDUCED OSTEOPOROSIS

Although several investigators have long addressed the question of how DM induces osteopenia and osteoporosis, the exact underlying mechanism is still elusive. However, it is widely accepted that hyperglycemia is a salient factor that has direct and indirect deleterious effects on osteoblast function and bone formation (Figure 1). At

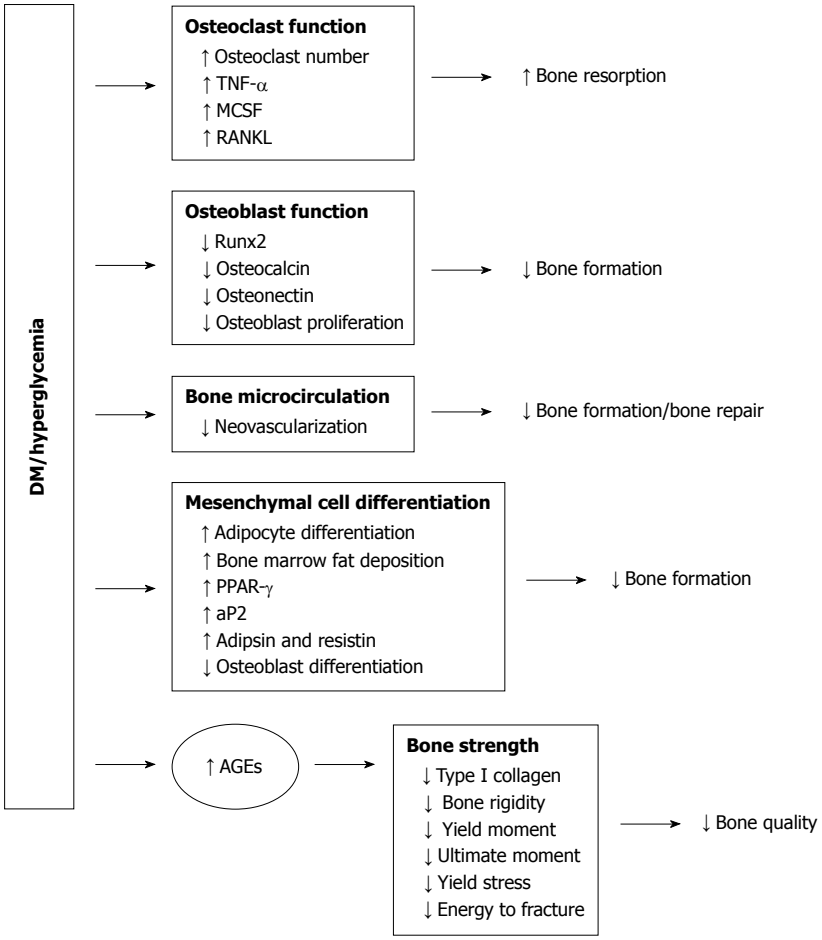

Figure 1 Possible deleterious effects of diabetes mellitus on bone metabolism and bone quality. Diabetes mellitus (DM) increases osteoclast function but decreases osteoblast function, thereby leading to accelerated bone loss, osteopenia and osteoporosis. DM/hyperglycemia induces production of macrophage colony stimulating factor (MCSF), tumor necrosis factor (TNF)-α and receptor activator of nuclear factor-κB ligand (RANKL), all of which are osteoblast-derived activators of osteoclast proliferation and differentiation. Moreover, DM/hyperglycemia suppresses osteoblast proliferation and function, in part, by decreasing *runt*-related transcription factor (Runx)-2, osteocalcin and osteopontin expressions. Adipogenic differentiation of mesenchymal stem cells is increased as indicated by the overexpression of adipocyte differentiation markers, including peroxisome proliferator-activated receptor (PPAR)-γ, adipocyte fatty acid binding protein (aP2), adipsin and resistin. A decrease in neovascularization may further aggravate bone loss. Bone quality is also reduced as a result of advanced glycation end products (AGE) production, which may eventually result in low-impact or fragility fractures.

the cellular level, a recent *in vitro* study in osteoblast-like MG63 cells demonstrated that high glucose concentrations markedly suppressed cell growth, mineralization, and expression of various osteoblast-related markers, including *runt*-related transcription factor-2 (Runx2), type I collagen, osteocalcin and osteonectin, while stimulating the expression of adipogenic markers, such as peroxisome proliferator-activated receptor (PPAR)-γ, adipocyte fatty acid binding protein (aP2), resistin and adipsin[43,44]. Consistent with the *in vitro* findings, a histomorphometric analysis in streptozotocin-induced DM mice showed increases in osteoclast numbers and expression of osteoclastogenic mediators, including TNF-α, MCSF, RANKL and vascular endothelial growth factor (VEGF)-A[45]. Moreover, there were upregulations of PPAR-γ, aP2 and resistin mRNAs, as well as increases in lipid-dense adipocytes in the bone marrow of these streptozotocin-induced DM mice, whereas adipose tissues at other sites, such as liver and peripheral areas, were decreased[44]. It is thus plausible that, in addition to direct interference with osteoblast function and bone formation, DM also induces lipid accumulation in the marrow of long bones, thereby leading to the expansion of marrow cavity and thinning of cortical envelope. The osteoblast-to-adipocyte shift might also reduce the number of differentiated osteoblasts available for bone formation.

Other cell types, such as endothelial progenitor cells (EPCs) lining the blood vessels, are also affected by hyperglycemia. It was shown that the streptozotocin-induced DM mice exhibit a reduction in circulating bone marrow-derived EPCs when compared to non-DM control mice[46]. Such decreases in circulating EPCs could retard angiogenesis essential for the repair process at fracture sites. Moreover, as demonstrated by the three-point bending mechanical test, DM was found to be associated with a reduction in parameters, such as bone rigidity, yield moment, ultimate moment, yield stress and energy to fracture, all of which are related to bone strength or "bone quality"[47,48]. Regarding the possible mechanisms underlying impaired mechanical properties, several investigations have demonstrated an increase in advanced glycation end products (AGE) or non-enzymatic cross-links within collagen fibers, which, in turn, lead to deterioration in the structural and mechanical properties of bone, and eventually to a decrease in bone strength[47]. *In vivo* studies in both type 1 and type 2 DM rats have confirmed that an increase in AGE production is negatively correlated with BMD and bone strength[49,50].

In addition to hyperglycemia, dysautonomia and impaired leptin function may indirectly contribute to osteopenia and osteoporosis in DM since both the sympathetic nervous system and leptin are known to modulate bone remodeling in a complex interdependent manner (for review Reference[51]). The final outcome of sympathetic stimulation (bone loss *vs* bone gain) depends on the relative distribution of activated adrenergic receptor subtypes

(β1, β2 or β3), expressed in osteoblasts[52]. β2-adrenergic receptor and leptin receptor knockout mice showed an increase in bone mass compared to normal mice, suggesting that β2 agonists and leptin are activators of bone resorption[53,54]. In contrast, osteoblast-like UMR106 cells exhibited the lower expression ratio of RANKL and OPG after exposure to β3-adrenergic agonist, suggesting a protective effect of β3-adrenergic receptor activation against bone resorption[52]. However, the possible direct link between the DM-induced autonomic neuropathy and impaired bone remodeling remains to be elucidated experimentally.

Several lines of evidence also suggest that DM-induced bone loss could be mediated, in part, by the humoral factors, kinins, which normally regulate blood circulation, inflammation and pain. Kinin dysfunctions could be responsible for several DM complications, such as hyperalgesia, cardiomyopathy and retinopathy[55-58]. In diabetic Akita mice with mutation in the insulin-2 gene, the lack of bradykinin receptor-1 (B1R) and receptor-2 (B2R) (i.e. B1R/B2R double knockout) induces profound diabetic complications, including massive albuminuria, glomerulosclerosis, reduction of nerve conduction velocity, and marked bone mineral loss[59]. It is thus possible that B1R/B2R and their related kinin signaling participate in the DM-induced bone loss.

PERSPECTIVES ON THE PREVENTION OF DM-INDUCED OSTEOPOROSIS

Since it is evident that most detrimental effects of DM on bone emanate from hyperglycemia and its consequences (e.g. AGE production and impaired vascularization), effective glycemic control and restoration of proper intraosseous blood supply should be of paramount importance for treatment and prevention of diabetic osteoporosis. The appropriate uses of antidiabetic agents should further help promote bone formation and/or prevent bone resorption. Recombinant insulin therapy might be a promising choice for diabetic intervention with its direct osteogenic effect through its receptors on osteoblasts. An *in vitro* study of insulin-treated bone marrow mesenchymal stem cells (BMSC; progenitors of both osteoblasts and adipocytes) cultured in high-glucose condition showed a significant increase in the activity of alkaline phosphatase, a representative of osteoblast differentiation, when compared to the control BMSC[60]. In addition, insulin also elicited synergistic effect when combined with supplementary 17β-estradiol by increasing type I collagen production and bone mineralizing nodules *in vitro*[60]. Furthermore, insulin should indirectly benefit bone by reducing the negative effects of chronic hyperglycemia[61]. Besides lowering plasma glucose levels and promoting anabolic bone function, insulin also enhances production of proteoglycans, the components of the gel-like extracellular matrix of cartilage, in the articular cartilage of streptozotocin-induced DM mice, suggesting that insulin might also protect against osteoarthritis in overweighed DM patients[61].

Among the wide variety of antidiabetic drugs, some have been reported to be favourable to osteogenesis, through their direct actions on osteoblasts or BMSC, while reducing adipogenesis. For instance, a recent investigation in metformin-treated streptozotocin-induced DM rats showed positive effects of metformin on osteoblast differentiation and function, including upregulation of Runx2 and osteocalcin protein expression, as well as increases in alkaline phosphatase activity, type I collagen synthesis and bone calcium accretion[62]. Similarly, glimepiride has been shown to stimulate proliferation and differentiation of primary rat osteoblasts *in vitro*[63]. In addition to synthetic drugs, certain herbal preparations, such as cinnamon bark extract, have been found to increase serum insulin levels and improve insulin sensitivity in adipose tissue by increasing serum adiponectin levels as well as upregulating PPAR-α and -γ mRNA expression[64], thereby inducing both antihyperglycemic and antihyperlipidemic actions. Thus, cinnamon extract probably helps reduce fat accumulation in bone marrow and indirectly facilitates bone formation[64].

In contrast, thiazolidinediones antidiabetic drugs, such as rosiglitazone, should be used with caution especially in postmenopausal DM patients since they may contribute to bone loss and fracture. Thiazolidinediones may decrease bone formation and BMD, while increasing bone resorption, as indicated by the reduced syntheses of alkaline phosphatase, osteocalcin, and procollagen type I N-terminal propeptide[33,65]. However, further investigations are needed to better understand the effects of thiazolidinediones on bone remodeling in DM patients at the cellular and molecular level.

Alleviation of microangiopathy and restoration of microcirculation in diabetic bone may be additional benefits of insulin and antihyperglycemic drugs. Xu and coworkers (2009) demonstrated that injection of BMSC treated with pancreatic extract into streptozotocin-induced DM rats not only normalized plasma glucose and prevented apoptosis of islet cells, but also elevated production of VEGF, IGF-1 and basic fibroblast growth factor (bFGF), all of which are known to have anti-apoptotic and angiogenic effects[66]. A recent *in vivo* study in type 2 DM (db^-/db^-) mice with ischemic hind limbs showed that injection of epidermal growth factor (EGF)-treated BMSC into the affected hind limbs increased angiogenesis by over 90%[67]. Such angiogenesis was due to the fact that the injected BMSC differentiated into new microvessels (neovascularization), using intercellular adhesion molecule-1 and vascular cell adhesion protein-1 for adhesion and migration[67].Overall, it is possible that antidiabetic agents with angiogenic activity could be used to enhance blood flow to fracture sites, which may in turn accelerate bone healing, and might also prevent osteopenia/osteoporosis. Conversely, certain rheological drugs, such as pentoxifylline, which increase blood flow and osteoblast activity, might be promising as anti-osteoporotic agents in both DM and non-DM patients[68].

In addition to medications, alternative interventions often prescribed to DM patients, such as exercise/physical activity, may be indirectly useful since they are expected to mitigate microangiopathy in bone by increasing neovascularization and blood flow. *In vivo* investigation in swimming rats showed higher bone capillary vascularity compared with sedentary controls[69]. Such higher vasculogenesis following exercise has been postulated to result from an increase in circulating EPCs[70,71]. Adams and colleagues demonstrated the elevation of EPC levels after single-exercise stress in patients with coronary artery disease[70]. An increase in EPC level was accompanied by an elevation of plasma VEGF[70,71], a crucial growth factor for EPC proliferation, differentiation and migration[70,71]. Thus, certain physical activities/interventions, such as appropriate endurance exercise, should improve perfusion in bone and alleviate bone loss in DM patients. Nevertheless, in "high-risk" individuals, including DM patients with very low BMD, previous low-impact/non-traumatic fractures and/or chronic use of corticosteroids, specific treatments for osteoporosis are still necessary (for reviews regarding the treatments of osteoporosis in DM patients, please see Refrences [15,21]).

CONCLUSION

In addition to neurovascular, ocular and renal complications, osteopenia and osteoporosis are important debilitating problems in DM patients. Osteoporosis and several other DM complications (e.g. visual impairment and gait imbalance) increase the risk of falls, fragility and low-impact fractures. It is apparent that hyperglycemia in DM directly suppresses osteoblast-mediated bone formation, while conversely promoting osteoclast-mediated bone resorption, adipogenic differentiation of mesenchymal stem cells (also precursors of osteoblasts), and fat accumulation in the marrow cavity, all of which deteriorate bone quality and strength and increase susceptibility to fracture. Therefore, an effective glycemic control should be the hallmark of prevention and treatment of DM-induced osteoporosis. Lowering of plasma glucose by appropriate antidiabetic drugs, recombinant insulin, herbal medications and/or lifestyle interventions (e.g. exercise) should help promote osteoblast function, angiogenesis (neovascularization) and bone perfusion, and help reduce fat accumulation in the marrow cavity, all of which eventually lead to better bone health for the DM patients.

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REFERENCES

1 **Sims NA**, Gooi JH. Bone remodeling: Multiple cellular interactions required for coupling of bone formation and resorption. *Semin Cell Dev Biol* 2008; **19**: 444-451
2 **Teitelbaum SL**. Bone resorption by osteoclasts. *Science* 2000; **289**: 1504-1508
3 **Matsuo K**, Irie N. Osteoclast-osteoblast communication. *Arch Biochem Biophys* 2008; **473**: 201-209
4 **Seriwatanachai D**, Thongchote K, Charoenphandhu N, Pandaranandaka J, Tudpor K, Teerapornpuntakit J, Suthiphongchai T, Krishnamra N. Prolactin directly enhances bone turnover by raising osteoblast-expressed receptor activator of nuclear factor κB ligand/osteoprotegerin ratio. *Bone* 2008; **42**: 535-546
5 **Zaidi M**. Skeletal remodeling in health and disease. *Nat Med* 2007; **13**: 791-801
6 **Asagiri M,** Takayanagi H. The molecular understanding of osteoclast differentiation. *Bone* 2007; **40**: 251-264
7 **Supanchart C**, Kornak U. Ion channels and transporters in osteoclasts. *Arch Biochem Biophys* 2008; **473**: 161-165
8 **Dobnig H**, Turner RT. Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. *Endocrinology* 1995; **136**: 3632-3638
9 **Jilka RL**. Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. *Bone* 2007; **40**: 1434-1446
10 **Mohan S**, Baylink DJ. Impaired skeletal growth in mice with haploinsufficiency of IGF-I: genetic evidence that differences in IGF-I expression could contribute to peak bone mineral density differences. *J Endocrinol* 2005; **185**: 415-420
11 **Zhang M**, Xuan S, Bouxsein ML, von Stechow D, Akeno N, Faugere MC, Malluche H, Zhao G, Rosen CJ, Efstratiadis A, Clemens TL. Osteoblast-specific knockout of the insulin-like growth factor (IGF) receptor gene reveals an essential role of IGF signaling in bone matrix mineralization. *J Biol Chem* 2002; **277**: 44005-44012
12 **Yang J**, Zhang X, Wang W, Liu J. Insulin stimulates osteoblast proliferation and differentiation through ERK and PI3K in MG-63 cells. *Cell Biochem Funct* 2010; **28**: 334-341
13 **Inzerillo AM**, Epstein S. Osteoporosis and diabetes mellitus. *Rev Endocr Metab Disord* 2004; **5**: 261-268
14 **Pogoda P**, Priemel M, Rueger JM, Amling M. Bone remodeling: new aspects of a key process that controls skeletal maintenance and repair. *Osteoporos Int* 2005; **16** Suppl 2: S18-S24
15 **Chau DL**, Edelman SV. Osteoporosis and diabetes. *Clin Diabetes* 2002; **20**: 153-157
16 **Schousboe JT**, Taylor BC, Ensrud KE. Assessing fracture risk: who should be screened? In: Favus MJ, editor Primer on the metabolic bone diseases and disorders of mineral metabolism. 6ed: American Society for Bone and Mineral Research, 2006: 262-267
17 **Merlotti D**, Gennari L, Dotta F, Lauro D, Nuti R. Mechanisms of impaired bone strength in type 1 and 2 diabetes. *Nutr Metab Cardiovasc Dis* 2010; **20**: 683-690
18 **Han SY**, Lee NK, Kim KH, Jang IW, Yim M, Kim JH, Lee WJ, Lee SY. Transcriptional induction of cyclooxygenase-2 in osteoclast precursors is involved in RANKL-induced osteoclastogenesis. *Blood* 2005; **106**: 1240-1245
19 **Ragab AA**, Nalepka JL, Bi Y, Greenfield EM. Cytokines synergistically induce osteoclast differentiation: support by immortalized or normal calvarial cells. *Am J Physiol Cell Physiol* 2002; **283**: C679-C687
20 American diabetes association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2009; **32** Suppl 1: S62-S67
21 **Brown SA**, Sharpless JL. Osteoporosis: an under-appreciated complication of diabetes. *Clin Diabetes* 2004; **22**: 10-20
22 **Hamilton EJ**, Rakic V, Davis WA, Chubb SA, Kamber N, Prince RL, Davis TM. Prevalence and predictors of osteopenia and osteoporosis in adults with type 1 diabetes. *Diabet Med* 2009; **26**: 45-52

23 **Mastrandrea LD**, Wactawski-Wende J, Donahue RP, Hovey KM, Clark A, Quattrin T. Young women with type 1 diabetes have lower bone mineral density that persists over time. *Diabetes Care* 2008; **31**: 1729-1735
24 **Saha MT**, Sievänen H, Salo MK, Tulokas S, Saha HH. Bone mass and structure in adolescents with type 1 diabetes compared to healthy peers. *Osteoporos Int* 2009; **20**: 1401-1406
25 **Lumachi F**, Camozzi V, Tombolan V, Luisetto G. Bone mineral density, osteocalcin, and bone-specific alkaline phosphatase in patients with insulin-dependent diabetes mellitus. *Ann N Y Acad Sci* 2009; **1173** Suppl 1: E64-E67
26 **Yamaguchi T**, Kanazawa I, Yamamoto M, Kurioka S, Yamauchi M, Yano S, Sugimoto T. Associations between components of the metabolic syndrome versus bone mineral density and vertebral fractures in patients with type 2 diabetes. *Bone* 2009; **45**: 174-179
27 **Petit MA**, Paudel ML, Taylor BC, Hughes JM, Strotmeyer ES, Schwartz AV, Cauley JA, Zmuda JM, Hoffman AR, Ensrud KE. Bone mass and strength in older men with type 2 diabetes: the Osteoporotic Fractures in Men Study. *J Bone Miner Res* 2010; **25**: 285-291
28 **Soto N**, Pruzzo R, Eyzaguirre F, Iñiguez G, López P, Mohr J, Pérez-Bravo F, Cassorla F, Codner E. Bone mass and sex steroids in postmenarcheal adolescents and adult women with type 1 diabetes mellitus. *J Diabetes Complications* 2011; **25**: 19-24
29 **Heilman K**, Zilmer M, Zilmer K, Tillmann V. Lower bone mineral density in children with type 1 diabetes is associated with poor glycemic control and higher serum ICAM-1 and urinary isoprostane levels. *J Bone Miner Metab* 2009; **27**: 598-604
30 **Yaturu S**, Humphrey S, Landry C, Jain SK. Decreased bone mineral density in men with metabolic syndrome alone and with type 2 diabetes. *Med Sci Monit* 2009; **15**: CR5-CR9
31 **Vestergaard P**. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes-a meta-analysis. *Osteoporos Int* 2007; **18**: 427-444
32 **Zofková I**. Pathophysiological and clinical importance of insulin-like growth factor-I with respect to bone metabolism. *Physiol Res* 2003; **52**: 657-679
33 **Adami S**. Bone health in diabetes: considerations for clinical management. *Curr Med Res Opin* 2009; **25**: 1057-1072
34 **Tuominen JT**, Impivaara O, Puukka P, Rönnemaa T. Bone mineral density in patients with type 1 and type 2 diabetes. *Diabetes Care* 1999; **22**: 1196-1200
35 **Melton LJ 3rd**, Leibson CL, Achenbach SJ, Therneau TM, Khosla S. Fracture risk in type 2 diabetes: update of a population-based study. *J Bone Miner Res* 2008; **23**: 1334-1342
36 **Bowman BM**, Miller SC. Skeletal adaptations during mammalian reproduction. *J Musculoskelet Neuronal Interact* 2001; **1**: 347-355
37 **Charoenphandhu N**, Wongdee K, Krishnamra N. Is prolactin the cardinal calciotropic maternal hormone? *Trends Endocrinol Metab* 2010; **21**: 395-401
38 **Kovacs CS**. Calcium and bone metabolism during pregnancy and lactation. *J Mammary Gland Biol Neoplasia* 2005; **10**: 105-118
39 **Suntornsaratoon P**, Wongdee K, Goswami S, Krishnamra N, Charoenphandhu N. Bone modeling in bromocriptine-treated pregnant and lactating rats: possible osteoregulatory role of prolactin in lactation. *Am J Physiol Endocrinol Metab* 2010; **299**: E426-E436
40 **Ofluoglu O**, Ofluoglu D. A case report: pregnancy-induced severe osteoporosis with eight vertebral fractures. *Rheumatol Int* 2008; **29**: 197-201
41 American Diabetes Association. What is gestational diabetes? 2010 [cited 2010 October, 11]; Available from: URL: http://www.diabetes.org/diabetes-basics/gestational/what-is-gestational-diabetes.html
42 **To WW**, Wong MW. Bone mineral density changes in gestational diabetic pregnancies-a longitudinal study using quantitative ultrasound measurements of the os calcis. *Gynecol Endocrinol* 2008; **24**: 519-525
43 **Wang W**, Zhang X, Zheng J, Yang J. High glucose stimulates adipogenic and inhibits osteogenic differentiation in MG-63 cells through cAMP/protein kinase A/extracellular signal-regulated kinase pathway. *Mol Cell Biochem* 2010; **338**: 115-122
44 **Botolin S**, Faugere MC, Malluche H, Orth M, Meyer R, McCabe LR. Increased bone adiposity and peroxisomal proliferator-activated receptor-γ2 expression in type I diabetic mice. *Endocrinology* 2005; **146**: 3622-3631
45 **Kayal RA**, Tsatsas D, Bauer MA, Allen B, Al-Sebaei MO, Kakar S, Leone CW, Morgan EF, Gerstenfeld LC, Einhorn TA, Graves DT. Diminished bone formation during diabetic fracture healing is related to the premature resorption of cartilage associated with increased osteoclast activity. *J Bone Miner Res* 2007; **22**: 560-568
46 **Kang L**, Chen Q, Wang L, Gao L, Meng K, Chen J, Ferro A, Xu B. Decreased mobilization of endothelial progenitor cells contributes to impaired neovascularization in diabetes. *Clin Exp Pharmacol Physiol* 2009; **36**: e47-e56
47 **Saito M**, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporos Int* 2010; **21**: 195-214
48 **Silva MJ**, Brodt MD, Lynch MA, McKenzie JA, Tanouye KM, Nyman JS, Wang X. Type 1 diabetes in young rats leads to progressive trabecular bone loss, cessation of cortical bone growth, and diminished whole bone strength and fatigue life. *J Bone Miner Res* 2009; **24**: 1618-1627
49 **Tomasek JJ**, Meyers SW, Basinger JB, Green DT, Shew RL. Diabetic and age-related enhancement of collagen-linked fluorescence in cortical bones of rats. *Life Sci* 1994; **55**: 855-861
50 **Saito M**, Fujii K, Mori Y, Marumo K. Role of collagen enzymatic and glycation induced cross-links as a determinant of bone quality in spontaneously diabetic WBN/Kob rats. *Osteoporos Int* 2006; **17**: 1514-1523
51 **Elefteriou F**. Regulation of bone remodeling by the central and peripheral nervous system. *Arch Biochem Biophys* 2008; **473**: 231-236
52 **Nuntapornsak A**, Wongdee K, Thongbunchoo J, Krishnamra N, Charoenphandhu N. Changes in the mRNA expression of osteoblast-related genes in response to β3-adrenergic agonist in UMR106 cells. *Cell Biochem Funct* 2010; **28**: 45-51
53 **Elefteriou F**, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, Kondo H, Richards WG, Bannon TW, Noda M, Clement K, Vaisse C, Karsenty G. Leptin regulation of bone resorption by the sympathetic nervous system and CART. *Nature* 2005; **434**: 514-520
54 **Shi Y**, Yadav VK, Suda N, Liu XS, Guo XE, Myers MG Jr, Karsenty G. Dissociation of the neuronal regulation of bone mass and energy metabolism by leptin in vivo. *Proc Natl Acad Sci USA* 2008; **105**: 20529-20533
55 **Phipps JA**, Feener EP. The kallikrein-kinin system in diabetic retinopathy: lessons for the kidney. *Kidney Int* 2008; **73**: 1114-1119
56 **Westermann D**, Walther T, Savvatis K, Escher F, Sobirey M, Riad A, Bader M, Schultheiss HP, Tschöpe C. Gene deletion of the kinin receptor B1 attenuates cardiac inflammation and fibrosis during the development of experimental diabetic cardiomyopathy. *Diabetes* 2009; **58**: 1373-1381
57 **Gabra BH**, Sirois P. Role of bradykinin B(1) receptors in diabetes-induced hyperalgesia in streptozotocin-treated mice. *Eur J Pharmacol* 2002; **457**: 115-124
58 **Marceau F**, Regoli D. Bradykinin receptor ligands: therapeutic perspectives. *Nat Rev Drug Discov* 2004; **3**: 845-852
59 **Kakoki M**, Sullivan KA, Backus C, Hayes JM, Oh SS, Hua K, Gasim AM, Tomita H, Grant R, Nossov SB, Kim HS, Jennette JC, Feldman EL, Smithies O. Lack of both bradykinin B1 and B2 receptors enhances nephropathy, neuropathy, and bone mineral loss in Akita diabetic mice. *Proc Natl Acad Sci USA* 2010; **107**: 10190-10195

60 **Gopalakrishnan V**, Vignesh RC, Arunakaran J, Aruldhas MM, Srinivasan N. Effects of glucose and its modulation by insulin and estradiol on BMSC differentiation into osteoblastic lineages. *Biochem Cell Biol* 2006; **84**: 93-101
61 **Cai L**, Okumu FW, Cleland JL, Beresini M, Hogue D, Lin Z, Filvaroff EH. A slow release formulation of insulin as a treatment for osteoarthritis. *Osteoarthritis Cartilage* 2002; **10**: 692-706
62 **Molinuevo MS**, Schurman L, McCarthy AD, Cortizo AM, Tolosa MJ, Gangoiti MV, Arnol V, Sedlinsky C. Effect of metformin on bone marrow progenitor cell differentiation: in vivo and in vitro studies. *J Bone Miner Res* 2010; **25**: 211-221
63 **Ma P**, Gu B, Ma J, E L, Wu X, Cao J, Liu H. Glimepiride induces proliferation and differentiation of rat osteoblasts via the PI3-kinase/Akt pathway. *Metabolism* 2010; **59**: 359-366
64 **Kim SH**, Choung SY. Antihyperglycemic and antihyperlipidemic action of Cinnamomi Cassiae (Cinnamon bark) extract in C57BL/Ks db/db mice. *Arch Pharm Res* 2010; **33**: 325-333
65 **Debiais F**. Thiazolidinediones: antidiabetic agents with effects on bone. *Joint Bone Spine* 2009; **76**: 221-223
66 **Xu YX**, Chen L, Hou WK, Lin P, Sun L, Sun Y, Dong QY, Liu JB, Fu YL. Mesenchymal stem cells treated with rat pancreatic extract secrete cytokines that improve the glycometabolism of diabetic rats. *Transplant Proc* 2009; **41**: 1878-1884
67 **Amin AH**, Abd Elmageed ZY, Nair D, Partyka MI, Kadowitz PJ, Belmadani S, Matrougui K. Modified multipotent stromal cells with epidermal growth factor restore vasculogenesis and blood flow in ischemic hind-limb of type II diabetic mice. *Lab Invest* 2010; **90**: 985-996
68 **Kinoshita T**, Kobayashi S, Ebara S, Yoshimura Y, Horiuchi H, Tsutsumimoto T, Wakabayashi S, Takaoka K. Phosphodiesterase inhibitors, pentoxifylline and rolipram, increase bone mass mainly by promoting bone formation in normal mice. *Bone* 2000; **27**: 811-817
69 **Viboolvorakul S**, Niimi H, Wongeak-in N, Eksakulkla S, Patumraj S. Increased capillary vascularity in the femur of aged rats by exercise training. *Microvasc Res* 2009; **78**: 459-463
70 **Adams V**, Lenk K, Linke A, Lenz D, Erbs S, Sandri M, Tarnok A, Gielen S, Emmrich F, Schuler G, Hambrecht R. Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia. *Arterioscler Thromb Vasc Biol* 2004; **24**: 684-690
71 **Miller-Kasprzak E**, Jagodziński PP. Endothelial progenitor cells as a new agent contributing to vascular repair. *Arch Immunol Ther Exp (Warsz)* 2007; **55**: 247-259

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WJD World Journal of Diabetes

Contents

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World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a monthly, open-access, peer-reviewed journal supported by an editorial board of 323 experts in diabetes mellitus research from 38 countries.

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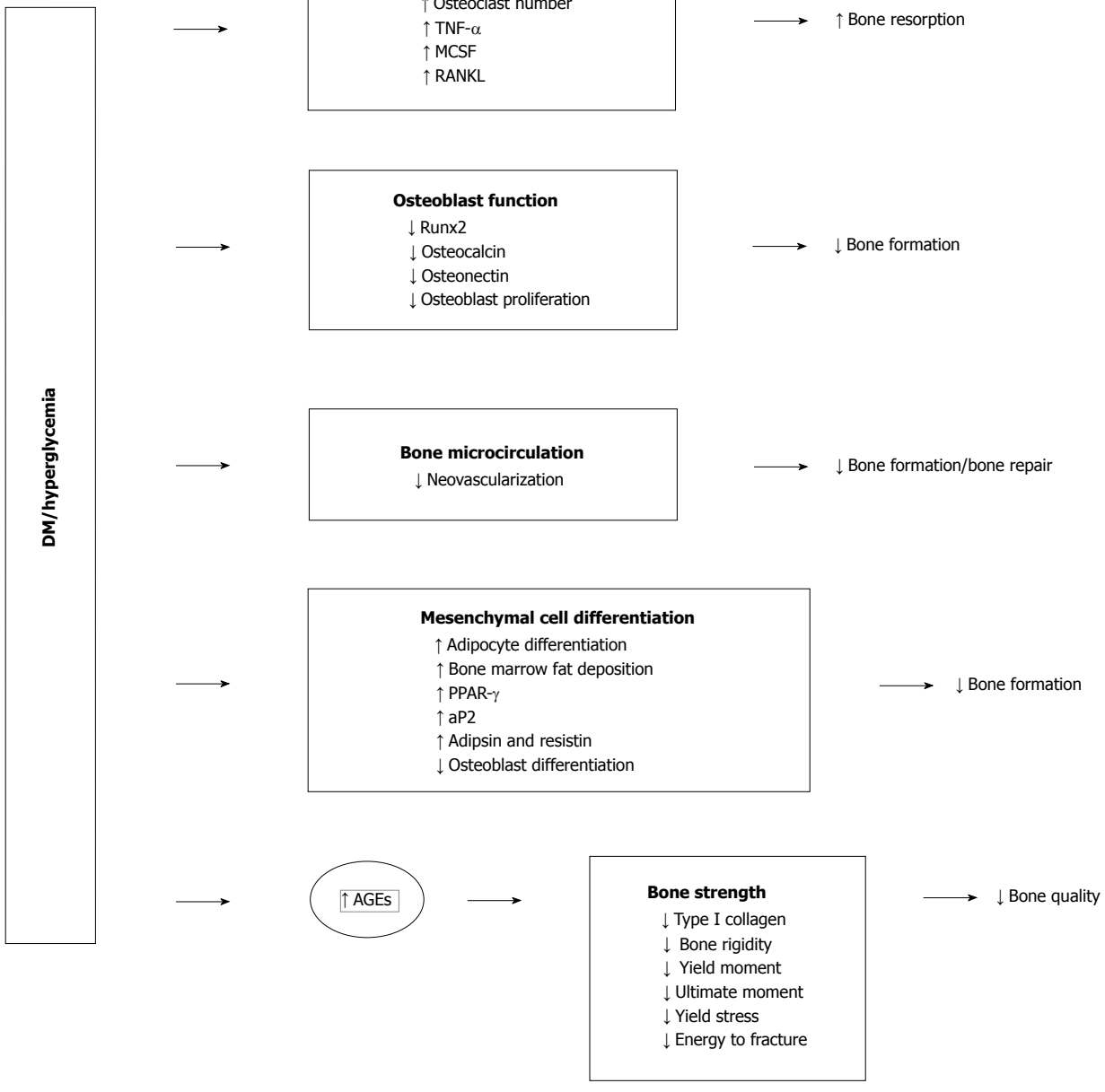

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Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

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Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
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Meetings

Events Calendar 2011

January 14-15, 2011
AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011 Miami
FL, United States

January 28, 2011
Diabetes UK and External Conferences
Diabetes Awareness Training
London, United Kingdom

January 28-29, 2011
9. Gastro Forum München
Munich, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference
Sydney, NSW, Australia

February 16-19, 2011
The 4th International Conference on Advance Technologies & Treatments for Diabetes
London, United Kingdom

February 24-26, 2011
2nd International Congress on Abdominal Obesity
Buenos Aires, Brazil

February 26-March 1, 2011
Canadian Digestive Diseases Week, Westin Bayshore, Vancouver
British Columbia, Canada

February 28-March 1, 2011
Childhood & Adolescent Obesity: A Whole-system Strategic Approach
Abu Dhabi, United Arab Emirates

March 3-5, 2011
42nd Annual Topics in Internal Medicine
Gainesville, FL, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011, Birmingham
England, United Kingdom

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology
Jacksonville, FL , United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform
Sacramento, CA, United States

March 25-27, 2011
MedicReS IC 2011 Good Medical Research
Istanbul, Turkey

March 28–30, 2011
The Second World Congress on Interventional Therapies for Type 2 Diabetes
New York, United States

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition
Riyadh, Saudi Arabia

May 7-10, 2011
Digestive Disease Week
Chicago, IL, United States

June 2-5, 2011
The 1st Asia Pacific Congress on Controversies to Consensus in Diabetes, Obesity and Hypertension
Shanghai, China

June 11-12, 2011
The International Digestive Disease Forum 2011
Hong Kong, China

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer
Barcelona, Spain

August 3-6, 2011
AADE 38th Annual Meeting
Las Vegas, United States

October 16-18, 2011
ISPAD Science School for Health Professionals
Miami, Unites States

October 19-22, 2011
ISPAD 36th Annual Meeting
Miami, United States

October 22-26, 2011
19th United European Gastroenterology Week
Stockholm, Sweden

October 26-29, 2011
CDA/CSEM Professional Conference and Annual Meetings
Toronto, Ontario, Canada

October 28-November 2, 2011
ACG Annual Scientific Meeting & Postgraduate Course
Washington, DC, United States

November 10-12, 2011
The Second International Diabetes & Obesity Forum
Istanbul, Turkey