World Journal of Diabetes

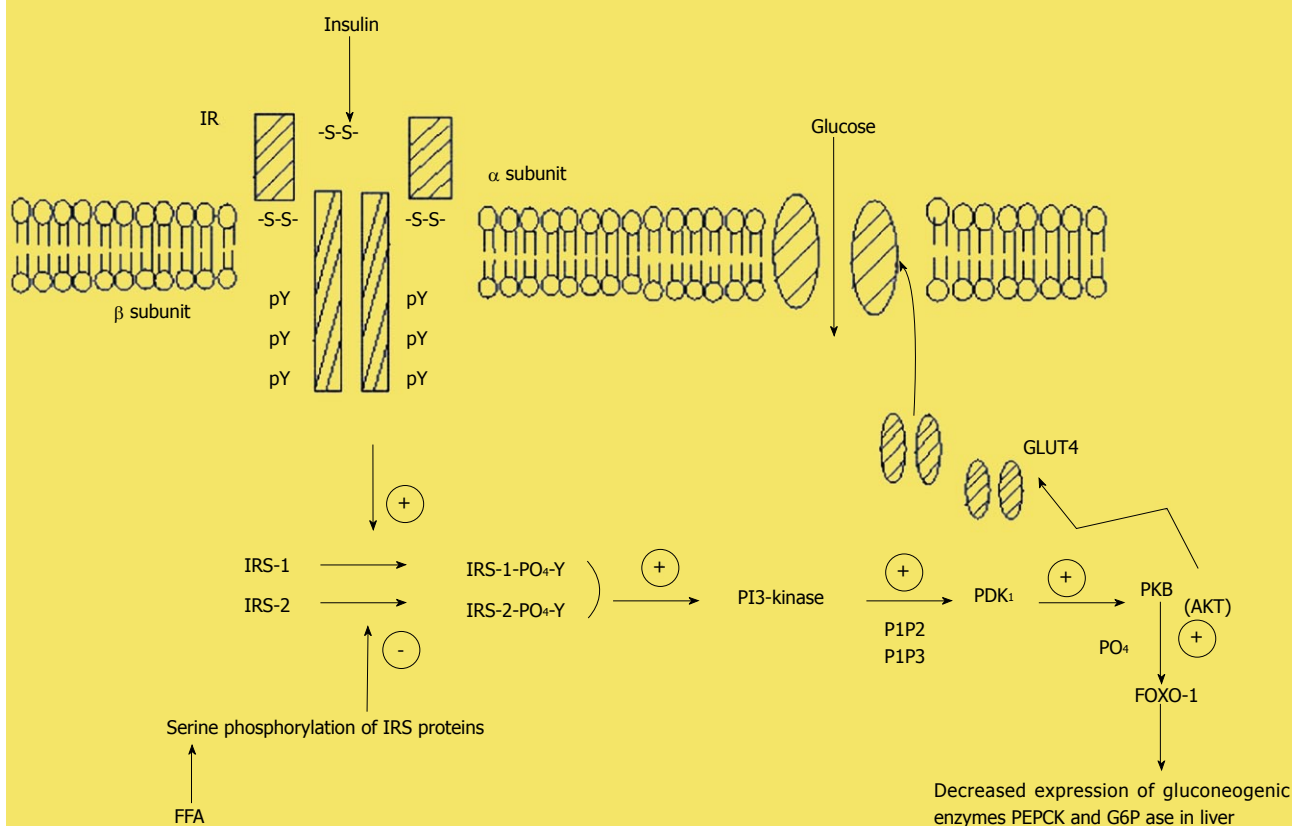*A peer-reviewed, online, open-access journal of diabetes*

Insulin signaling pathway showing binding of insulin with the insulin receptor leading to the activation of glucose transporter 4 which imports glucose into the cell. Binding of insulin to the IR activates PI3-k which produces PI4, 5P2 and PI3, 4, 5P3. These serve as docking sites for PDK1 which then mediates activation of PKB. Activated PKB can regulate transcription of target genes-PEPCK and G6Pase *via* Foxo-1. Increased free fatty acids may cause serine phosphorylation of IRS proteins, which in turn decreases IRS-tyrosine phosphorylation, impairing downstream effectors.

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World Journal of *Diabetes*

Contents

Contents

AIM AND SCOPE

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

The major task of *WJD* is to report rapidly the most recent results in basic and clinical research on diabetes including: metabolic syndrome, functions of α, β, δ and PP cells of the pancreatic islets, effect of insulin and insulin resistance, pancreatic islet transplantation, adipose cells and obesity, clinical trials, clinical diagnosis and treatment, rehabilitation, nursing and prevention. This covers epidemiology, etiology, immunology, pathology, genetics, genomics, proteomics, pharmacology, pharmacokinetics, pharmacogenetics, diagnosis and therapeutics. Reports on new techniques for treating diabetes are also welcome.

EDITORIAL

Mediterranean diet and diabetes prevention: Myth or fact?

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Abstract

Type 2 diabetes is a major, non-communicable disease with increasing prevalence at a global level. Therefore, in order to prevent this condition action should be taken regarding the modifiable factors that influence its development - lifestyle and dietary habits. As the Mediterranean dietary pattern has beneficial effects on both human health and regarding the development and treatment of type 2 diabetes, promoting adherence to this pattern is of considerable public health importance.

INTRODUCTION

Diabetes mellitus, one of the major non-communicable diseases at a global level, is a condition difficult to treat and expensive to manage[1]. Individuals with type 2 diabetes are at a high risk of developing a range of debilitating complications that can lead to disability and premature death: cardiovascular disease, peripheral vascular disease, nephropathy, changes to the retina and blindness; imposing important medical and economic burdens. Genetic susceptibility and environmental influences seem to be the most important factors responsible for the development of this condition. However, a drastic increase of physical inactivity, obesity and type 2 diabetes has been recently observed; a fact which indicates that obesity and physical inactivity may constitute the main reasons for the increasing burden of diabetes in the developed world[2]. Fortunately, because environmental factors are modifiable, disease manifestation from these factors is largely preventable[3].

MEDITERRANEAN DIET AND DIABETES

The Mediterranean diet was first described in the 1960s by Ancel Keys based on his observation of food habits of some populations in the Mediterranean region. The Mediterranean dietary pattern emphasizes a consumption of fat primarily from foods high in monounsaturated fatty acids and mainly olive oil and encourages daily consumption of fruits, vegetables, low fat dairy products and whole grains, weekly consumption of fish, poultry, tree nuts, legumes, monthly consumption of red meat, as well as a moderate consumption of alcohol, normally with meals but the proportions of macronutrients may vary. There is no single Mediterranean diet although the dietary patterns that prevail in the Mediterranean region have many common characteristics. Total lipid intake may be high as in Greece (around 40% of total energy intake), or moderate as in Italy (around 30% of total energy intake)[4,5].

The Mediterranean diet is one of the best known dietary patterns for its beneficial effects on human

health and seems to have pleiotropic effects that may act beneficially against the development of type 2 diabetes, including reduced oxidative stress and insulin resistance. High consumption of vegetables, fruits, legumes, nuts, fish, cereals and olive oil together with moderate consumption of alcohol, predominantly wine, leads to a high ratio of monounsaturated fatty acids to saturated fatty acids, a low intake of trans fatty acids and high ingestion of dietary fiber, antioxidants, polyphenols and magnesium[3]. Therefore, the Mediterranean diet could serve as an anti-inflammatory dietary pattern which could protect from or even treat diseases that are related to chronic inflammation including type 2 diabetes[6]. At this point it should be mentioned that several large epidemiological studies have shown that diets characterized by a low degree of energy density overall such as the Mediterranean diet, prevent weight gain and exert a protective effect on the development of type 2 diabetes, a condition that is partially mediated through weight maintenance[3].

Results from epidemiological studies show the beneficial effect of the Mediterranean dietary pattern on diabetes mellitus and glucose metabolism in general. According to a large prospective study of 13 380 Spanish university graduates, a traditional Mediterranean food pattern was associated with a significant reduction of 83% in the risk of developing type 2 diabetes[7]. An inverse association has been also found between adherence to Mediterranean diet and indices of glucose homeostasis in a Greek adult population[6,8], among elderly people[9] and high-risk patients[10]. Greater adherence to the Mediterranean diet in combination with light physical activity was associated with lower odds of having diabetes after adjustment for various factors according to the ATTICA Study[11]. Finally, according to a recent study in Italy in 901 outpatients with type 2 diabetes, greater adherence to the traditional Mediterranean diet was associated with lower HbA_{1c} levels and 2 h postmeal glucose levels independently of other confounding factors[12]. On the other hand, a Paleolithic diet (i.e. a diet consisting of lean meat, fish, shellfish, fruits and vegetables, roots, eggs and nuts, but not grains, dairy products, salt or refined fats and sugar) was associated with marked improvement of glucose tolerance while control subjects who were advised to follow a Mediterranean-like diet did not significantly improve their glucose tolerance despite decreases in weight and waist circumference[13].

Results from clinical trials also support the protective role of the Mediterranean diet on type 2 diabetes. According to a multicenter randomized primary prevention trial, subjects without diabetes allocated to a Mediterranean diet either focused on olive oil or nuts had lower fasting glucose levels, lower fasting insulin levels and insulin resistance compared to those assigned to a low fat diet. However, in this study, nutritional education was more intense for the participants assigned to the Mediterranean diet groups[14]. According to Shai *et al*,

participants with diabetes assigned to the Mediterranean diet had lower levels of fasting plasma glucose and insulin than those assigned to the low fat diet. Insignificant changes in plasma glucose were observed for subjects without diabetes while insulin levels decreased significantly in all groups[15]. Finally, according to another intervention dietary study in a young healthy normolipidemic population, *in vivo* insulin sensitivity was improved when saturated fatty acids were replaced by carbohydrates or monounsaturated fatty acids in an enriched Mediterranean diet[16].

Furthermore, it is worth mentioning that certain individual components of the Mediterranean dietary pattern may also protect against the development of diabetes. High consumption of olive oil, fruits and vegetables, whole grain cereals, fish and moderate consumption of alcohol leads to a low glycaemic index diet and to a higher intake of monounsaturated fatty acids, n-3 fatty acids, dietary fiber and antioxidant and anti-inflammatory factors[5,17]. Finally, due to the high fiber content of the Mediterranean diet, favourable changes may occur in the composition of the gut microbiota which may be another link for the protective effect of this pattern[18].

Nevertheless, dietary habits in the developed world and in developing countries at "nutrition transition", in particular India and China, are changing towards the opposite direction despite the nutritional recommendations for a healthy diet and lifestyle[19-21]. Even in Mediterranean countries, more fat, meat, egg, dairy products and sugar and less cereals, legumes, vegetables and seafood are being consumed[22]. Therefore, lifestyle measures for the prevention of obesity and diabetes are of significant public health importance.

CONCLUSION

In conclusion, effective lifestyle modifications including counseling on weight loss, adoption of a healthy dietary pattern like the Mediterranean diet, together with physical activity are the cornerstone in the prevention of type 2 diabetes. Therefore emphasis must be given to promoting a healthier lifestyle[1] and finding solutions in order to increase adherence and compliance to the lifestyle modifications, especially for high-risk individuals. Results from epidemiological studies and clinical trials evaluating the role of the Mediterranean dietary pattern regarding the development and treatment of type 2 diabetes indicate the protective role of this pattern. As a result, promoting adherence to the Mediterranean diet is of considerable public health importance as this dietary pattern, apart from its various health benefits, is tasty and easy to follow in the long term.

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TOPIC HIGHLIGHT

Sarika Arora, MD, *Series Editor*

Molecular mechanisms of insulin resistance in type 2 diabetes mellitus

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Abstract

Free fatty acids are known to play a key role in promoting loss of insulin sensitivity in type 2 diabetes mellitus but the underlying mechanism is still unclear. It has been postulated that an increase in the intracellular concentration of fatty acid metabolites activates a serine kinase cascade, which leads to defects in insulin signaling downstream to the insulin receptor. In addition, the complex network of adipokines released from adipose tissue modulates the response of tissues to insulin. Among the many molecules involved in the intracellular processing of the signal provided by insulin, the insulin receptor substrate-2, the protein kinase B and the forkhead transcription factor Foxo 1a are of particular interest, as recent data has provided strong evidence that dysfunction of these proteins results in insulin resistance *in vivo*. Recently, studies have revealed that phosphoinositidedependent kinase 1-independent phosphorylation of protein kinase Cε causes a reduction in insulin receptor gene expression. Additionally, it has been suggested that mitochondrial dysfunction triggers activation of several serine kinases, and weakens insulin signal transduction. Thus, in this review, the current developments in understanding the pathophysiological processes of insulin resistance in type 2 diabetes have been summarized. In addition, this study provides potential new targets for the treatment and prevention of type 2 diabetes.

Saini V. Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. *World J Diabetes* 2010; 1(3): 68-75 Available from: URL: http://www.wjgnet.com/1948-9358/full/v1/i3/68.htm DOI: http://dx.doi.org/10.4239/wjd.v1.i3.68

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder in man, currently affecting over 170 million people world-wide and, potentially, over 365 million in the year 2030[1]. Type 2 DM is rapidly emerging as one of the greatest global health challenges of the 21st century. This looming epidemic is also expected to trigger a steep rise in the complications associated with diabetes, such as ischemic heart disease, stroke, neuropathy, retinopathy, and nephropathy. Besides β cell failure, the major pathophysiological event contributing to the development of type 2 DM is the resistance of target tissues to insulin, which is usually associated with abnormal insulin secretion. Clinically, the term "insulin resistance" implies that higher-than-normal concentrations of insulin are required to maintain normoglycemia. On a cellular level, it defines the inadequate strength of insulin signaling from the insulin receptor downstream to the final substrates of insulin action involved in multiple metabolic and mitogenic aspects of cellular function[2].

The pathogenesis of type 2 diabetes involves abnor-

malities in both insulin action and secretion[3]. Although the precise pathophysiological sequence which leads to insulin resistance is still largely unknown, recent studies have contributed to a deeper understanding of the underlying molecular mechanisms. This review deals with the mechanisms related to type 2 diabetes. A detailed understanding of these basic pathophysiological mechanisms is critical for the development of novel therapeutic strategies to treat diabetes.

NORMAL INSULIN SIGNALING

The insulin receptor (IR) is a heterotetramer consisting of two α subunits and two β subunits that are linked by disulphide bonds. Insulin binds to the α subunit of the insulin receptor and activates the tyrosine kinase in the β subunit. Once the tyrosine kinase of insulin receptor is activated, it promotes autophosphorylation of the β subunit, where phosphorylation of three tyrosine residues (Tyr-1158, Tyr-1162, and Tyr-1163) is required for amplification of the kinase activity[4]. Most of the metabolic and antiapoptotic effects of insulin are mediated by the signaling pathway involving the phosphorylation of the insulin receptor substrate (IRS) proteins, and the activation of the phosphatidylinositol (PI) 3-kinase, Akt (also known as protein kinase B), the molecular target of rapamycin (mTOR), and p70 S6 kinase[5,6]. The insulin receptor tyrosine kinase phosphorylates the IRS proteins, and phosphotyrosine residues on IRS proteins become good targets for the p85 regulatory subunit of PI3-kinase. The activated PI3-kinase generates 3'-phosphoinositides [phosphatidyl-inositol-3,4-bisphosphate (PIP2) and phosphatidyl-inositol-3,4,5-trisphosphate (PIP3)][7], which bind to the phosphoinositidedependent kinase 1 (PDK1). Known substrates of the PDKs are the protein kinase B (PKB) and also atypical forms of the protein kinase C (PKC)[8].

PKB

Downstream from PI3-kinase, the serine/threonine kinase Akt (also called PKB), triggers insulin effects on the liver, such as glycogen synthesis and the suppression of hepatic glucose production. Akt plays an important role by linking glucose transporter (GLUT4), the insulin-dependent glucose transporter protein, to the insulin signaling pathway. It activates GLUT4 which moves to the cell surface to transport glucose into the cell[9-11]. Recent data from PKB knockout animal models offer a clearer answer to the question of whether PKB is required for normal glucose homeostasis. While disruption of PKB/Akt1 isoform in mice did not cause any significant perturbations in metabolism, mice with a knock-out of the PKB (Akt2) isoform show insulin resistance, ending up with a phenotype closely resembling type 2 diabetes in humans[12-13]. Consistently, recent studies on inherited insulin post-receptor mutations in humans detected a missense mutation in the kinase domain of PKB (Akt2) in a family of severely insulin resistant patients. The mutant kinase was unable to phosphorylate downstream targets and to mediate inhibition of phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic key enzyme[14]. This suggests that the impairment of insulin activity leading to insulin resistance is linked to insulin signaling defects. These insulin signaling pathways are shown in Figure 1.

MUTATIONS IN IRS PROTEINS

In humans, rare mutations of the IRS-1 protein are associated with insulin resistance[15]. Disruption of the *IRS-1* gene in mice results in insulin resistance, mainly of muscle and fat[16]. Interesting results are obtained by studying IRs in knockout mice. Heterozygous knockout mice lacking a single allele of *IRS-1* gene lack any significant phenotype, whereas homozygous disruption of the *IRS-1* gene results in a mild form of insulin resistance[17]. IRS-1 homozygous null mice (IRS-1$^{-/-}$) do not show a clear diabetic phenotypic expression, presumably because of pancreatic β cell compensation. IRS-2$^{-/-}$ mice, on the other hand, developed diabetes as a result of severe insulin resistance paired with β cell failure[18,19].

INCREASE IN SERINE PHOSPORYLATION OF IRS PROTEINS

Recent studies have made it apparent that serine phosphorylation of IRS proteins can reduce the ability of IRS proteins to attract PI3-kinase, thereby minimizing its activation[20-25], and can also lead to an accelerated degradation of the IRS-1 protein[26]. This serine phosphorylation in turn decreases IRS-1 tyrosine phosphorylation, impairing downstream effectors[27]. Serine phosphorylation of IRS proteins can occur in response to a number of intracellular serine kinases[28]. The causes of serine phosphorylation of IRS-1 proteins are shown in Table 1.

Recent studies have demonstrated hyper-serine phosphorylation of IRS-1 on Ser^{302}, Ser^{307}, Ser^{612}, and Ser^{632} in several insulin-resistant rodent models[23,29-31] as well as in young lean insulin-resistant offspring of type 2 diabetic parents[32]. Further evidence for this hypothesis stems from recent studies in a muscle-specific triple serine to alanine mutant mouse (IRS-1 Ser → Ala^{302}, Ser → Ala^{307}, and Ser → Ala^{612}), which has been shown to be protected from high-fat diet-induced insulin resistance *in vivo*[33]. Based on *in vitro* studies, serine phosphorylation may lead to dissociation between the insulin receptor/IRS-1 and/or IRS-1/PI3-kinase, preventing PI3-kinase activation[34,35] or increased degradation of IRS-1[36]. Furthermore, there are data linking IRS dysfunction in skeletal muscle to adipocyte biology and lipotoxicity. For example, circulating free fatty acids (FFA) and the adipokine tumour necrosis factor (TNF) may increase serine phosphorylation of IRS proteins, thereby causing impaired insulin signal transduction[37].

Figure 1 Insulin signaling pathway showing binding of insulin with the insulin receptor leading to the activation of glucose transporter 4 which imports glucose into the cell. Binding of insulin to the IR activates PI3-k which produces PI4, 5P2 and PI3, 4, 5P3. These serve as docking sites for PDK1 which then mediates activation of PKB. Activated PKB can regulate transcription of target genes-PEPCK and G6Pase *via* Foxo-1. Increased free fatty acids may cause serine phosphorylation of IRS proteins, which in turn decreases IRS-tyrosine phosphorylation, impairing downstream effectors. pY: phosphorylated tyrosine; IR: insulin receptor; IRS: insulin receptor protein; PI3-k: phosphatidylinositol 3-kinase; PDK1: phosphoinositidedependent kinase 1; PKB: protein kinase B; Foxo-1: forkhead box protein O; PEPCK: phosphoenolpyruvate carboxykinase; G6Pase: glucose-6-phosphatase; FFA: free fatty acids; PIP2: phosphatidyl-inositol-3,4-bisphosphate; PIP3: phosphatidyl-inositol-3,4,5-tris-phosp-hate; GLUT4: glucose transporter 4.

Table 1 Causes of insulin receptor substrate-1 serine phosphorylation
mTOR
p70S6 kinase
Amino acids
Hyperinsulinemia
JNK
Stress
Hyperlipidemia
Inflammation
IKK
Inflammation
TNF-α
Obesity
Inflammation
Mitochondrial dysfunction
PKCθ
Hyperglycemia
Diacylglycerol
Inflammation

mTOR: molecular target of rapamycin; JNK: c-Jun N-terminal kinase; IKK: IκB kinase; TNF: tumour necrosis factor; PKC: protein kinase C.

FORKHEAD BOX PROTEIN O-1

The fasting hyperglycemia in patients with type 2 diabetes is the clinical correlate of the increased glucose production by the liver because of insulin resistance. This is the result of the lack of inhibition of the two key gluconeogenic enzymes, PEPCK and the glucose-6-phosphatase (G6Pase) catalytic subunit. There is increasing evidence that Foxo-proteins are critically involved in the insulin dependent regulation of gluconeogenic gene expression and insulin-resistance *in vivo*[38,39]. Studies in hepatoma cells[40,41] suggest that transcription of reporter genes containing insulin response elements from the PEPCK and G6Pase promoters are regulated by forkhead box protein o (Foxo)-1 and 3. Furthermore, Foxo1 is phosphorylated in an insulin-responsive manner by Akt. Reduced activity of Akt2 results in decreased phosphorylation of Foxo protein, allowing it to enter the nucleus and activate the transcription of these rate-controlling enzymes of gluconeogenesis[40,42].

PI3 KINASE

A molecular mechanism that may potentially lead to insulin resistance is a disruption in the balance between the amounts of the PI3-kinase subunits[43]. The PI3-kinase family is divided into three different classes, of which class 1a[44] exists as heterodimers, consisting of a regulatory subunit p85, which is tightly associated

with a catalytic subunit, p110. Normally, the regulatory subunit exists in stoichiometric excess to the catalytic one, resulting in a pool of free p85 monomers not associated with the p110 catalytic subunit. However, there exists a balance between the free p85 monomer and the p85-p110 heterodimer, with the latter being responsible for the PI3-kinase activity[45,46]. Because the p85 monomer and the p85-p110 heterodimer compete for the same binding sites on the tyrosine-phosphorylated IRS proteins, an imbalance could cause either increased or decreased PI3-kinase activity[47]. This is recently suggested by studies which showed that human placental growth hormone causes severe insulin resistance by specifically increasing the expression of the p85α subunit and subsequently affecting the ability of insulin to stimulate the association of the p85-p110 heterodimer with IRS-1, thus reducing the PI3-kinase insulin signaling[48]. Other studies in insulin-resistant states induced by obesity, type 2 diabetes[49] and short-term overfeeding of lean nondiabetic women[50] have also supported these findings. Additionally, Barbour and colleagues[51] have demonstrated that pregnancy-induced insulin resistance is probably due to increased expression of skeletal muscle p85 in response to increased concentrations of the human placental growth hormone. Furthermore, women who remain insulin resistant postpartum have been found to display higher levels of p85 in their muscle tissue[52].

PKC

The underlying mechanism of FFA-induced impairment of insulin signals is still unclear. The molecular mechanism underlying defective insulin-stimulated glucose transport activity can be attributed to increases in intramyocellular lipid metabolites such as fatty acyl CoAs and diacylglycerol, which in turn activate a serine/threonine kinase cascade, thus leading to defects in insulin signaling through the Ser/Thr phosphorylation of the insulin receptor substrate-1[53]. Diacylglycerol (DAG) has been shown to increase in muscle during both lipid infusions and fat feeding and it is also a known activator of novel PKC isoforms[53]. Some of the PKC isoforms represent such signaling molecules. PKC isoforms are classified as classical (cPKCα, βI, βII, γ), novel (nPKC δ, ε, θ, η) or atypical (aPKC ζ, λ). cPKCs are activated by Ca^{2+} and DAG, nPKCs are activated only by DAG and aPKCs respond to neither Ca^{2+} nor DAG[54]. Among all these PKC isoforms, nPKCs are said to have a modulatory role in insulin signaling. Recent reports also demonstrate a link between nPKCs and FFA induced insulin resistance; lipid infusion in rats and humans impaired insulin-stimulated glucose disposal into the muscle and concomitantly activated PKCθ and PKCδ[55,56]. The latter has been shown to be a possible candidate for phosphorylation of the IR on serine residues[57], resulting in defects in the insulin signaling pathway and imposing insulin resistance.

Clearly, the IR is one of the major targets in FFA-induced impairment of insulin activity. Studies performed *in vivo* have suggested that glucose uptake rather than intracellular glucose metabolism is the rate-limiting step for fatty acid-induced insulin resistance in humans[58]. This indicates a mechanism in which accumulation of intracellular fatty acids or their metabolites results in an impairment of signaling through the IRS/PI3-kinase and a decrease in the recruitment of GLUT4 transporters to the cell membrane.

PDK1 can directly phosphorylate all PKCs including nPKCs[59]. The PKCε isotype has been shown to be related to insulin resistance. PKCε has also shown PDK1-independent phosphorylation due to FFA[60,61]. This may be due to constitutive phosphorylation of PKCε by FFA in a PDK1-independent manner. It was shown that myristic acid incubation of HEPG2 cells causes myristoylation of PKCε which results in constitutive phosphorylation of PKCε at thr566/ser729 in the kinase domain required for PKCε activity. This phosphorylation was totally independent of PDK1, which was demonstrated by the researchers by using PDK1 knockout cells. In the same way, addition of palmitate to skeletal muscle cells or adipocytes may affect palmitoylation of PKCε, resulting in constitutive phosphorylation of PKCε[60,61]. Taken together, it is clear that FFA causes PDK1-independent phosphorylation of PKCε, which in turn translocates to the nucleus, and its time of entry into the nucleus coincides with the inhibition of IR gene transcription.

MOLECULAR MECHANISM OF INHIBITION OF IR GENE TRANSCRIPTION

In order to understand the molecular basis of the regulation of IR gene expression, the promoter region of the human IR gene has been identified and studied by several groups[62]. Two unique AT-rich sequences, C2 and E3, within the IR gene promoter have been identified, and both these sequences are positively regulated by transcription factor *HMGA1* (earlier known as *HMG1-Y*)[63]. *HMGA1* interacts with the AT rich regions and regulates transcriptional activation of many genes by modifying DNA conformation, which permits recruitment of transcriptional factor to the transcription start site[64,65]. *HMGA1* induces transcriptional activation of the human IR gene by permitting the recruitment of *SP1* and *cEBPβ*, the ubiquitously expressed transcription factors, to the promoter region. A recent report demonstrates that a genetic flaw which reduces the intracellular expression of *HMGA1* protein can adversely affect IR expression in cells and tissues from subjects with insulin resistance and type 2 diabetes[66]. There is also a possibility that activated PKCε phosphorylates *HMGA1*, which inhibits its mobilization to the promoter region IR gene. It has been shown that phosphorylation of the HMGA1 protein reduces its DNA-binding ability[67]. Without the mobilization of *HMGA1* to the IR promoter there is no recruitment of additional transcription factors to the promoter region of the IR gene and therefore no expression of the IR gene.

PGC-1

The PPARγ co-activator-1 (PGC-1) has been recognized as playing a major role in glucose homeostasis of the organism. Work mainly by Spiegelman's group demonstrated the crucial role of PGC-1 in the regulation of GLUT4 gene expression in muscle cells. They showed that PGC-1 powerfully induces the expression of the endogenous GLUT4 gene in cultured myotubes, resulting in expression comparable to that seen in muscle *in vivo*[68]. In addition, PGC-1, a factor integrating the effects of glucocorticoids and cAMP on gluconeogenic gene expression in the liver[69,70] is also regulated by Akt and Foxo-1[71]. PGC-1 may also play a role in the regulation of genes involved in the process of oxidative phosphorylation which commonly show reduced expression in the muscles of diabetic patients[72].

OTHER CAUSES OF INSULIN RESISTANCE

Mitochondrial dysfunction

It has been known for many years that severe mitochondrial dysfunction can result in diabetes[73]. In a study using $^{13}C/^{31}P$ MRS, it was found that in healthy lean elderly volunteers with severe muscle insulin resistance, there is ~40% reduction in the rates of oxidative phosphorylation activity associated with increased intramyocellular and intrahepatic lipid content[74]. This study suggests that an acquired loss of mitochondrial function associated with aging predisposes elderly subjects to intramyocellular lipid accumulation, which results in insulin resistance[53]. Further, it was found that mitochondrial density was reduced by 38%, intramyocellular lipid content was increased by 60% and serine phosphorylation of IRS-1 was increased by 50% in the young insulin-resistant offspring of type 2 diabetes parents[32].

Adipokines

Insulin has three major target tissues-skeletal muscle, adipose tissue and the liver. Not only is IR overexpressed in the cells of these tissues, but these are also the three places where glucose is deposited and stored; no other tissue can store glucose. About 75% of insulin-dependent postprandial glucose disposal occurs into the skeletal muscle[75]; it is therefore the major target organ. Patients suffering from insulin resistance and type 2 diabetes frequently display signs of abnormal lipid metabolism, increased circulatory concentration and elevated deposition of lipids in the skeletal muscle[76]. Increase in plasma FFA reduces insulin-stimulated glucose uptake, whereas a decrease in plasma lipid content improves insulin activity in the skeletal muscle cells, adipocytes and liver[77]. Studies have shown that raising plasma fatty acids in both rodents[78] and humans[79] abolishes insulin activation of IRS-1-associated PI3-kinase activity in skeletal muscle where IRS-1 is most prevalent. Lipid-associated insulin

resistance has also been shown to be linked to GLUT4 translocation defects[9].

Adipose tissue also acts as an endocrine organ producing adipokines which modulate glucose homeostasis[80]. Currently, those most intensely discussed are TNF-α, leptin, adiponectin and resistin. At a molecular level, TNF-α increases serine phosphorylation of IRS-1 and down-regulates GLUT4 expression, thereby contributing to insulin resistance[81]. Furthermore, mice lacking functional TNF-α were protected from obesity-induced insulin resistance[82]. The role of leptin in regulating food intake and energy expenditure is well established. Humans with leptin deficiency or leptin receptor mutations are severely obese[83,84]. In addition, it has direct effects on insulin sensitivity and may also reverse insulin resistance in mice with congenital lipodystrophy[85]. Adiponectin has insulin-sensitizing effects, as it enhances inhibition of hepatic glucose output as well as glucose uptake and utilization in fat and muscle. The expression of adiponectin is decreased in obese humans and mice[86]. Thus, in humans, adiponectin levels correlate with insulin sensitivity. Because of its insulin-antagonistic effects, the adipokine resistin has attracted a lot of primarily preclinical research interest. Resistin decreases insulin-dependent glucose transport *in vitro* and increases fasting blood glucose concentrations and hepatic glucose production *in vivo*[87,88].

CONCLUSION

In this review, we have summarized current developments contributing to our understanding of insulin resistance, and to the pathogenesis of type 2 diabetes. Among the many molecules involved in the intracellular processing of the signal provided by insulin, IRS-2, PKB, the Foxo protein and p85 regulatory subunit of PI-3 kinase have attracted particular interest, because their dysfunction results in insulin resistance *in vivo*. The identification of signaling defects and an understanding of the complex relationship of the different factors modulating insulin sensitivity is an important prerequisite for the development of novel and more specific anti-diabetic compounds. By elucidating the cellular and molecular mechanisms responsible for insulin resistance, these studies provide potential new targets for the treatment and prevention of type 2 diabetes.

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Metabolic effects of obesity: A review

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Abstract

With the many recent advances in the biomedical world, vast changes are taking place in our growing knowledge of the physiological aspects of almost all the tissues and organs of the human body. One of the most prevalent topics of discussion is the question of obesity and its effect on the metabolic changes in the human body. The original classical role of adipose tissue as an energy storage organ has been greatly modified. We now know that it is an endocrine organ, producing adipokines like leptin, adiponectin, visfatin, resistin, apelin, *etc*, which modulate metabolic processes in the body. Since obesity is associated with an increase in the adipose tissue mass, these hormones may be expected to be produced in increased concentrations and may thus have a significant impact on the macronutrient metabolism. Further, these adipokines may interact with long term energy modulators like insulin. Even though the scientific community has started unravelling the mysteries of the close linkage between obesity, its hormones and their physiological effects, a lot still remains to be discovered. The present discussion makes an attempt to trace the basic modern day concepts of the role of obesity in various metabolic processes.

INTRODUCTION

Genetic predisposition is a key contributing factor in obesity, as has been demonstrated by familial aggregation, twin and adoption studies[1,2]. Estimates for a genetic basis for obesity range from approximately 40% to 70%. The idea that genetic loci alter body fat content has been substantiated by the identification of mutations that cause low- or high-fat phenotypes in rodents and humans[3]. Obesity comes about when energy intake, principally stored as triglycerides, exceeds energy expenditure[4]. Obesity is a complex trait influenced by diet, developmental stage, age, physical activity and genes[5]. Many recent epidemiological studies have documented the rapid increase in the prevalence of obesity. According to data from the Center for Disease Control Behavioural Risk Factor Surveillance System, in the United States the prevalence of obesity [body mass index (BMI) > 30 kg/m^2] has increased from < 20% a decade earlier to 30% in 2006[6]. Along with the increase in obesity there is a parallel increase in the prevalence of type 2 diabetes, impaired glucose tolerance[7,8], and other complications of obesity, such as hypertension, sleep apnoea, and arthritis. A recent study

has suggested that, due to the increase in obesity, future life expectancy may even decrease[9].

REGULATION OF ENERGY HOMEOSTASIS AND OBESITY

Obesity is characterized by an excess of adipose tissue. The increase of food intake (hyperphagia) triggered by a period of fasting is a simple but compelling example of food-intake regulation. The balance between energy intake (food consumption) and energy expenditure (basal metabolic rate, i.e. biochemical processes required to maintain cellular viability, physical activity and adaptive thermogenesis) is tightly regulated. A homeostatic network maintains energy stores through a complex interplay between the feeding regulatory centres in the central nervous system (CNS), particularly in the hypothalamus and the regulated storage and mobilization of fat stores that maintains the body energy stores[10,11]. Thus, genes that encode the molecular components of this system may underlie obesity and related disorders. A number of recent research groups have encoded the molecular and genetic mysteries that underlie obesity and its related disorders.

Signalling pathways in the hypothalamus

The hypothalamus is the major nervous centre controlling food intake. It has two major areas which play important role in maintaining the normal energy homeostasis of the body by controlling the hunger and satiety centres. The ventro-medial hypothalamic nucleus (VMN), a portion of the hypothalamus, is known as the 'satiety centre'. Stimulation of the VMN causes suppression of food intake, whereas a bilateral VMN lesion induces hyperphagia and obesity. The lateral hypothalamic area is known as the 'hunger centre', and its stimulation or any lesion induces the opposite set of responses[12,13]. Various neuropeptides (e.g. the melanocortin system, neuropeptide Y) and neurotransmitters (e.g. serotonin, dopamine and noradrenaline) along with insulin and leptin molecules function in the hypothalamus and thus coordinate the behavioural, physiological and metabolic responses. These response elements maintain the energy balance *via* both the intake and the expenditure pathways[14].

In addition to these long-term adiposity signals, short-term meal-related signals are also transmitted to the CNS through afferent nerves or gut-secreted peptides (e.g. cholecystokinin, ghrelin[15]). Neurons in the CNS also directly sense carbohydrates and fats[16,17].

Melanocortins

Melanocortins are peptides that are cleaved from the proopiomelanocortin precursor molecule, and thus exert their effects by binding to members of a family of melanocortin receptors[18].

Melanocortins also promote negative energy balance. Among the growing list of melanocortins, the signifi-

cant ones for these purposes are the α-melanocyte-sti mulating hormone[19], the corticotropin-releasing hormone[20], the thyrotropin-releasing hormone[21], cocaine and the amphetamine-regulated transcript[22] along with interleukin-1β. Neuronal synthesis of these peptides increases in response to increased adiposity signalling in the brain[23].

The role of melanocortin signalling in the control of energy homeostasis first emerged after the cloning of the MC3- and MC4-receptor genes, and the discovery that they are expressed primarily in the brain[24]. This discovery was followed by evidence that a synthetic agonist of these receptors suppresses food intake whereas a synthetic antagonist leads to the opposite effect[25].

Neuropeptide effectors

Neuropeptide Y (NPY) is a prominent orixgenic molecule and belongs to the class of anabolic effector pathways. Experimental studies have shown that repeated injection of NPY into cerebral ventricles or directly into the hypothalamus stimulates food intake, decreases the total body energy expenditure and leads to obesity[26-29]. It also induces lipogenic enzymes in the liver and white adipose tissue[27]. NPY gene expression and secretion of the NPY peptide in the hypothalamus are increased during active depletion of body fat stores[30,31] and leptin inhibits arcuate nucleus NPY gene expression[32,33]. NPY meets the criteria for an anabolic signalling molecule. Moreover, the Agouti-related protein (AGRP), orexin (also known as 'hypocretin') and the melanin-concentrating hormone (MCH) have all been added to the list of candidate anabolic effector signalling molecules.

Ghrelin

Ghrelin, a peptide hormone secreted from the stomach, is now known to have a potent appetite-stimulating activity. It has also been suggested that the primary location for the orexigenic activity of ghrelin is through the neuropeptide Y/Agouti-related peptide (NPY/AGRP) neurons within the arcuate nucleus of the hypothalamus[31]. Recently, it has been shown that area postrema, a caudal brain stem center that lacks a blood-brain barrier, is a key site of activity for ghrelin in stimulating appetite and regulating pancreatic protein secretion[32]. Ghrelin is suggested to play a role in long-term regulation of energy balance, as chronic administration of ghrelin causes weight gain by reducing fat utilization as an energy source[33]. Circulating levels of ghrelin are increased in the fasting state and in anticipation of food, and are attenuated by feeding and the presence of nutrients in the stomach. Ghrelin also plays a role in the digestion of food and the stimulation of gastric motility, acid secretion, and pancreatic protein secretion .

ROLE OF ADIPOSE TISSUE AS AN ENDOCRINE ORGAN

Adipose tissue is a major endocrine organ, producing

various hormones that regulate body metabolism. An increase in the fat cell mass leads to imbalances in its release of hormones, which can have various metabolic effects. The metabolic complications of obesity, often referred to as the metabolic syndrome, consist of insulin resistance, often culminating in β-cell failure, impaired glucose tolerance and type 2 diabetes, dyslipidemia, hypertension, and premature heart disease. Abdominal obesity, ectopic lipid accumulation, hepatic steatosis, and sleep apnea can also be included in the metabolic complications of obesity[34].

In mammals, white adipose tissue functions as the main depot for fuel storage. In the past decade, identification of myriad lipid and protein signals secreted from this tissue has led to its recognition as a major endocrine organ[35,36]. Adipocytes secrete a variety of biologically active molecules, termed as adipocytokines[37]. Adipose tissue has been found to be an important source of various hormones. Of these, the hormones which play an important role in body weight regulation are mainly leptin, visfatin, apelin, resistin, and adiponectin.

ROLE OF LEPTIN IN BODY WEIGHT REGULATION

Leptin

Leptin, the 167 amino acid protein, is a cytokine-like hormone secreted from white adipose tissue. It was the first adipocytokine identified, encoded by the *ob* gene. Leptin receptors are expressed in a number of different tissues. Adipocytes have been identified as the primary site for leptin expression, however it is also expressed in the gastric wall, vascular wall, placenta , ovary , in skeletal muscle, and the liver[38-41]. Leptin has several roles, including growth control, metabolic control, immune regulation, insulin sensitivity regulation, and reproduction[42-44]. However, its most important role is in body weight regulation.

Leptin and insulin

The mechanisms involved in leptin secretion are all quite different. The rate of insulin-stimulated glucose utilization in adipocytes is a key factor linking leptin secretion to body fat mass[45]. Although the mechanism is incompletely understood, it may involve glucose flux through the hexosamine pathway[46]. In addition, various observations indicate that leptin has a more important role than insulin in the CNS control of energy homeostasis. Insulin is secreted from the endocrine pancreas and exerts potent effects on peripheral nutrient storage. Insulin is an afferent signal to the CNS that causes long-term inhibitory effects on energy intake. Leptin receptors and insulin receptors are expressed by brain neurons involved in energy intake[47-49], and administration of either peptide directly into the brain reduces food intake[50-52] whereas deficiency of either hormone does the opposite[53-54]. Leptin deficiency causes severe obesity, with hyperphagia

that persists despite high insulin levels. In contrast, obesity is not induced by insulin deficiency. However, such comparisons are complicated by the critical role that insulin has in promoting both fat storage and leptin synthesis by fat cells.

Leptin and obesity

Leptin is the chief regulator of the "brain gut axis", which provides a satiety signal through its action on the CNS receptors within the hypothalamus[41,55] Activation of hypothalamic leptin receptors suppresses food intake and promotes energy expenditure pathways[56]. Leptin levels decrease with weight reduction.

The hypothesis that leptin resistance can occur in association with obesity was first suggested by the finding of elevated plasma leptin levels in obese humans[57]. This hypothesis suggests that some cases of human obesity may be due to reduced leptin action in the brain, and affected individuals are unlikely to respond to pharmacological treatment with leptin. Several mechanisms contribute to leptin resistance.

Leptin uptake into the brain is facilitated by leptin receptors expressed by endothelial cells[58] in the blood-brain barrier that function as leptin transporters. Impaired leptin transport across endothelial cells of the blood-brain barrier is one potential mechanism leading to leptin resistance. Whether dysfunction of this transport process can lead to obesity remains to be determined, but it has been seen that in obese humans cerebrospinal fluids demonstrate low levels of leptin in comparison to plasma[59].

Upon activation of leptin receptors in the brain, a series of integrated neuronal responses required for food intake and energy balance are activated, and these neuronal effector pathways play a key role in energy homeostasis. Failure of one or more of these pathways in response to the leptin signalling will manifest as leptin resistance[60].

Reduced leptin-receptor signal transduction is another potential cause of leptin resistance. Like other cytokine receptors, activation of the leptin receptor induces expression of a protein that inhibits any further leptin signal transduction, termed 'suppressor of cytokine signalling-3' (SOCS-3)[61]. The potential contribution of SOCS-3 to acquired forms of leptin resistance and obesity is an active area of study.

Leptin and inflammation

The role of Leptin in inflammation can be summarized as: (1) Pro-inflammatory; (2) Increase in T cell activation, and cytokine release proliferation; (3) Promotes Th1 response; (4) Increases NK cell activation; (5) Increases macrophage activation and cytokine release [tumor necrosis factor (TNF)-α/interleukin (IL-6) *etc*]; and (6) Activates neutrophils and increases their chemotaxis and oxidative burst.

Leptin acts on the monocytes and induces the release of cytokines such as TNF-α or IL-6 as well as CCL2 and VEGF[58]. It leads to increased proliferation and

differentiation of the monocytes. Acting on the neutrophils, leptin leads to an increased expression of CD 11b, as well as increased neutrophil chemotaxis and oxidative burst[58,62,63], all of which are very important in innate immune responses and regulation of pathogen colonization of the skin and mucosa[64].

Visfatin

Visfatin is also known as pre-B cell colony enhancing factor (PBEF). Visfatin also possess nicotinamide phos phoribosyltransferase (Nampt) activity. It is produced by the visceral adipose tissue. The expression of visfatin is increased in abdominal obesity and type 2 DM. Visfatin binds to the insulin receptors at a site distinct from insulin and mimics insulin in exerting a hypoglycemic effect by reducing glucose release from the hepatocytes, and stimulating the glucose utilization in the peripheral tissues[65].

However, recent studies indicate the association of visfatin with obesity alone and make its metabolic role debatable. Revello *et al* demonstrated that the extracellular form of Nampt (eNampt/Visfatin/PBEF) secreted through the non-classical secretory pathway had nicotinamide adenine dinucleotide (NAD) biosynthetic activity. Haplodeficiency and chemical inhibition of Nampt re sulted in decreased NAD biosynthesis and glucose-stimulated insulin secretion in pancreatic islets *in vitro* and *in vivo*. It has been suggested that supplementation of nicotinamide mononucleotide, a Nampt reaction product, results in an amelioration of these defects. Revello and his co-workers also demonstrated that visfatin does not mimic insulin[66].

Apelin

Apelin is an adipocytokine whose plasma concentration is increased in obesity, insulin resistance and hyperinsulinemia[66]. In the cardiovascular system, apelin elicits endothelium dependent, nitric oxide mediated vasorelaxation and reduces arterial blood pressure[67], along with a positive inotropic activity.

Resistin

Resistin is thus named because it renders resistance to the action of insulin[68]. It is made up of 114 amino acids. It has been observed that circulating resistin levels are increased in obese humans. It is considered a pro-inflammatory molecule. It activates NFκB-dependent cytokine release and adhesion molecule expression including TNF-α and IL-6. It also plays an important role in the pathogenesis of diabetes and its complications. The release of resistin is often associated with stimulation by the inflammatory process, IL-6, hyperglycemia and hormones like the growth hormone and the gonadal hormones. The role of resistin in obesity and insulin resistance in humans is controversial[69].

Adiponectin

Adiponectin is an important adipocytokine, present within cells and in the circulation, in multimeric forms: trimers, hexamers, high-MW oligomers and full-length adiponectin multimers (fAd). The fAd may cleave to liberate a fragment containing the C-terminal globular domain (gAd), which plays an important role in adipose tissue metabolism[70]. Adiponectin oligomers act *via* two receptor subtypes (AdipoR1 and AdipoR2), the stimulation of which results in increased AMP-activated protein kinase (AMP-kinase), PPAR-α ligand activities and activation of a NF-κB signaling pathway[71-73].

Adiponectin has the following metabolic functions in the body: (1)Enhances hepatic insulin actions and suppresses fatty acid influx into the liver[74,75]; (2) Enhances glucose uptake in the liver and skeletal muscles[71]; and (3) Increases fatty acid oxidation[76].

The main difference between adiponectin and the other hormones is that, whereas the other hormones are related to insulin resistance and are increased in obesity, adiponectin production and concentration decreases in obesity[77].

Combined efforts of various researchers have led to the discovery that the adiponectin levels in humans is less in obese individuals than in the lean subjects[78]. In another recent study it has been observed that plasma MMW and LMW adiponectin levels decrease in Type 2 diabetics as compared to the non-diabetic individuals[79]. Various other studies have demonstrated the inverse relationship between plasma adiponectin and serum triglyceride levels as well as fasting and post-prandial plasma glucose concentrations.

Adiponectin and inflammation: The role of adiponectin has been defined beneficial to the body. (1) It is anti-inflammatory[80]; (2) It decreases T cell activation and proliferation; (3) It inhibits NFkB dependent cytokine release and molecule expression including TNF-α/IL-6[81]; (4) It increases IL-10; and (5) It inhibits phagocytosis oxidative burst.

In obesity, concentrations of inflammatory mediators like TNF-α and IL-6 increases. This leads to a decrease in adiponectin expression and release. The main function of adiponectin in an immune metabolism is *via* the NFκB pathway[73]. In the immune system, adiponectin inhibits T cell activation and proliferation.

Adiponectin also inhibits B-cell lymphopoiesis[82]. Adi ponectin induces the production of the anti-inflammatory mediators IL-10 in human monocytes, monocyte-derived macrophages, and dendritic cells. In addition, adiponectin significantly impairs the production of the proinflammatory cytokine IFN-γ. Moreover, adiponectin-treated macrophages exhibit reduced phagocytic capacity[83].

Adiponectin and cardiovascular function: Adiponectin has been shown to have various vasculoprotective effects. In obesity, the adiponectin level decreases and it leads to an increase in cardiovascular risk.

Various studies have made an effort to correlate this

adipocytokine to the plasma lipoprotein concentration and its implication on CAD[84-86] but without any conclusive results.

The beneficial role of adiponectin on the cardiovascular system might be related to the following factors: (1) Adipo-vascular axis: It proposes the mechanism of adiponectin induced vascular protection *via* the epidermal growth factor and other endothelial growth factors by augmenting endothelial cell proliferation[87]; and (2) Adiponectin also decreases human aortic smooth muscle cell growth and migration response to growth factors[88].

Adiponectin and the hepatic system: In a recent study on Japanese subjects it was seen that adiponectin concentrations are lower in subjects with increased transaminase activities, indicating that hypoadiponectinemia contributes to an increase in transaminase activity. This may signify that liver diseases could be worsened if associated with metabolic diseases[89]. Moreover, it has been observed that high levels of adiponectin can protect against steato hepatitis[90]: (1) Hypoadiponectinemia→Insulin resistance + Hyperlipidemia→Fatty Liver, and (2) Hypoadiponectinemia→Increase hepatic fatty acid influx→Fatty Liver.

Adiponectin and bone: In recent studies, it has been observed that adiponectin and its receptors are also expressed in osteoblasts[91-93]. Further studies suggest that adiponectin stimulates the proliferation, differentiation and mineralization of osteoblasts *via* the AdipoR1 and AMP kinase signaling pathways in autocrine and/or paracrine fashions[94]. It has been found that the suppression of AdipoR1 expression by its siRNA inhibited the differentiation and mineralization of the cells and that adiponectin promoted these processes through the action of AdipoR1 on the osteoblasts. Recent studies have also shown that adiponectin promotes osteoblast proliferation[91-93] and exerts an enhancing action on human osteoblast differentiation and mineralization[93].

However, despite intensive research being carried out on adiponectin, various issues still remain to be explored further such as the complete identification of the adiponectin receptor and the exact mechanism of adiponectin action.

Acute-phase serum amyloid A: Circulating acute-phase serum amyloid A (A-SAA) levels are elevated in obese individuals as compared to lean[95,96], and the expression of A-SAA is strictly correlated with the BMI and fat cell size. It is a pro-inflammatory and lipolytic adipokine in humans. A-SAA may act locally to alter cytokine production and fat metabolism, as well as systemically on liver, muscle, and cells of the immune metabolism and the vasculature, to impact insulin resistance and atherosclerosis. Research on A-SAA has shown that the increased expression of A-SAA by adipocytes in obesity suggests that it may play a critical role in local and systemic inflammation and free fatty acid production and could be a direct link between obesity and its co-morbities, such as insulin resistance and atherosclerosis[97].

The signaling pathays of the A-SAA mediated inflammation response are not well studied. However, in neutrophils, SAA has been found to induce IL-8 production through the formyl peptide receptor like 1/lipoxin A4 receptor and activates nuclear factor kappa B[98]. The same signaling pathways has recently been shown to be an important mediator of inflammation associated with insulin resistance[99,100].

Since energy balance involves this complex interplay between multiple tissues and signaling pathways, an integrated view of feeding behavior, neuroendocrine signaling, nutrient uptake, transport, storage and utilization is required for understanding fat regulation.

EFFECTS OF OBESITY ON THE MACRONUTRIENT METABOLISM ARE MAINLY MEDIATED BY INSULIN RESISTANCE

Several adipokines like Leptin[101], adiponectin[102] and visfatin[103] have been shown to stimulate insulin sensitivity. On the other hand, resistin[104] and the retinol binding protein[105] induce insulin resistance. Adiponectin is proposed to be essential in insulin sensitivity[106]. In obesity there is a decrease in the Adipo R expression levels, thereby reducing adiponectin sensitivity and enhancing insulin resistance[107].

The term "insulin resistance" is defined as resistance to the effects of insulin on glucose uptake, metabolism, or storage. Insulin resistance in obesity is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle, and by impaired suppression of hepatic glucose output[108].

Insulin is a critical regulator of virtually all aspects of adipocyte biology, and adipocytes are one of the most highly insulin-responsive cell types. The physiological role of insulin includes the metabolism of all 3 macronutrients (carbohydrates, lipids, and proteins) as well as cellular growth. Insulin's action on lipid metabolism is analogous to its role in glucose metabolism, i.e. promoting anabolism and inhibiting catabolism. Insulin stimulates glucose transport and triglyceride synthesis (lipogenesis), as well as inhibiting lipolysis[109]. Specifically, insulin upregulates LPL and stimulates gene expression of intracellular lipogenic enzymes, such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). In addition, insulin inhibits adipocyte HSL through inhibition of its phosphorylation[110,111]. It has also been demonstrated that insulin influences the secretion of proteins from mature adipocytes probably by increasing the production of enzymes necessary for the processing of secreted protein precursors[112].

Mechanism of insulin resistance
Insulin's metabolic effects are mediated by a broad array of tissue-specific actions that involve rapid changes in protein phosphorylation and function, as well as changes

in gene expression. The initial molecular signal for insulin action involves activation of the insulin receptor tyrosine kinase, which results in phosphorylation of insulin receptor substrates (IRSs) on multiple tyrosine residues. These phosphotyrosine residues act as docking sites for many SH2 domain-containing proteins, including the p85 regulatory subunit of phosphoinositide 3' kinase (PI3K). Binding of the p110 catalytic subunit of PI3K to p85 activates the lipid kinase that promotes glucose transport[113].

Insulin action in adipocytes also involves changes in gene transcription. The transcription factor ADD-1/SREBP-1c (adipocyte determination and differentiation factor-1/sterol regulatory element-binding protein-1c) may play a critical role in the actions of insulin to regulate adipocyte gene expression[114-116], by inducing genes involved in lipogenesis and repressing those involved in fatty acid oxidation.

Functional defects in insulin resistance may be due to impaired insulin signalling in all three target tissues i.e. in adipose tissue, skeletal muscle and liver. In both muscle and adipocytes, insulin binding to its receptor, receptor phosphorylation and tyrosine kinase activity, and phosphorylation of IRSs are reduced. Recent studies have indicated that defective signaling from the insulin receptor is an important component of insulin resistance associated with obesity in humans.

There are also tissue-specific alterations observed in adipocytes of obese humans, IRS-1 expression is reduced, resulting in decreased IRS-1-associated PI3K activity, and IRS-2 becomes the main docking protein for PI3K[117]. In contrast, in skeletal muscle of obese individuals, IRS-1 and IRS-2 protein levels are normal but PI3K activity associated with both IRSs are impaired[118].

Mechanism for signaling defects in insulin pathways in obesity

It has been suggested that there is increased expression and activity of several protein tyrosine phosphatases (PTPs), which dephosphorylate and thus terminate signalling propagated through tyrosyl phosphorylation events. Some data indicate that there is increased expression and/or activity of the three PTPs i.e. PTP1B, leukocyte antigen-related phosphatase (LAR), and src-homology-phosphatase 2, in the muscle and adipose tissue of obese humans[119]. PTP1B and LAR have been shown to dephosphorylate the insulin receptor and IRS-1 *in vitro*[120,121]. PTP1B not only has a regulatory role in insulin action, but also has a role in energy homeostasis.

Role of TNF-α in insulin resistance in obesity

TNF-α is a pluripotent cytokine produced from macrophages[122]. Fat tissue is a significant source of endogenous TNF-α production and the expression of this cytokine in adipose tissue is elevated in obesity[123]. This abnormal expression of TNF-α in adipose tissue plays a critical role in peripheral insulin resistance in obesity. It has been studied that neutralization of TNF-α in obese and insulin-resistant animals results in significant increases in peripheral insulin sensitivity[124]. Various studies have demonstrated that TNF-α induces insulin resistance through its ability to inhibit intracellular signalling through serine phosphorylation of IRS-1, and can reduce GLUT4 expression. Moreover, this inhibition can be reversed by neutralizing TNF-α *in vivo*[125,126]. The increased expression of TNF-α is significantly correlated with the hyperinsulinemia in the presence of normoglycemia. It has been demonstrated as a marker of insulin resistance[127]. In addition to its role in host defense, TNF-α also has important effects on whole body lipid and glucose metabolism[128].

Effect of obesity on carbohydrate metabolism

The action of insulin in lowering blood glucose levels results from the suppression of hepatic glucose production and the increased glucose uptake into muscle and adipose tissue *via* GLUT4. Muscle has long been considered the major site of insulin-stimulated glucose uptake *in vivo*, with adipose tissue contributing relatively little to total body glucose disposal. On the other hand, various transgenic studies have raised the possibility of a greater role for glucose uptake into fat in systemic glucose homeostasis. Over-expression of GLUT4 selectively in fat tissue enhances whole body insulin sensitivity and glucose tolerance[129], and knocking out GLUT4 selectively from fat tissue results in a degree of insulin resistance similar to that seen with muscle-specific knockout of GLUT4. In all forms of obesity, there is downregulation of GLUT4, a major factor contributing to the impaired insulin-stimulated glucose transport in adipocytes[130]. However, in the skeletal muscle of obese humans, GLUT4 expression is normal. It has also been suggested that defective glucose transport may be due to impaired translocation, docking, or fusion of GLUT4-containing vesicles with the plasma membrane[131]. With obesity there is reduced glucose disposal in adipose tissue. It has been suggested that obesity leads to the development of hyperglycemia[130], hyperlipemia[132], hyperinsulinemia[133], and insulin resistance[134]. Molecules like FFA, leptin, or TNF-α, all of which are released from adipose tissue, are known to affect glucose homeostasis indirectly. Undoubtedly there are other, as yet undiscovered, molecules from adipose tissue that influence systemic metabolism.

Effect of obesity on lipid metabolism

Obesity is associated with increased basal lipolysis in adipose tissue, and elevated circulating FFAs[135]. Acute-phase serum amyloid A (SAA), a lipolytic adipokine in humans, stimulates basal lipolysis. The lipolysis has been postulated to be an autocrine feedback mechanism by which increased SAA production from enlarged adipocytes A into the circulation may contribute to insulin resistance. The SAA act through the CLA-1 and the extra-cellular signal regulated kinase signaling pathway to stimulate lipolysis directly[136]. Alternatively, increased

lipolysis by SAA may be indirect, i.e. through the stimulation of the lipolytic cytokines viz IL-6 and TNF-α.

Plasma triglyceride (TG) concentration is another metabolic variable, most affected in obesity. It has been suggested that there is tissue resistance to insulin mediated glucose uptake, which in turn accelerates the very low density lipoprotein (VLDL), TG production rate and leads to endogenous hypertriglyceridemia[137-139]. In obesity there is decreased Lipoprotein lipase-mediated lipolysis of chylomicron-TG and ineffective inhibition of hormone-sensitive lipase-mediated lipolysis in adipose tissue[140]. Postprandial lipemia and elevated plasma FA levels are well-recognized abnormalities in obesity. Excess fatty acid availability early in the postprandial period (when it is normally suppressed by insulin) is estimated to influence glucose uptake by as much as 50%[141]. SAA has also a direct effect on cholesterol metabolism. Being an apolipoprotein by nature, it is the apoprotein of high-density lipoprotein (HDL)[142]. The inter-action of SAA with HDL may impair the function of HDL as an anti-atherogenic molecule[143] and facilitate its degradation[144]. The increase of adipose tissue derived SAA in obesity may be a link between obesity, low HDL and increased coronary Artery Disease risk.

Effect of obesity on protein metabolism

It is a well established fact that human obesity is accompanied by abnormalities in both glucose and lipid metabolism[145-149]. However, it is controversial whether protein metabolism is also disturbed in overweight individuals. Some researchers have reported that moderate obesity and difference in body fat distribution are associated with abnormalities in protein metabolism and have hypothesized that moderate obesity is associated with increased proteolysis, an increased rate of basal leucine turnover[150-152], and the impairment of insulin's antiproteolytic action , whereas others have found similar rates of basal leucine turnover in nonobese and obese subjects[153-156]. Conflicting reports also have appeared about the effect of insulin on protein anabolism. Some studies have indicated that the insulin resistance of obesity extends to protein metabolism[150,157], whereas other reports have challenged this conclusion[152,156].

ROLE OF OBESITY IN INFLAMMATION

Adipose tissue-derived proteins have been defined as adipokines, and have been implicated in the pathogenesis of chronic inflammation in obesity. The study of adipose tissue on inflammation was considerably impacted by the demonstration of resident macrophages in adipose tissue[158]. The possible mechanisms underlying the infiltration of macrophages into adipose tissue may be the chemokines by adipocytes, which would then attract resident macrophages[159]. Recent studies have suggested that macrophages infiltrate adipose tissue as part of a scavenger function in response to adipocyte necrosis[160]. The adipose tissue of obese humans contains an increa-

sed number of macrophages, and once activated, these macrophages secrete a host of cytokines, such as TNF-α, IL-6, and IL-1. The adipose tissue-resident macrophages are responsible for the expression of most of the tissue TNF-α and IL-6. The expression of macrophage markers in human adipose tissue was high in subjects with obesity and insulin resistance, and was also correlated with the expression of TNF-α and IL-6[161,162].

With obesity and progressive adipocyte enlargement, the blood supply to adipocytes may be reduced, and the induction of adipocyte hypoxia *in vitro* results in the expression of a number of inflammatory cytokines[163]. Obesity is associated with elevated levels of circulating proinflammatory cytokines such as plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP), TNF-α, and IL-6 and monocyte chemoattractant protein-1 (MCP-1)[164]. Many of these factors are produced by adipose tissue, such as circulating levels of TNF-α, IL-6, and MCP-1. Adipocytes express low levels of the MCP-1, and increased expression has been seen in obese subjects[165]. Adiponectin acts as a key regulator of adipocyte secretory function *via* its autocrine action, which correlates with adiposity and insulin resistance[166].

PAI-1 is elevated in subjects with metabolic complications of obesity, and is expressed in the stromal fraction of adipose tissue, including endothelial cells[167-169]. PAI-1 inhibits both the tissue-type plasminogen activator and the urokinase-type plasminogen activator through its serine protease inhibitor function, and this inhibition of fibrinolysis may contribute to a pro-thrombotic state[170].

The cell types involved in the inflammatory response in obesity are not fully delineated. Recent attention has focused on adipose tissue macrophages (ATMs) as a mediator of inflammatory responses in adipose tissue. In addition to the production of pro-inflammatory cytokines that promote metabolic complications, adipose tissue is the sole source of adiponectin, which is anti-inflammatory and associated with protection from atherosclerosis[171]. From an evolutionary perspective, adipose macrophages may have represented an important part of the host defense against injury or infection.

ROLE OF DIFFERENT FAT DEPOTS IN METABOLISM

Many studies have shown that excess fat in the upper part of the body, i.e. central or abdominal (android or male-type obesity) correlates with increased mortality and risk for disorders such as diabetes, hyperlipidemia, hypertension, and atherosclerosis of coronary, cerebral, and peripheral vessels more than the lower body or gluteo-femoral or peripheral depot (gynoid i.e. female-type of fat distribution)[172,173]. Abdominal fat is composed of abdominal subcutaneous fat and intraabdominal fat (which includes visceral or intraperitoneal fat). The visceral fat is associated with disturbances in insulin-glucose homeostasis, alterations in plasma lipoprotein-lipid levels[174], particularly increased plasma triglycerides and low HDL

cholesterol concentrations. These effects on the lipid profile may be due to the association of insulin resistance with disturbances in plasma lipid transport and lipoprotein levels[175]. Mobilization of FFAs is more rapid from visceral than from subcutaneous fat cells because of the higher lipolytic activity in visceral adipocytes compared to subcutaneous adipose tissue. This variation may be due to the increased expression and function of β-adrenoreceptors and a decreased insulin receptor affinity and signal transduction in visceral adipocytes[176,177]. This in turn results in a variation in the action of lipolysis-regulating hormones, catecholamines and insulin[176].

CONCLUSION

The newly identified function of the adipocytes has progressed from a simple energy storage tissue to a major endocrine system. The hormones secreted from adipose tissue influence energy homeostasis, glucose and lipid metabolism, vascular homeostasis, immune response, and reproductive functions. Newly discovered roles include the production of the cytokines IL-6, TNF-α, and leptin, which all play decisive roles in the development of obesity and insulin resistance. Thus, the enlargement of the adipose mass has pleiotropic effects on endocrine and metabolic events at whole body level that may contribute to the pathogenesis of the detrimental complications of obesity.

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Mechanisms of developmental programming of the metabolic syndrome and related disorders

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Abstract

There is consistent epidemiological evidence linking low birth weight, preterm birth and adverse fetal growth to an elevated risk of the metabolic syndrome (obesity, raised blood pressure, raised serum triglycerides, lowered serum high-density lipoprotein cholesterol and impaired glucose tolerance or insulin resistance) and related disorders. This "fetal or developmental origins/programming of disease" concept is now well accepted but the "programming" mechanisms remain poorly understood. We reviewed the major evidence, implications and limitations of current hypotheses in interpreting developmental programming and discuss future research directions. Major current hypotheses to interpret developmental programming include: (1) thrifty phenotype; (2) postnatal accelerated or catch-up growth; (3) glucocorticoid effects; (4) epigenetic changes; (5) oxidative stress; (6) prenatal hypoxia; (7) placental dysfunction; and (8) reduced stem cell number. Some hypothetical mechanisms (2, 4 and 8) could be driven by other upstream "driver" mechanisms. There is a lack of animal studies addressing multiple mechanisms simultaneously and a lack of strong evidence linking clinical outcomes to biomarkers of the proposed programming mechanisms in humans. There are needs for (1) experimental studies addressing multiple hypothetical mechanisms simultaneously; and (2) prospective pregnancy cohort studies linking biomarkers of the proposed mechanisms to clinical outcomes or surrogate biomarker endpoints. A better understanding of the programming mechanisms is a prerequisite for developing early life interventions to arrest the increasing epidemic of the metabolic syndrome, type 2 diabetes and other related disorders.

Key words: Fetal origins; Developmental programming mechanisms; Metabolic syndrome; Insulin resistance; Type 2 diabetes

INTRODUCTION

The metabolic syndrome is commonly defined as a combination of at least three of the following five con-

ditions: obesity, elevated blood pressure, elevated serum triglycerides, low serum high-density lipoprotein (HDL) cholesterol and impaired glucose tolerance or insulin resistance[1]. The clustering of these risk factors predisposes an individual to non-insulin-dependent (type 2) diabetes, cardiovascular morbidity and mortality. There is general consensus regarding the five components of the syndrome but definitions differ regarding cutoffs and mandatory criteria. Recently, central obesity [waist circumference > 102 cm in males or > 88 cm in females or body mass index (BMI) > 30] was proposed as a mandatory component by the International Diabetes Federation[2].

Epidemiological and experimental evidence suggest an association between an adverse prenatal environment and the risk of developing the metabolic syndrome and related disorders. This "fetal origins of disease" hypothesis was first proposed by Barker and Hales' group to explain the associations between low birth weight (LBW < 2500 grams) and increased risk of impaired glucose tolerance and cardiovascular disease in retrospective cohort studies[3-5]. Subsequent epidemiological studies in different populations largely confirmed this "fetal origins" phenomenon[3-8]. In recent years, the term "fetal origins/ programming" has been replaced by "developmental origins/programming" to accommodate the increasingly accepted concept that "programming" may continue in the early postnatal period.

A number of hypotheses have been proposed to interpret developmental programming[9-14] but none have received unanimous recognition. It is now worth reflecting on what is known about the mechanisms of developmental programming after decades of research. We critically reviewed the key evidence, implications and limitations of current hypotheses to interpret developmental programming of the metabolic syndrome and discuss the directions for future research. The evidence acquisition was based on a literature review based on a PubMed search of publications between January 1970 and February 2010.

MAJOR HYPOTHESES

We use the term "major hypotheses" to refer to those supported by substantial epidemiological and experimental evidence. Two competing major hypotheses have been proposed: "thrifty phenotype" and "postnatal accelerated growth".

Thrifty phenotype

Rationale: Hales and Barker proposed the thrifty phenotype hypothesis (Figure 1)[11,12]. The hypothesis suggests that fetal and early postnatal malnutrition may induce poor development of pancreatic β-cell mass. Malnutrition may have a selective impact on the growth of different organs with protection of the most vital (e.g. the brain). Because altered growth during critical periods permanently changes the structure and functional capacity of pancreatic β-cell mass, such changes may "program" the metabolic system

Figure 1 The thrifty phenotype hypothesis (reproduced with permission, Hales and Barker[12]).

which increases the fetus' chance of survival in poor nutritional environments but results in difficulty in coping with nutritional abundance later in life. The development of the metabolic syndrome following malnutrition in early life may depend on the superimposition of other postnatal risk factors, notably physical inactivity and obesity.

Epidemiological evidence: Barker and colleagues observed in 1986 that the geographical distribution of heart disease in the United Kingdom was closely related to a person's place of birth[15], suggesting that early life events could cause permanent changes in physiology predisposing to chronic heart disease. LBW has been strongly linked with impaired glucose tolerance and type 2 diabetes in adulthood[3-5,16-18]. Reduced fetal growth was related to increased plasma concentrations of 32-33 split proinsulin, a sign of beta-cell dysfunction[19-21], and was linked to high blood pressure[22,23]. Children small in birth size may predispose to metabolic abnormalities upon exposure to postnatal environmental risk factors such as low physical activity and/or high-energy intake[24]. Prenatal exposure to famine during the Dutch Hunger Winter of 1944-1945 was associated with impaired glucose tolerance and insulin secretion in adulthood[25,26]. The Pune Maternal Nutrition Study correlated prenatal specific micronutrient (vitamin B12) deficiency with increased insulin resistance in childhood[27]. Changes resulting from fetal and early postnatal malnutrition include: (1) metabolic adaptations in hepatic enzymes[28], lipoprotein profiles[29] and clotting factors[30]; (2) anatomical adaptations that affect end-organ glucose uptake[31] and renal solute metabolism[32]; and (3) endocrine

adaptations that affect the hypothalamic-pituitary-adrenal (HPA) axis[33], insulin signaling[34] and leptin levels[35]. These changes could collectively lead to the metabolic syndrome and related disorders[36].

Experimental evidence: In animal models, fetal mal-nutrition has been associated with marked structural and physiological alterations[37-39]. Gestational calorie restriction and protein deprivation in rats led to hypertension in adult offspring[40-42] and to altered glucose metabolism in sheep[43,44]. Malnutrition-associated changes in fetal leptin levels may alter the programming of appetite and eating behaviors leading to an increased risk of cardiovascular and metabolic diseases[45-48]. The pattern of dietary response of inbred mouse strains was similar to that expected under the thrifty phenotype hypothesis[49]. Permanent reductions in pancreatic cells and insulin secretion have been observed in protein-malnourished fetuses[50].

Implications and limitations: The thrifty phenotype is the most widely accepted hypothesis to interpret developmental programming. The hypothesis emphasizes the etiological role of poor fetal and early postnatal nutrition and implicitly advocates promoting fetal and infant nutrition and growth[51]. It may well explain the increasing prevalence of obesity and type 2 diabetes in India and South Asian countries where malnutrition was previously common but has become less so in recent decades[52]. However, in virtually all human studies, maternal and fetal nutritional status, as determined either directly by specific nutrient biomarkers or indirectly by weight gain during pregnancy, were not available for linkage to clinical outcomes. The hypothesis does not match the trends of increasing birth weights and declining LBW rates in many countries in recent decades[53-55], raising concerns as to whether poor fetal nutrition or "thrifty phenotype" is a major driver of developmental programming. Furthermore, poor fetal growth is a mere proxy for various perinatal insults. It is plausible that adverse insults may drive both poor fetal growth and developmental programming.

"Postnatal accelerated growth" or "catch-up growth" hypothesis
Rationale: The "postnatal accelerated growth" hypothesis was proposed by Drs. Singhal and Lucas to explain the association between faster early postnatal growth and surrogate endpoints in childhood and adolescence indicative of metabolic and cardiovascular risks based on follow-ups of preterm infants in two early neonatal feeding/nutritional intervention trials[13,14]. Increased infant growth rate by a nutrient enriched diet, even for only a few weeks postnatally, was associated with long-term adverse metabolic effects[6-8,56]. Fasting concentrations of 32-33 split proinsulin (a marker of insulin resistance) in adolescents born preterm were significantly elevated among those who had received a nutrient-enriched diet in early postnatal life *vs* placebo[7]. The authors concluded that early postnatal accelerated growth rather than pre-

maturity *per se* may be the culprit in programming insulin resistance and related disorders[14].

Cianfarani proposed a similar "catch-up growth" hypothesis[9]. At birth, infants with intrauterine growth retardation (IUGR) have low concentrations of insulin, insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein (IGFBP)-3; and high concentrations of growth hormone, IGFBP-1 and IGFBP-2. Normalization of these hormones occurs during the first trimester of postnatal life[57,58]. During this early postnatal catch-up growth period when suddenly exposed to increased concentrations of insulin and IGF-1, tissues chronically depleted of these two hormones during fetal life may counteract the hike by developing insulin resistance as a metabolic defense against developing hypoglycemia[9]. Therefore, IUGR infants who show early and complete growth recovery could be at higher risk for the occurrence of the metabolic syndrome in adulthood.

Dr. Gluckman proposed another similar hypothesis, the "predictive adaptive response"[10]. Based on the "predicted" postnatal environment, the fetus would make adaptations in utero or in the early postnatal period[10]. IUGR fetuses would thus predict a poor postnatal nutritional environment. When mismatch occurred between predicted and actual, disease would manifest[10,59].

Epidemiologic evidence: Early childhood growth acceleration has been associated with later insulin resistance[60], obesity[61] and cardiovascular disease[8]. In low birth weight infants, early growth acceleration has been associated with metabolic disturbances including dyslipidemia and elevated concentrations of insulin and IGF-1[9,62,63]. IUGR infants often experience compensatory accelerated growth after a period of poor fetal nutrition followed by the removal of such nutritional deficiency postnatally[64]. The most rapid growth occurs in early infancy[65] in the first few weeks after birth[66,67]. Factors promoting neonatal growth such as enhanced neonatal nutrition could permanently affect or program long-term health[68]. Such accelerated growth may have adverse consequences later in life[64], increase the propensity to cardiovascular disease[69] and its risk factors such as insulin resistance[70], obesity[65] and higher blood pressure[71]. Patients with impaired glucose tolerance or diabetes typically had a low BMI in infancy, an early adiposity rebound in childhood and an accelerated increase in BMI until adulthood[72].

Experimental evidence: In rats, accelerated early postnatal growth impaired glucose tolerance and shortened the lifespan[73]. Small neonatal rats temporarily overfed during the brief suckling period had permanent elevations in plasma insulin and cholesterol levels in adulthood[74]. Postnatal accelerated growth can adversely affect glucose tolerance in rats[75]. Even in rats without IUGR, overfeeding during the brief suckling period permanently increased later plasma insulin and cholesterol concentrations[76,77] with the propensity to obesity, high blood pressure and diabetes[53,77].

Implications and limitations: An important implication of the "postnatal accelerated growth" or "catch-up growth" hypothesis is that it questions the current practice of promoting postnatal catch-up growth of small babies[14]. Enhancing infant growth rate by a nutrient-enriched diet may actually do more harm than good in the long run. However, this hypothesis has been well tested only in preterm infants in Dr. Lucas's studies. It remains unclear whether the findings hold for catch-up growth in IUGR infants born at term. The increasing birth weights in most countries in recent decades[54,78-80] indicate that there are unlikely substantial increases in the incidence of postnatal catch-up growth. Consequently, it appears difficult to explain the substantial rise in the incidence of the metabolic syndrome. Also, the hypothesis does not match the epidemiological evidence of an elevated risk of the metabolic syndrome among macrosomic infants who often show catch-down rather than catch-up growth during the early postnatal period.

MINOR HYPOTHESES

We use the term "minor hypotheses" to refer to those supported by insufficient research data, especially in humans.

Glucocorticoids programming

A product of the activation of the HPA axis, glucocorticoids have potent effects on tissue development especially the maturation of organs such as the lung[81]. One outcome of fetal malnutrition is the exposure of the fetus to excess glucocorticoids which may restrict fetal growth and program the cardiovascular, endocrine and metabolic systems[82]. Normally, fetal glucocorticoid levels are much lower than maternal levels due to the placental barrier[83,84] - the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) catalyzes glucocorticoids (cortisol and corticosterone) into inert forms (cortisone, 11-dehydro corticosterone)[80,85]. However, synthetic glucocorticoids (betamethasone, dexamethasone) commonly administered to pregnant women at risk of preterm delivery to reduce neonatal pulmonary, renal and cerebral morbidities[86], are poor substrates of 11β-HSD2. Prenatal glucocorticoid overexposure through external sources or inhibition of placental 11β-HSD-2 may induce fetal HPA axis dysfunction - a potential link between adverse fetal environment and insulin resistance and hypertension in adulthood[87].

There is strong evidence of glucocorticoid programming in animal models. Many studies have reported decreased birth weights and abnormal levels of plasma HPA-axis hormones in rats prenatally exposed to synthetic glucocorticoids or inhibition of 11β-HSD2 with increased blood pressures and glucose intolerance in adulthood[87-89]. Hypertension in rats whose mothers were fed a low-protein diet during pregnancy was preventable by chemical blockade of maternal glucocorticoid synthesis[90], suggesting that the link between maternal protein deprivation and adult-onset hypertension may be mediated by maternal glucocorticoids. However, there is weak and inconsistent evidence regarding antenatal exposure to synthetic glucocorticoids and components of the metabolic syndrome in humans. Studies have reported no change, slight increase or decrease in blood pressure[91-94]. A Cochrane meta-analysis showed no differences in adult blood pressure[95]. In contrast, a recent follow-up study of 534 adults whose mothers had participated in a randomized controlled trial reported increased insulin resistance associated with antenatal betamethasone treatment[93]. LBW adults had much higher urinary glucocorticoid[96] and plasma cortisol concentrations[97] and showed greater responsiveness to adrenocorticotropic hormone[98,99]. Prenatal glucocorticoids may be the link between LBW and increased risk of glucose intolerance and hypertension[87]. Elevated blood pressure after antenatal exposure to glucocorticoids may result from altered renal renin-angiotensin system development[100] or from epigenetic changes affecting the expression of specific transcription factors, especially the glucocorticoid receptor[87].

However, there is a lack of strong evidence of glucocorticoid programming in humans. It remains unknown whether glucocorticoids drive both IUGR and the programming of metabolic syndrome components as observed in animal models.

Epigenetic programming

Experimentally, transmission to the next generation of a "programmed" phenotype has been demonstrated for birth weight, metabolic dysfunction[101-103], blood pressure and vascular dysfunction[104]. Wild type mice born to hypertensive heterozygous nitric oxide synthase-3 knockout mice displayed hypertension and vascular dysfunction[104]. Such transmission can be attributed to the fact that the programmed mother provided a deprived intrauterine environment, thus perpetuating the cycle of fetal (mal) adaptations. An alternate possibility is that epigenetic modification of the germline by stable DNA methylation or histone acetylation transmitted the prenatal experience of one generation to future generations.

Many candidate and confirmed players able to induce developmental programming can modify gene methylation. For example, Rees showed hypermethylation in the fetal liver of low protein fed dams[105]. Peroxisomal proliferator-activated receptor (PPAR) alpha and glucocorticoid receptor genes were hypomethylated and their expression increased in the liver of the offspring of protein-restricted rats[106]. Reactive oxygen species can modify methylation leading to changes in gene transcription and expression[107]. However, there are relatively little data demonstrating epigenetic changes after adverse perinatal conditions in genes closely implicated in cardiovascular and metabolic disorders. Bogdarina reported modifications in the methylation status of the angiotensin Ⅱ AT1b receptor gene in the adrenal gland of the offspring of low-protein fed dams[108]. Neonatal overfeeding in rats led to permanent dysregulation of the

central circuitry of food intake inhibition with resistance to signals triggered by insulin and leptin[109-111]. Circulating leptin and insulin stimulate the expression of the main anorexigenic neurohormone - proopiomelanocortin (P OMC) while inhibiting the orexigenic neuropeptide Y[112,113]. Plagemann recently demonstrated hypermethylation of the hypothalamic POMC gene promoter region in neonatal overfeeding animals within two specific protein-1 (Sp-1) binding sequences. This led to blunted POMC expression despite hyperleptinemia and hyperinsulinemia and demonstrated that a nutritionally acquired alteration of the methylation pattern could modify the set point of a gene promoter critical for body weight regulation[114].

The availability of methyl donor micronutrients may affect epigenetic programming. Dietary protein restriction in pregnancy induced and folic acid supplementation prevented epigenetic modifications of hepatic glucocorticoid receptor gene expression in rat offspring[106]. Folate supplementation of low-protein fed dams prevented elevation of blood pressure in adult offspring[115]. Restricting the supply of vitamin B12, folate and methionine even within normal physiological ranges during the periconceptional period was associated with widespread epigenetic alternations, insulin resistance and elevated blood pressure in sheep[116]. In contrast, maternal high folate status in pregnancy was associated with *increased* insulin resistance in children[27], indicating the need for caution in applying results from animal models to humans. Dietary methyl supplementation with folic acid and B12 may have unintended deleterious consequences on epigenetic regulation[117]. More human data are needed in this nascent research area.

It should be pointed out that epigenetic programming is a physiological process in normal fetal development; the epigenetic changes dictate cell differentiation. The question is, are some epigenetic changes "pathological" secondary to certain perinatal insults? There is a lack of human data linking perinatal "programming" insults to developmental epigenetic changes and later risk of the metabolic syndrome and related disorders. Improved understanding of epigenetic changes may be helpful in designing interventions to prevent or possibly reverse adverse programming.

Oxidative stress programming

Because many known or suspected causes of or conditions associated with adverse fetal growth or preterm birth have been associated with oxidative stress, it is plausible that oxidative stress may be the underlying common link to elevated risks of the metabolic syndrome[118]. Oxidative stress programming may act directly through modulation of gene expression (perhaps epigenetic) or indirectly *via* the effects of certain oxidized molecules. Experimental investigations have well demonstrated the role of redox balance in modulating gene expression[119,120]. There is considerable experimental evidence indicating that both the insulin function axis and blood pressure regulation

could be sensitive targets to oxidative stress programming during the prenatal and early postnatal periods[121-126]. However, there remains a lack of epidemiological data relating biomarkers of perinatal oxidative stress to the metabolic syndrome. Validation of the oxidative stress hypothesis would suggest new early interventions to stem the modern epidemic of the metabolic syndrome.

Prenatal hypoxia programming

There is some evidence linking prenatal hypoxia to increased vulnerability to metabolic and cardiovascular diseases[127]. Chronic hypoxia is a common insult to the fetus and reduced uteroplacental blood flow can result in fetal IUGR independently of malnutrition[128-130]. Chronic prenatal hypoxia has been shown to increase the susceptibility of the adult heart to ischemia-reperfusion injury[131]. Human studies at high altitude also suggest that prenatal hypoxia can result in LBW[132-134]. Chronic hypoxia has profoundly adverse effects on cardiac development and function in the fetus[129] and enhanced β1-adrenergic receptor signaling may induce cardiomyocyte apoptosis *via* a protein kinase A-dependent mechanism[132]. Animal studies show that chronic hypoxia could increase the heart-to-body weight ratio in the fetus, suggesting an asymmetric growth of the heart[133]. Chronic hypoxia significantly increased the levels of cytochrome C, a mitochondrial marker protein, in the fetal heart[134]. The increased cytochrome C levels are likely a metabolic adaptation in the myocardium during asymmetric enlargement of the heart in hypoxic fetuses. Animal experiments showed that mitochondrial biogenesis played an important role in the early stages of cardiac hypertrophy[128]. Hypoxia could induce apoptosis in cultured neonatal rat cardiomyocytes[135]. In response to chronic hypoxia during gestation, many genes related to cell signaling and survival are down- or up-regulated in the fetal heart and other tissues[127]. No epidemiological data are available as to whether these effects are transient or permanent.

Placental dysfunction

It has been proposed that adult cardiovascular and metabolic diseases originate via developmental plasticity and adaptations arising from failure of the maternal-placental nutrient supply to match fetal requirements[136]. This hypothesis emphasizes the role of the placenta in fetal programming. Maternal nutrition was associated with fetal development and programming of human cardiovascular and metabolic disease[137]. Maternal body composition and nutrition intake may affect fetal development by direct effects on substrate availability to the fetus and indirect effects via changes in placental function and structure[136]. Fetal adaptations may result from alterations in placental growth and vascular resistance, altered nutrient and hormone metabolism in the placenta and changes in nutrient transfer and partitioning between mother, placenta and fetus[138-140]. Fetal cardiovascular adaptations, alterations in fetal body composition and changes in fetal endocrinology and metabolism may have long-term effects

on postnatal health. However, no data are available on the epidemiological associations between pathological placental changes and the metabolic syndrome in the offspring. The hypothesis implies that improving placental function may have lifelong health benefits.

Reduced stem cell number

After organogenesis, the ability of cells to divide substantially for self-renewal and repair is limited although some stem cells remain in various tissues in postnatal life. The stem cell hypothesis suggests that IUGR is associated with a reduced number of tissue stem cells, leading to an early exhaustion of organ function when demands are increased greatly[141]. The time windows for stem cell proliferation may represent critical periods. Diabetes patients often have fewer β-cells prior to the onset of disease or the pancreas failed to generate more β-cells in response to an increased demand for insulin[142]. In rats, fetal and neonatal nutritional deprivation caused permanent reductions in β-cell mass and functional efficiency, resulting in glucose intolerance in adulthood[143]. IUGR induced by bilateral uterine artery ligation in pregnant rats caused postnatal glucose intolerance and insulin resistance in offspring; the β-cells mass of IUGR rats was reduced by one-third[144]. There is a lack of data on stem cell number as it relates to metabolic syndrome programming in humans.

OVER-NUTRITION PROGRAMMING?

Although most research is focused on adverse programming associated with poor fetal growth, there is evidence that maternal overnutrition or fetal overgrowth may result in an offspring phenotype susceptible to the metabolic syndrome[145]. Maternal high fat or cholesterol overfeeding during pregnancy and lactation in rodents resulted in a phenotype of the offspring that closely resembled the human metabolic syndrome[145,146]. Gestational diabetes is associated with glucose oversupply to the fetus and consequently fetal macrosomia and may have adverse metabolic programming effects[147]. The effects of gestational diabetes seem independent of genetic factors[148]. Experimental evidence indicates that overnutrition may program obesity and metabolic syndrome through epigenetic changes[114]. A meta-analysis showed a U-shaped relationship between birth weight and type 2 diabetes risk in humans[149]. The hypothesis is concordant with the increasing birth weights over recent decades and may partly explain the increasing prevalence of the metabolic syndrome. It is unclear whether the effects of fetal overgrowth programming could be largely explained by impaired maternal glucose tolerance in humans.

CONCLUSION

The various hypotheses for interpreting developmental programming could be interrelated, indicating the need for research to address multiple mechanisms simultaneously.
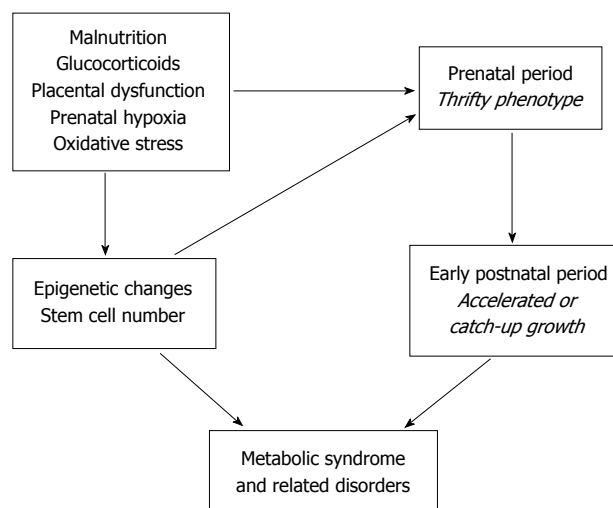

Figure 2 Pieces of the puzzle - potential links between current hypotheses to interpret the mechanisms of developmental programming of the metabolic syndrome and related disorders.

Some mechanisms could be driven by other "driver" mechanisms (Figure 2). Multiple overarching drivers may exist: malnutrition, glucocorticoids, oxidative stress, prenatal hypoxia and placental dysfunction. These drivers may act alone or in combinations to induce epigenetic changes or reduce stem cell number, leading to the thrifty phenotype often followed by catch-up growth and the propensity to metabolic syndrome. Thus, adverse programming may occur in the absence of poor fetal growth.

The current prevailing theory is that fetal programming effects are magnified over the life course. However, the postnatal environment may either mask or magnify the true effects of programming - the direction of effect modifications is unknown. The strongest evidence supporting the various hypothetical mechanisms comes from animal models. However, we cannot assume that findings from animal models are applicable to humans as human pregnancy physiology is much more complex. For example, preeclampsia (gestational hypertension with proteinuria) is a gestational complication unique to humans[150]. Even the commonly used operating definitions for retarded or excessive fetal growth in humans are largely arbitrary and need re-evaluations[151]. There is a need for studies to address multiple mechanisms simultaneously in animal models and a need for prospective pregnancy cohort data linking intrauterine environmental biomarkers of the proposed programming mechanisms to clinical outcomes or surrogate biomarker endpoints in humans. A better understanding of the programming mechanisms is a prerequisite for developing early life interventions to halt the worldwide increasing epidemic of the metabolic syndrome, type 2 diabetes and other related disorders.

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LETTERS TO THE EDITOR

Dipeptidyl peptidase-4 inhibitor for steroid-induced diabetes

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Abstract

The addition of the dipeptidyl peptidase-4 (DDP-4) inhibitor has been reported to achieve greater improvements in glucose metabolism with fewer adverse events compared to increasing the metformin dose in type 2 diabetic patients. We present a patient with steroid-induced diabetes whose blood glucose levels were ameliorated by the use of the DPP-4 inhibitor, showing that the DPP-4 inhibitors may be an effective and safe oral anti-diabetic drug for steroid-induced diabetes.

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Key words: Dipeptidyl peptidase-4; Nateglinide; Sitagliptin; Steroid-induced diabetes

TO THE EDITOR

In the March 2010 issue of *World Journal of Diabetes*, Filozof *et al*[1] demonstrated that the addition of the dipeptidyl peptidase-4 (DDP-4) inhibitor, vildagliptin, achieved greater improvements in glucose metabolism with fewer adverse events compared to increasing the metformin dose, suggesting the effectiveness of the DPP-4 inhibitor for type 2 diabetes mellitus. We agree with their suggestion and will introduce a patient with steroid-induced diabetes whose blood glucose levels were ameliorated by the use of the DPP-4 inhibitor, sitagliptin.

An 81-year-old female patient was treated daily with 20 mg prednisolone due to polymyalgia rheumatica (PMR) and developed steroid-induced diabetes. Her hemoglobin A_{1c} level was 8.3% and 30-40 units of insulin aspart daily were used to treat hyperglycemia. Her symptoms of PMR were ameliorated and the daily dose of prednisolone was decreased. Since she refused to use insulin when out of hospital, we started oral anti-diabetic drugs. Her fasting blood glucose levels were normal and postprandial glucose levels and daily C-peptide levels in urine (141 mg/d) were remarkably elevated. Therefore, we started to use metformin, nateglinide and pioglitazone. However, her postprandial glucose levels did not decrease and 6-8 units of insulin aspart were needed (Figure 1). Higher doses of metformin and α-glucosidase inhibitor could not be used because of her abdominal symptoms. Then we changed from nateglinide to sitagliptin. After the change of therapy, her postprandial glucose levels were significantly decreased and finally the addition of insulin was not needed in
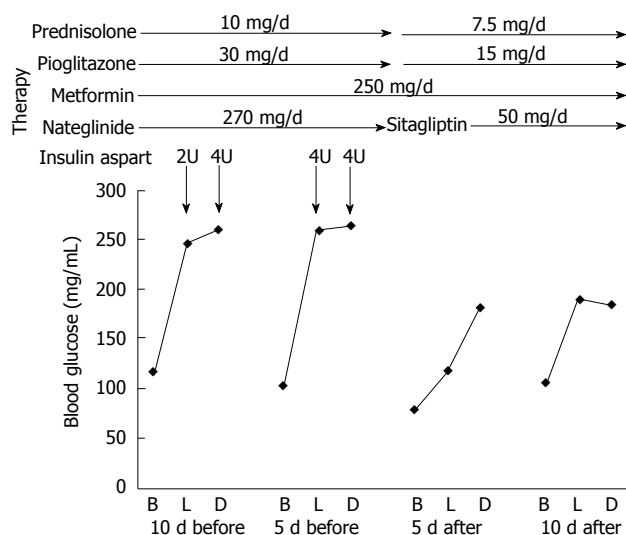
Figure 1 Changes of blood glucose levels before breakfast, lunch, and dinner, at 5 and 10 d before and after the modification of therapy. B: Breakfast; L: Lunch; D: Dinner.

spite of the reduced dose of pioglitazone due to lower limb edema (Figure 1).

Some patients treated with steroids show hyperglycemia, develop diabetes and sometimes need insulin therapy for marked hyperglycemia. The underlying mechanisms for steroid-induced diabetes may include increased gluconeogenesis and hepatic glucose output and insulin resistance. The characteristics for steroid-induced diabetes have been reported to be normal fasting plasma glucose levels and postprandial hyperglycemia[2]. The DPP-4 inhibitors prevent the inactivation of the incretin hormones which is released from the gut following food ingestion and in turn stimulates insulin secretion, inhibits glucagon secretion, improves hyperglycemia and insulin resistance and rarely induces hypoglycemia[3,4]. Furthermore, treatment with the DPP-4 inhibitors has been reported to increase pancreatic islet β-cell density and stimulate islet β-cell proliferation while preventing apoptosis and islet fibrosis and decreasing superoxide production and nitrotyrosine formation[5].

The DPP-4 inhibitors-mediated mechanisms for improvement of hyperglycemia may ameliorate steroid-induced postprandial hyperglycemia and not induce fasting hypoglycemia.

Our study has limitations. Since about 2 wk of routine treatment of thiazolidinediones is necessary to reach their obvious effect on insulin sensitivity, it is hard to distinguish the effect of blood glucose reduction that is attributed to the use of sitagliptin or that of pioglitazone. In view of this, it would be better to include a control in parallel. It should also be considered that steroid-induced diabetes has a tendency of self recovery after termination or decreased dose of glucocorticoids. This observation is based on only one patient. To elucidate the effectiveness of the DPP-4 inhibitor for steroid-induced diabetes, further studies, preferably with larger numbers of subjects, will be needed.

In conclusion, α-glucosidase inhibitor and thiazolidinediones have been reported as effective oral anti-diabetic drugs for steroid-induced diabetes[6,7]. The DPP-4 inhibitors may also be an effective and safe oral anti-diabetic drug for steroid-induced diabetes.

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14th Pan Arab Conference on
Diabetes PACD14

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Digestive Disease Week Annual
Meeting

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June 25-29
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Instructions to authors

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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/eid.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.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1948-9358/g_info_20100107145507.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: t time or temperature, c concentration, A area, l length, m mass, V volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.
Biology: *H. pylori*, *E coli*, *etc*.

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