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Molecular Basis of Insulin Resistance and Its Relation to Metabolic Syndrome

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1. Introduction

The metabolic syndrome is an agglomeration of interrelated risk factors that is associated with nearly 5-fold increased risk for type 2 diabetes mellitus (DM) and a 2-fold increased risk of coronary artery disease (CAD) [1]. Reaven first suggested this cluster of metabolic abnormalities in 1988. It is characterized by insulin resistance, visceral adiposity, dyslipidemia and a systemic pro-inflammatory and pro-coagulant state [2]. Insulin resistance is defined as reduced insulin action in metabolic and vascular target tissues, hence higher than normal concentration of insulin is required to maintain normoglycemia. On a cellular level, it indicates an 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 [3].

The development of insulin resistance leads to many of the metabolic abnormalities associated with this syndrome. Patients with insulin resistance tend to have impaired fasting plasma glucose levels, which increase the prevalence of more atherogenic, small dense low-density lipoprotein (LDL) particles. The growing incidence of insulin resistance and metabolic syndrome (MS) is seriously threatening human health globally. Individuals with MS have a 30%–40% probability of developing diabetes and/or CVD within 20 years, depending on the number of components present [4].

In the United States (US), the prevalence of the MS in the adult population was estimated to be more than 25%. Similarly, the prevalence of MS in seven European countries was approximately 23%. It was estimated that 20%–25% of South Asians have developed MS and many more may be prone to it [5,6]. The main reason why MS is attracting scientific and commercial interest is that the factors defining the syndrome are all factors associated with increased morbidity and mortality in general and from CVD in particular [7].

Though, Insulin resistance has been recognized as a basis of CVD and diabetes type II, its etiology still remains elusive. Recent studies have contributed to a deeper understanding of the underlying molecular mechanisms of Insulin resistance. This review provides a detailed understanding of these basic pathophysiological mechanisms which may be critical for the development of novel therapeutic strategies to treat/ prevent metabolic syndrome.

2. Signalling through Insulin receptor and its downstream Pathways

Insulin action is initiated by an interaction of insulin with its cell surface receptor [8]. 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 extracellular α subunit of the insulin receptor and activates the tyrosine kinase in the β subunit (figure 1). Binding of insulin to IR effects a series of intramolecular transphosphorylation reactions, where one β subunit phosphorylates its adjacent partner on a specific tyrosine residue. Once the tyrosine kinase of insulin receptor is activated, it promotes autophosphorylation of the β subunit itself, where phosphorylation of three tyrosine residues (Tyr-1158, Tyr-1162, and Tyr-1163) is required for amplification of the kinase activity [9]. It then recruits different substrate adaptors such as the Insulin Receptor Substrate (IRS) family of proteins. Although IRs are present on the surface of virtually all cells, their expression in classical insulin target tissues, i.e. muscle, liver and fat, is extremely high [10]. Tyrosine phosphorylated IRS then displays binding sites for numerous signaling partners. Phosphorylated IRS proteins serve as multisite docking proteins for various effector molecules possessing *src* homology 2 (SH2) domains, including phosphatidylinositol 3-kinase (PI 3-kinase) regulatory subunits (p85, p55, p50, p85, and p55^{PIK}), the tyrosine kinases Fyn and Csk, the tyrosine protein phosphatase SHP-2/Syp, as well as several smaller adapter molecules such as the growth factor receptor binding proteins Grb-2, Crk, and Nck [11]. Activation of these SH2 domain proteins initiates signaling cascades, leading to the activation of multiple downstream effectors that ultimately transmit the insulin signal to a branching series of intracellular pathways that regulate cell differentiation, growth, survival, and metabolism. Four members of the IRS family have been identified that are considerably similar in their general architecture [12-15]. IRS proteins share a similar structure characterized by the presence of an NH₂-terminal pleckstrin homology (PH) domain adjacent to a phosphotyrosine-binding (PTB) domain followed by a variable-length COOH-terminal tail that contains a number of Tyr and Ser phosphorylation sites. The PH domain is critical for IR-IRS interactions. Plasma membrane phospholipids, cytoskeletal elements, and protein ligands mediate these interactions [16, 17]. In contrast, the PTB domain interacts directly with the juxtamembrane (JM) domain of the insulin and IGF-I receptors [18, 19], and hindrance of these interactions (by Ser/Thr phosphorylation) negatively affects insulin signaling [19]. A third domain, the kinase regulatory loop binding (KRLB) is found only in IRS-2 [20, 21]. This domain interacts with the phosphorylated regulatory loop of the IR, whereas the phosphorylation of two Tyr residues within the KRLB are crucial for this interaction [22].

PI3 kinase is a target of the IRS proteins (IRS-1 and IRS-2) which phosphorylates specific phosphoinositides to form phosphatidylinositol 4,5 bisphosphate (PIP₂) to

phosphatidylinositol 3,4,5 triphosphate; in turn, this activates ser/thr kinase, i.e. phosphoinositide-dependent kinase-1 (PDK1) [23, 24]. Known substrates of the PDKs are the protein kinase B (PKB) and also atypical forms of protein kinase C (PKC) [25].

Downstream from PI 3-kinase, the serine/threonine kinase Akt (also called PKB) triggers insulin effects on the liver. Phosphatidylinositol-dependent kinase (PDK) and PKB/Akt have a pleckstrin homology domain that enables these molecules to migrate toward the plasma membrane [26]. Activated Akt induces glycogen synthesis, through inhibition of GSK-3; protein synthesis via mTOR and downstream elements; and cell survival, through inhibition of several pro-apoptotic agents (Bad, Forkhead family transcription factors, GSK-3). Insulin stimulates glucose uptake in muscle and adipocytes via translocation of GLUT4 vesicles to the plasma membrane [27- 29]. This suggests that the impairment of insulin activity leading to insulin resistance is linked to insulin signalling defects.

Recently, an alternative PI 3-kinase independent mechanism to enhance GLUT4 translocation and glucose uptake was described. According to this model, binding of insulin to its receptor finally activates the small G-protein TC10 via the scaffolding protein CAP (Cbl-associated protein) resulting in GLUT4 translocation and enhanced glucose uptake [30]. Insulin signaling also has growth and mitogenic effects, which are mostly mediated by the Akt cascade as well as by activation of the Ras/MAPK pathway. A negative feedback signal emanating from Akt/PKB, PKCZ, p70 S6K and the MAPK cascades results in serine phosphorylation and inactivation of IRS signaling [31, 32]. Insulin signalling molecules involved in metabolic and mitogenic action have been demonstrated to play a role in cellular insulin resistance. A few recent reports indicate that some PKC isoforms may have a regulatory effect on insulin signalling. The expression levels and activity of a few PKC isoforms are found to be associated with insulin resistance [33-35].

Recent data from PKB knockout animal models provide an insight into the role of PKB in normal glucose homeostasis. While disruption of PKB/Akt1 isoform in mice have not shown to 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 [36-37]. Subsequent studies [38- 40] in insulin-resistant animal models and humans have consistently demonstrated a reduced strength of insulin signaling via the IRS-1/PI 3-kinase pathway, resulting in diminished glucose uptake and utilization in insulin target tissues. Recent studies on inherited insulin post-receptor mutations in humans have detected a missense mutation in the kinase domain of PKB (Akt2) in a family of severely insulin resistant patients. The mutant PKB was unable to phosphorylate downstream targets and to mediate inhibition of phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic key enzyme [41]. Another recent study, involving the stimulation of PI3K and Akt-1, -2, and -3 by insulin and epidermal growth factors (EGFs) in skeletal muscles from lean and obese insulin-resistant humans showed that Insulin activated all Akt isoforms in lean muscles, whereas only Akt-1 was activated in obese muscles. Insulin receptor substrate (IRS)-1 expression was reduced in obese muscles, and this was accompanied by decreased Akt-2 and -3 stimulation. In contrast, insulin- or EGF-stimulated phosphotyrosine-associated PI3K activity was not different between lean and obese muscles.

These results showed that a defect in the ability of insulin to activate Akt-2 and -3 may explain the impaired insulin-stimulated glucose transport in insulin resistance [42].

This suggests that the impairment of insulin activity leading to insulin resistance is linked to insulin signalling defects. These insulin signalling pathways are shown in figure 1.

3. Mechanisms related to Insulin resistance

Two separate, but likely, complementary mechanisms have recently emerged as a potential explanation for Insulin resistance. First, changes in IRS-1 either due to mutations or serine phosphorylation of IRS proteins can reduce their ability to attract PI 3-kinase, thereby minimizing its activation. A number of serine kinases that phosphorylate serine residues of IRS-1 and weaken insulin signal transduction have been identified. Additionally, mitochondrial dysfunction has been suggested to trigger activation of several serine kinases, leading to a serine phosphorylation of IRS-1. Second, a distinct mechanism involving increased expression of p85 α has also been found to play an important role in the pathogenesis of insulin resistance. Conceivably, a combination of both increased expression of p85 α and increased serine phosphorylation of IRS-1 is needed to induce clinically apparent insulin resistance.

4. Mutations of IRS as a cause of Insulin resistance

IRS-1 protein is a gene product of IRS-1 gene. In humans, rare mutations of the IRS-1 protein are associated with insulin resistance [43] and disruption of the IRS-1 gene in mice results in insulin resistance mainly of muscle and fat [44]. The genetic analysis of the IRS-1 gene has revealed several base-pair changes that result in amino acid substitutions [45-47]. The most common amino acid change is a glycine to arginine substitution at codon 972 (G972R), which has an overall frequency of $\approx 6\%$ in the general population [48], with a carrier prevalence of 9% among Caucasians [49]. This mutation has been reported to significantly impair IRS-1 function in experimental models [50], and clinical studies have shown that this genetic variant is associated with reduced insulin sensitivity [51]. Expression of this variant in 32-D cells is associated with a significant (20-30%) impairment of insulin-stimulated PI3-kinase activity, as well as reduced binding of IRS-1 to the p85 regulatory subunit of PI3-kinase. Genotype/phenotype studies stratified according to body mass index (BMI) indicate that obese subjects who are heterozygous for the mutant allele have a 50% decrease in insulin sensitivity, compared with wild-type obese subjects. This suggests that there may be an interaction between the mutant allele and obesity, such that, in the presence of obesity, the mutant variant may aggravate the obesity-associated insulin resistance [49]. Moreover, earlier observations have indicated that the presence of a mutated IRS-1 gene is associated with dyslipidemia, further suggesting that this gene variant may have a significant effect on several risk factors for CAD [48, 50-52].

Interestingly, IRS-2 knockout mice not only show insulin resistance of muscle, fat and liver, but also manifest diabetes as a result of cell failure [53]. This phenotype with severe

hyperglycemia as a consequence of peripheral insulin resistance and insufficient insulin secretion due to a significantly reduced β -cell mass reveals many similarities to type 2 diabetes in man and outlines the role of IRS proteins for the development of cellular insulin resistance. Homozygous 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 [54]. 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 [55, 56]. Even though β cell mass was reduced in *IRS-2*^{-/-} mice, individual β cell showed normal or increased insulin secretion in response to glucose [55].

In regard to insulin signaling, experiments in immortalized neonatal hepatocytes show that the lack of IRS-2 is not compensated for by an elevation of IRS-1 protein content or an increase in tyrosine phosphorylation [57]. Previous experiments performed in peripheral tissues of *IRS-1*^{-/-} mice by Yamauchi et al. [44] suggested that IRS-2 could be a major player in hepatic insulin action. However, to what extent reduced IRS-2 contributes to insulin resistance in the liver remains uncertain. In humans, a number of polymorphisms have been identified in the *IRS-2* gene. Among those, the amino acid substitution Gly1057Asp has been found in various populations with a prevalence sufficiently high to modulate a population's risk of type 2 diabetes. In Caucasians, Finns, and Chinese, however, this variant has not shown an association with type 2 diabetes [58, 59]. Although the polymorphism was associated with decreased insulin sensitivity and impaired glucose tolerance in women with polycystic ovary syndrome [60], it showed no association with insulin sensitivity in other studies [59, 61, 62]. In contrast, another study in women with polycystic ovary syndrome found that homozygous carriers of the Gly1057 allele had higher 2-h plasma glucose concentrations during an oral glucose tolerance test (OGTT) [63]. Decreased serum insulin and C-peptide concentrations during an OGTT were reported in middle-aged glucose-tolerant Danish males carrying the Asp1057 allele [62]. However, using formal β -cell function tests, associations with insulin secretion were not reproduced in German, Finnish, and Swedish populations [59, 61, 62].

5. Serine phosphorylation of IRS as a cause of Insulin resistance

IRS-1 contains 21 putative tyrosine phosphorylation sites, several of which are located in amino acid sequence motifs that bind to SH-2 domain proteins, including the p85 regulatory subunit of PI 3-kinase, Grb-2, Nck, Crk, Fyn, Csk, phospholipase C γ , and SHP-2 [64]. IRS-1 contains also > 30 potential serine/threonine phosphorylation sites in motifs recognized by various kinases such as casein kinase II, protein kinase C, protein kinase B/Akt, and mitogen-activated protein (MAP) kinases [12, 64].

Human IRS-2 contains 22 potential tyrosine phosphorylation sites, but only 13 are conserved in IRS-1. The amino acid sequence identity between IRS-1 and IRS-2 is 43%, with some domains such as the PH and PTB domains exhibiting higher degrees of identity (65 and 75%, respectively). The COOH-terminal domains of IRS-1 and IRS-2 are poorly conserved,

displaying only 35% identity, which arises largely from similar tyrosine phosphorylation motifs surrounded by variable stretches of amino acid sequence. The middle of IRS-2 possesses a unique region comprising amino acids 591–786 that interacts specifically with the kinase regulatory loop binding (KRLB) domain of the insulin receptor β subunit [65]. Since this region is absent in IRS-1, this domain may contribute to the signaling specificity of IRS-2. In addition, IRS-1 and IRS-2 may regulate unique signaling pathways because of different tissue distribution, subcellular localization, kinetics of activation/deactivation, or specificity of interaction with downstream effectors [66–68]. For example, it has been shown that IRS-1 and IRS-2 differ in their subcellular localization since IRS-1 is twofold more concentrated in the intracellular membrane compartment than in cytosol, whereas IRS-2 is twofold more concentrated in cytosol than in the intracellular membrane compartment [69]. Further studies have shown that IRS-2 is dephosphorylated more rapidly and activates PI 3-kinase more transiently than IRS-1, thus indicating that differences in kinetics of activation may contribute to the diversity of the insulin signaling transduced by IRS-1 and IRS-2 [69,70].

Since, IRS-1 and IRS-2 have the longest tails, which contain ~20 potential Tyr phosphorylation sites. Many of the Tyr residues gather into common Tyr-phosphorylated consensus motifs (YMXM or YXXM) that bind SH2 domains of their effector proteins. Spatial matching is required for successful protein-protein interaction. Ser/Thr phosphorylation of IRS proteins in close proximity to their PTB (receptor-binding) region impedes the binding of the SH2 domains of these effectors, thus inhibiting insulin signaling [71].

Serine phosphorylation of IRS proteins can occur in response to a number of intracellular serine kinases [72]. The causes of IRS-1 serine phosphorylation are-

1. mTOR- p70S6 kinase, Amino acids, Hyperinsulinemia
2. JNK- Stress, Hyperlipidemia, Inflammation
3. IKK- Inflammation
4. TNF α - Obesity, Inflammation
5. Mitochondrial dysfunction
6. PKC θ - Hyperglycemia, Diacylglycerol, Inflammation

Recent studies have demonstrated hyper-serine phosphorylation of IRS-1 on Ser³⁰², Ser³⁰⁷, Ser⁶¹², and Ser⁶³² in several insulin-resistant rodent models [73–76] as well as in lean insulin-resistant offspring of type 2 diabetic parents [77]. Further evidence for this hypothesis stems from recent studies in a muscle-specific triple serine to alanine mutant mouse (IRS-1 Ser \rightarrow Ala³⁰², Ser \rightarrow Ala³⁰⁷, and Ser \rightarrow Ala⁶¹²), which has been shown to be protected from high-fat diet-induced insulin resistance in vivo [78]. Based on in vitro studies, serine phosphorylation may lead to dissociation between insulin receptor/IRS-1 and/or IRS-1/PI 3-kinase, preventing PI 3-kinase activation [79, 80] or increasing degradation of IRS-1 [81].

Ser³¹⁸ of IRS-1 is a potential target for PKC ζ [82], JNK, and kinases along the PI3K-mTOR pathway [83]. It is located in close proximity to the PTB domain. Therefore, its phosphorylation presumably disrupts the interaction between IR and IRS-1.

Phosphorylation of Ser³¹⁸ is not restricted to insulin stimulation. Elevated plasma levels of leptin, an adipokine produced by adipocytes [84], also stimulates the phosphorylation of Ser³¹⁸. This down regulates insulin-stimulated Tyr phosphorylation of IRS-1 and glucose uptake.

In a recent study using skeletal muscle biopsies from 11 humans, the mTOR-S6K pathway was shown to negatively modulate glucose metabolism under nutrient abundance [151]. In agreement with previous studies, phosphorylation of Ser³¹² and Ser⁶³⁶ of IRS-1 was implicated as part of this negative regulation [85, 86]. Increased phosphorylation of Ser⁶³⁶ of IRS-1 was observed in myotubes of patients with type 2 diabetes. Inhibition of ERK1/2 with PD-98059 reduced this phosphorylation, thereby implicating ERK1/2 in the phosphorylation of Ser⁶³⁶ in human muscle [87].

To unveil the importance of phosphorylated Ser/Thr residues of human IRS-1, Yi et al. [88] adopted a mass spectrometry approach. More than 20 Ser residues of IRS-1 were found to undergo insulin-stimulated phosphorylation in human muscle biopsies, three of which were newly identified sites: Thr⁴⁹⁵, Ser⁵²⁷, and Ser¹⁰⁰⁵. This report validates previous in vitro and in vivo studies in animal models and suggests that the same strategy could be employed to identify phosphorylated Ser/Thr sites under conditions of insulin resistance, obesity, or type 2 diabetes.

Impaired hepatic glycogen storage and glycogen synthase activity is a common finding in insulin resistance [89] and polymorphisms in the glycogen synthase gene have been described in insulin resistant patients. The most frequent mutations are the so-called *Xba*I mutations and Met416Val within intron 14 and exon 10, respectively. Currently, there are conflicting data on the correlation of these polymorphisms with insulin resistance and Type 2 diabetes mellitus [90-92].

Recently, a hypothesis that mitochondrial dysfunction or reduced mitochondrial content accompanied by a decreased mitochondrial fatty acid oxidation and accumulation of fatty acid acyl CoA and diacylglycerol can cause insulin resistance has gained substantial experimental support [93- 95]. The mechanism of insulin resistance in these cases has been suggested to involve activation of a novel PKC that either by itself or via IKK β or JNK-1 could lead to increased serine phosphorylation of IRS-1. Severe mitochondrial dysfunction can result in diabetes that is typically associated with severe β -cell dysfunction and neurological abnormalities [96]. In a study ,using ¹³C/³¹P MRS, it was found that in the healthy lean elderly volunteers with severe muscle insulin resistance, there is ~40% reduction in rates of oxidative phosphorylation activity associated with increased intramyocellular and intrahepatic lipid content [94]. 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 [78]. Further, it was found that mitochondrial density was reduced by 38% in the insulin-resistant offspring [77].

[This topic has been dealt in details in subsequent chapter by Wang etal.]

The proinflammatory novel PKC θ has been found to cause serine phosphorylation of IRS-1 [97, 98], while PKC θ knockout mice have been shown to be protected from fat-induced insulin resistance [75]. Increased activity of PKC θ , along with increased activity of JNK, has also been found in skeletal muscle of obese and type 2 diabetic subjects [99, 100], supporting a potential role of these serine kinases in the pathogenesis of insulin resistance.

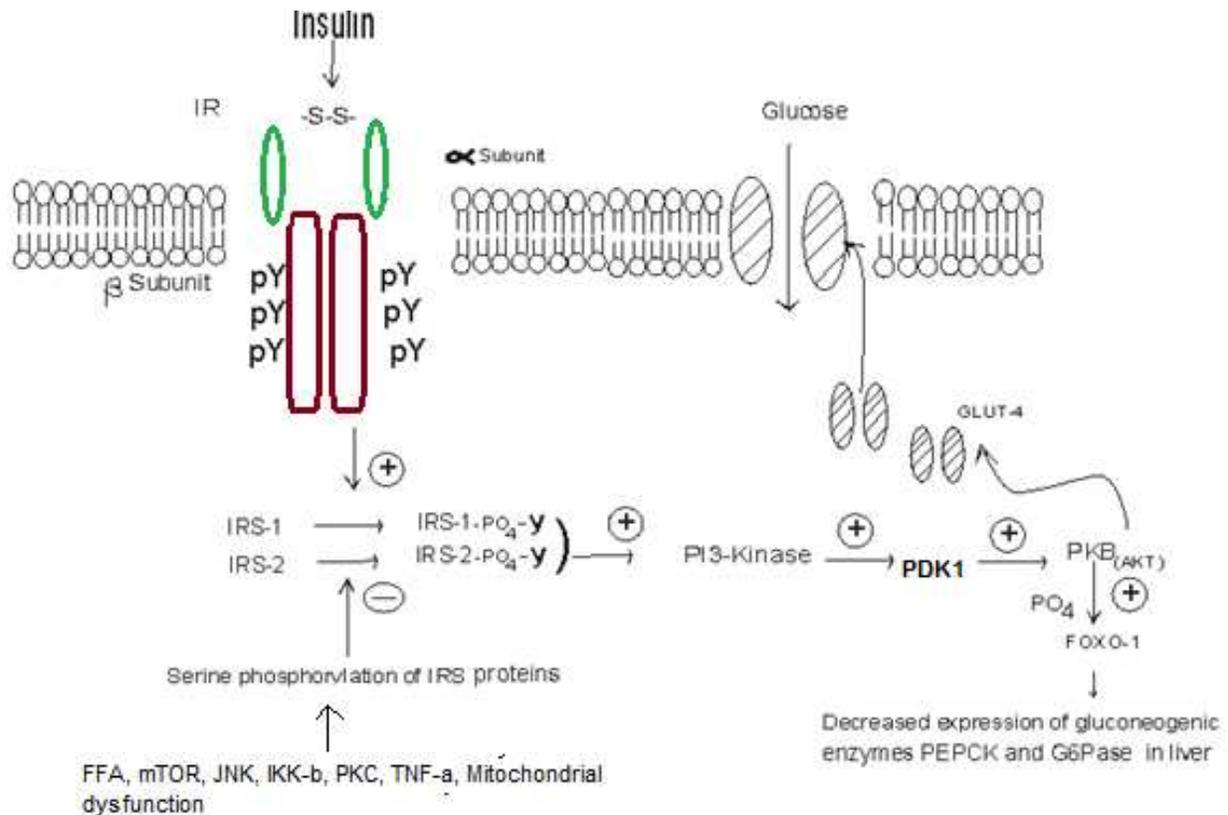


Figure 1. Insulin signaling pathway showing that the binding of insulin with Insulin receptor (IR) leads to phosphorylation of tyrosine residues followed by activation of downstream signalling pathways which result in recruitment of GLUT-4 transporter to the plasma membrane and entry of glucose molecules within the cell. Serine phosphorylation of IRS protein has an inhibitory effect on downstream pathways resulting in insulin Resistance.

6. Increased expression of p85

A molecular mechanism that can potentially lead to insulin resistance is a disruption in the balance between the amounts of the PI 3-kinase subunits [101]. PI 3-kinase belongs to the class 1a 3-kinases [102], which exist as heterodimers, consisting of a regulatory subunit p85, which is tightly associated with a catalytic subunit, p110. Most tissues express two forms of regulatory subunit, p85 α and p85 β , and two forms of catalytic subunit, p110 α and p110 β [102]. p85 α and p85 β share the highest degree of homology in the C-terminal half of the molecules, which contains two SH2 domains that bind to tyrosine-phosphorylated proteins and an inter-SH2 domain that interacts with the catalytic subunit. The N-terminal halves of p85 α and p85 β contain an SH3 domain, a BCR homology region, and two proline-rich domains, but these domains are less well conserved between the two molecules. Two

isoforms of p85 α truncated in the N-terminal region, identified as AS53 (or p55 α) [103, 104] and p50 α [105, 106], as well as p85 α itself, are derived from a single gene (*Pik3r1*). p85 β and another short isoform with limited tissue distribution termed p55 γ /p55^{PIK} are encoded by separate genes [107]. 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 PI 3-kinase activity [108-110]. 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 PI 3-kinase activity [111]. Increase or decrease in expression of p 85 would result in a shift in the balance either in the favour of free p85 or p85-p110 complexes [108-110].

One of the first indications that an imbalance between the abundance of p85 and p110 can alter PI 3-kinase activity came from experiments with l-6 cultured skeletal muscle cells treated with dexamethazone [111]. This treatment significantly reduced PI 3-kinase activity, despite an almost fourfold increase in expression of p85 α (no change in p85 β) and only a minimal increase in p110. The authors concluded that p85 α competes with the p85-p110 heterodimer, thus, reducing PI 3-kinase activity.

Subsequently, animals with a targeted disruption of *p85 α* (p85^{+/-} heterozygous mice) have been found to have a higher ratio of p85-p110 dimer to free p85 and to be more sensitive to insulin [101, 111-114].

The possibility of mismatch between free p85 and p85-p110 complexes has been recently supported by studies in insulin-resistant states induced by human placental growth hormone [115], obesity, and type 2 diabetes [100] and by short-term overfeeding of lean non-diabetic women [116]. Barbour et al [117] have demonstrated that insulin resistance of pregnancy is likely due to increased expression of skeletal muscle p85 in response to increasing concentrations of human placental growth hormone. Furthermore, women remaining insulin resistant postpartum have been found to display higher levels of p85 in the muscle [118].

Another small study of eight healthy lean women without a family history of diabetes, by Cornier et al showed that 3 days of overfeeding (50% above usual caloric intake) led to a significant increase in expression of p85 α , ratio of p85 α to p110, and a decline in insulin sensitivity. Within this experimental time frame, overfeeding did not cause any change in serine phosphorylation of either IRS-1 or S6K1, suggesting that increased expression of p85 α may be an early molecular step in the pathogenesis of the nutritionally induced insulin resistance [116].

7. Role of the adipose tissue in insulin resistance

Insulin has 3 major target tissues—skeletal muscle, liver and adipose tissue. It has been postulated that the insulin receptor (IR) is overexpressed in the cells of these tissues. Also only these three organs in the body are capable of glucose deposition and storage; no other cells can

store glucose. Removal of excess postprandial glucose by insulin occurs due to glucose uptake and storage in insulin sensitive target cells. About 75% of insulin-dependent postprandial glucose disposal occurs into the skeletal muscle [119]; therefore, it is the major target cell. While insulin-stimulated glucose disposal in adipose tissue is of little quantitative importance compared with that in muscle, regulation of lipolysis with subsequent release of glycerol and FFA into the circulation by insulin has major implications for glucose homeostasis.

It is widely accepted that increased availability and utilization of FFA contribute to the development of skeletal muscle insulin resistance [120-122]. Moreover, FFA have been shown to increase endogenous glucose production both by stimulating key enzymes and by providing energy for gluconeogenesis [123]. Finally, the glycerol released during triglyceride hydrolysis serves as a gluconeogenic substrate [124]. Consequently, resistance to the antilipolytic action of insulin in adipose tissue resulting in excessive release of FFA and glycerol would have deleterious effects on glucose homeostasis.

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 [125]. Increase in plasma FFA reduces insulin-stimulated glucose uptake, whereas a decrease in lipid content improves insulin activity in the skeletal muscle cells, adipocytes and liver [126]. Lipid-associated insulin resistance has also been shown to be linked to Glut4 translocation defects [27]. Studies have shown that raising plasma fatty acids in both rodents [75] and humans [127] abolishes insulin activation of IRS-1-associated PI 3-kinase activity in skeletal muscle where IRS-1 is most prevalent.

Adipose tissue can modulate whole body glucose metabolism by regulating levels of circulating free fatty acids (FFA) and also by secreting adipokines, thereby acting as an endocrine organ. However, 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 Ser/Thr phosphorylation of insulin receptor substrate-1 [78].

Some of the PKC isoforms represent such signalling molecules. PKC isoforms are classified as classical (cPKC α , β I, β II, γ), novel (nPKC δ , ϵ , θ , η) and atypical (aPKC ζ , λ). cPKCs are activated by Ca²⁺ and diacylglycerol (DAG), nPKCs are activated by only DAG and aPKCs respond to neither Ca²⁺ nor DAG [128]. Among all these PKC isoforms, nPKCs are said to have a modulatory role in insulin signalling. Recent reports also demonstrate a link between nPKCs and FFA induced insulin resistance.

Diacylglycerol is an attractive trigger for fat-induced insulin resistance in skeletal muscle, since it has been shown to increase in muscle during both lipid infusions and fat feeding and it is a known activator of novel protein kinase C (PKC) isoforms [78].

Recent studies have revealed that accumulation of intracellular lipid metabolites activate a serine kinase cascade involving PKC- ϵ , leading to decreased insulin receptor kinase activity

resulting in 1) lower insulin-stimulated IRS-2 tyrosine phosphorylation, 2) lower IRS-2-associated PI 3-kinase activity, and 3) lower AKT2 activity [129]. These fat-induced defects in insulin signalling in turn result in reduced insulin stimulation of glycogen synthase activity, resulting in decreased insulin-stimulated hepatic glucose uptake and reduced insulin stimulation of hepatic glucose production. Furthermore, reduced activity of AKT2 results in decreased phosphorylation of forkhead box protein O (FOXO), allowing it to enter the nucleus and activate the transcription of the rate-controlling enzymes of gluconeogenesis (phosphoenolpyruvate carboxykinase, glucose-6-phosphate phosphatase).

Increased gluconeogenesis further exacerbates hepatic insulin resistance and results in fasting hyperglycemia [129- 131]. Mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) is a key enzyme in de novo fat synthesis in liver, and recent studies in mtGPAT knockout mice have clearly implicated intracellular accumulation of diacylglycerol in triggering fat-induced insulin resistance in liver through activation of PKC- ϵ [132]. These data have important implications for the development of novel therapeutic agents to reverse and prevent hepatic insulin resistance associated with non-alcoholic fatty liver and type 2 diabetes [133].

Lipid infusion in rats and humans impaired insulin-stimulated glucose disposal into the muscle and concomitant activation of PKC θ and PKC δ [134, 135]. PKC δ has been shown to be a possible candidate for phosphorylation of the IR on serine residues [136]. These result in defects in the insulin signalling pathway imposing insulin resistance.

Recently, the PPAR γ 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 a crucial role of PGC-1 in the regulation of GLUT4 in muscle cells [137]. (PGC)-1 α and PGC-1 β are transcriptional factor co-activators that regulate mitochondrial biogenesis. In addition AMP kinase, which is activated during exercise and ischemia by a reduction in the ATP/AMP ratio, has been shown to be an important regulator of mitochondrial biogenesis, mediating its effects through MEF2- and CREB-mediated increased PGC-1 α expression [138-141]. Extracellular stimuli such as cold, thyroid hormone, and exercise stimulate mitochondrial biogenesis through PGC-1 in brown fat and skeletal muscle. Increased PGC-1 protein expression leads to increases in the target genes, including nuclear respiratory factor (NRF)-1. NRF-1 is a transcription factor stimulating many nuclear-encoded mitochondrial genes such as OXPHOS genes and also mitochondrial transcription factor A (mtTFA), a key transcriptional factor for the mitochondrial genome. mtTFA can bind to the D-loop of the mitochondrial genome and increase transcription of mitochondrial genes and replication of mitochondrial DNA [142].

A recent study by Ling et al. [143] demonstrated an age dependent decrease in muscle gene expression of PGC-1 α and PGC-1 β in young and elderly dizygotic and monozygotic twins without known diabetes

Adipose tissue also acts as an endocrine organ producing adipokines which modulate glucose homeostasis [144]. Currently, those most intensely discussed are tumor necrosis factor- α (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 [38]. Furthermore, mice lacking TNF α function were protected from obesity-induced insulin resistance [145]. 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 [146,147]. The 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 [148]. Thus, in humans, adiponectin levels correlate with insulin sensitivity. Because of its insulin-antagonistic effects, the adipocytokine resistin has attracted a lot of research interest. This is mainly based on data obtained *in-vitro* and from some animal models. Resistin decreases insulin-dependent glucose transport *in-vitro* and increases fasting blood glucose concentrations and hepatic glucose production *in-vivo* [149, 150].

8. Insulin resistance and Forkhead box protein O (FOXO)

The fasting hyperglycaemia in patients with Type 2 diabetes is the clinical correlate of the increased glucose production by the liver because of insulin resistance. This is as a result of the lack of inhibition of the two key gluconeogenic enzymes, phospho-enolpyruvate carboxykinase (PEPCK) and the glucose-6- phosphatase (G6Pase) catalytic subunit. Studies in hepatoma cells [151,152] suggest that Foxo1 and -3 regulate the transcription of reporter genes containing insulin response elements from the PEPCK and G6Pase promoters. Furthermore, Foxo1 is phosphorylated in an insulin-responsive manner by PIP3-dependent kinases, such as 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 [151,153]. There is increasing evidence that Foxo-proteins are critically involved in the insulin dependent regulation of gluconeogenic gene expression and insulin-resistance *in-vivo* [154, 155]. In addition, the PPAR γ co-activator-1 (PGC-1), a factor integrating the effects of glucocorticoids and cAMP on gluconeogenic gene expression in the liver [156, 157] is also regulated by PKB and Foxo1 [158].

9. FFA induced Inhibition of Insulin receptor (IR) gene expression by PKC ϵ

Clearly, the IR is one of the major targets in FFA-induced impairment of insulin activity. Recent studies performed *in-vivo* suggested that glucose uptake rather than intracellular glucose metabolism is the rate-limiting step for fatty acid induced insulin resistance in humans [159]. This indicates a mechanism in which accumulation of intracellular fatty acids or their metabolites results in an impairment of signaling through IRS/PI 3-kinase.

Recent evidence has shown that PDK1 can directly phosphorylate all PKCs including nPKCs [160]. The PKC ϵ isotype has recently been shown to be related to insulin resistance. Insulin stimulation of PDK1 phosphorylation is inhibited by an FFA, i.e. palmitate. PKC ϵ phosphorylation is dependent on PDK1; FFA incubation of skeletal muscle cells and adipocytes inhibited PDK1 phosphorylation but surprisingly increased PKC ϵ

phosphorylation. Inhibition of PDK1 by FFA is reflected in Akt phosphorylation as Akt phosphorylation is also dependent on PDK1 [161]. It has been shown that myristic acid incubation of HEPG2 cells causes myristoylation of PKC ϵ which results in constitutive phosphorylation of PKC ϵ at thr566/ser729 in the kinase domain required for PKC ϵ activity. This phosphorylation was totally independent of PDK1, which the workers demonstrated by using PDK1 knockout cells. In the same way, addition of palmitate to skeletal muscle cells or adipocytes may affect palmitoylation of PKC ϵ resulting in constitutive phosphorylation of PKC ϵ [162, 163]. Taken together, it is clear that FFA causes PDK1-independent phosphorylation of PKC ϵ which in turn translocates to the nucleus, and its time of entry into the nucleus coincides with inhibition of IR gene transcription.

10. Conclusion

In this review, current developments contributing to understanding of insulin resistance and to the pathogenesis of metabolic syndrome has been discussed. Among the many molecules involved in the intracellular processing of the signal provided by insulin, IRS-2, PKB, Foxo protein and p85 regulatory subunit of PI-3 kinase have attracted particular interest, because their dysfunction results in insulin resistance *in-vivo*. It has been well established that FFA are responsible for insulin resistance. This review focuses on the current trends in research in this important domain and throws light on certain possibilities regarding the manner in which FFA inhibits insulin activity.

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