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Insulin resistance, metabolic stress, and atherosclerosis

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1. ABSTRACT

Atherosclerosis, a pathological process that underlies the development of cardiovascular disease, is the primary cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM). T2DM is characterized by hyperglycemia and insulin resistance (IR), in which target tissues fail to respond to insulin. Systemic IR is associated with impaired insulin signaling in the metabolic tissues and vasculature. Insulin receptor is highly expressed in the liver, muscle, pancreas, and adipose tissue. It is also expressed in vascular cells. It has been suggested that insulin signaling in vascular cells regulates cell proliferation and vascular function. In this review, we discuss the association between IR, metabolic stress, and atherosclerosis with focus on 1) tissue and cell distribution of insulin receptor and its differential signaling transduction and 2) potential mechanism of insulin signaling impairment and its role in the development of atherosclerosis and vascular function in metabolic disorders including hyperglycemia, hypertension, dyslipidemia, and hyperhomocysteinemia. We propose that insulin signaling impairment is the foremost biochemical mechanism underlying increased cardiovascular morbidity and mortality in atherosclerosis, T2DM, and metabolic syndrome.

2. INTRODUCTION

Diabetes is a group of metabolic diseases marked by high blood glucose levels, either because of insufficient insulin production or impaired biological response to insulin, termed as insulin resistance (IR), a salient feature of type 2 diabetes mellitus (T2DM) (1, 2). It is reported that in the United States 11.3 percentage of adults age 20 years and older have T2DM, this percentage increases to 26.9 percentage in adults age 65 years and older (3). Death rates in adults having diabetes with pre-existing heart disease and stroke are about 2 to 4 times higher than adults without diabetes (4). Systemic IR is associated with impaired vascular insulin signaling (5) and blunted vascular effects of insulin (6). However, the molecular mechanisms linking IR to the development of atherosclerosis remain obscure.

Atherosclerosis is a multifactorial pathological process involving a wide range of cell types and tissues, including vascular, immune, and metabolic cells (7). Similarly, biological actions of insulin are mediated by its binding to cell surface insulin receptor, expressed in nearly every cell type in the body (8). Insulin has numerous effects on peripheral tissues that stimulate glucose uptake. The most sensitive tissues for the insulin-glucose uptake reaction are skeletal muscle and adipose tissue. However,

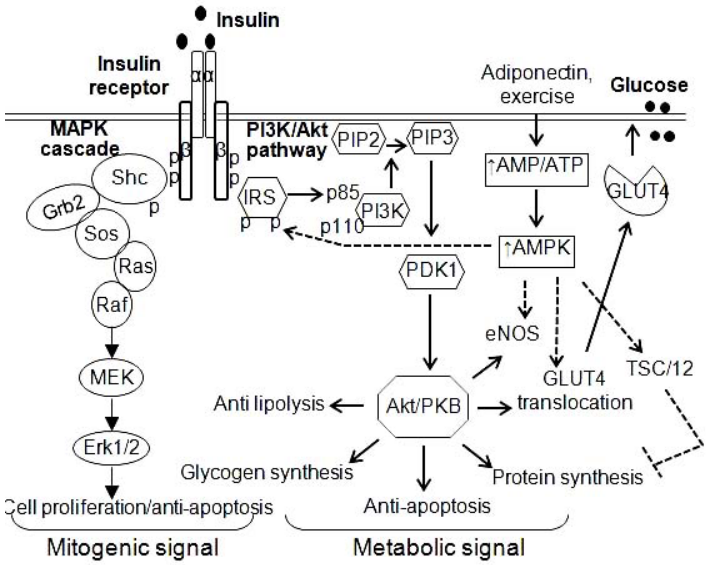

Figure 1. Insulin signaling. Insulin binds to its receptor causes a conformational change, autophosphorylation of the receptor, and activation of two pathways; The PI3K/Akt pathway leads to IRS tyrosine phosphorylation, which further phosphorylate PI3K subunit p85 and heterodimerization of p85 and p110. Akt activation upon the heterodimerization leads to PIP3 generation, which causes tyrosine phosphorylation/activation of PDK1; leading to serine phosphorylation/activation of Akt (also termed PKB). Activated Akt/PKB causes serine phosphorylation of eNOS/activation, rab-GTPase-activating protein-AS160, and HSL serine phosphorylation, inhibition of glycogen synthase kinase-3, threonine phosphorylation of S6K, and induction in Bcl-x gene expression; leading to NO generation, GLUT4 membrane translocation/glucose uptake, anti-lipolysis, glycogen synthesis, protein synthesis, and reduced-apoptosis. Exercise or insulin sensitizer adiponectin activates AMPK, which causes serine phosphorylation of IRS (at amino acid position 789- a positive regulation), eNOS (a positive regulation), and rab-GTPase-activating protein-AS160 and threonine phosphorylation of TSC1/2 (a negative regulation); leading to PI3K/Akt activation, NO production, glucose uptake, and protein synthesis inhibition. The MAPK cascade leads to activation of Ras signaling, cell proliferation, and inhibits apoptosis. IRS: insulin receptor substrate, PIP2: phosphatidylinositol 4,5-bisphosphate, PIP3: phosphatidylinositol 3,4,5-trisphosphate, PDK1: phosphoinositide-dependent kinase-1, PI3K: phosphatidylinositol 3-kinase, PKB: protein kinase B, eNOS: endothelial nitric oxide synthase, AS160: Akt substrate of 160 kDa, HSL: hormone sensitive lipase, S6K: ribosomal protein S6 kinase, NO: nitric oxide, GLUT4: glucose transporter4, AMP: adenosine monophosphate, ATP: adenosine triphosphate, AMPK: 5‘-adenosine monophosphate-activated protein kinase, TSC1/2: tuberous sclerosis protein 1 and 2- tumor suppressor, Shc: sh2 domain-containing alpha-2 collagen-related protein, Grb2: growth factor receptor-bound protein 2, Sos: son of sevenless, MAPK: mitogen-activated protein kinase, MEK: mitogen-activated protein kinase kinase, Erk1/2: extracellular signal-regulated kinase1/2.

insulin receptor signaling exerts important biological effects on vascular cells and regulates vessel dilation and contraction (9, 10). Moreover, insulin receptor signaling regulates monocyte differentiation into macrophages (11). Certainly, insulin and its receptor are expressed in metabolic organs like the skeletal muscle and pancreas as well as in liver and adipose tissue, which plays an important role in glucose and lipid metabolism (8).

Herein, we describe the tissue and cellular distribution of insulin receptor, and the role of its signaling in physiologic and pathophysiologic conditions. We emphasize the impact of impaired insulin signaling in vascular dysfunction, hypertension, hyperglycemia, dyslipidemia, and other metabolism disorders.

3. INSULIN RECEPTOR SIGNALING

Biological actions of insulin are initiated by its binding to its cell surface receptor, which results in autophosphorylation of the receptor and activation of its intrinsic tyrosine kinase activity (12, 13). The phosphorylated insulin receptor functions as a tyrosine kinase leading to activation of 2 distinct pathways (Figure 1). Via the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, it phosphorylates insulin receptor substrate (IRS) family members IRS-1 to 4 (14) at tyrosine residues. Evidence has demonstrated that IRS contains several tyrosine phosphorylation sites and about 50 serine/threonine (Ser/Thr) phosphorylation sites (15). Tyrosine phosphorylation sites such as those found at amino acid positions 608 and 628 (Tyr^{608} and Tyr^{628}); have been shown to positively regulate IRS function. Whereas, Ser/Thr phosphorylation sites such as those found at amino acid positions 307, 612, and 632 (Ser^{307}, Ser^{612}, and Ser^{632}) have been shown to negatively regulate IRS function by increasing release of IRS from its internal membrane pools and thus increasing proteosomal degradation. However, evidence shows that Ser/Thr phosphorylation of IRS at amino acid position 789 (Ser^{789}), can positively regulate

IRS function (16). Thus, the delicate balance that exists between positive tyrosine/serine phosphorylation sites and negative serine phosphorylation sites regulates IRS function (15).

Tyrosine-phosphorylated IRS then binds to the Src homology 2 (SH2) domain-containing adaptor protein p85, a regulatory subunit of PI3K, resulting in activation of the catalytic p110 subunit of PI3K (17, 18). Activated PI3K converts phosphatidylinositol 4, 5-bisphosphate (PIP2) to phosphatidylinositol 3, 4, 5-trisphosphate (PIP3). This initiates a cascade of serine kinases where phosphoinositide dependent kinase-1 (PDK-1) is phosphorylated and activated in order to phosphorylate v-akt murine thymoma viral oncogene (Akt), also known as protein kinase B (PKB), which further phosphorylates and activates downstream substrates (19). This cascade eventually culminates in the pleiotropic biological actions of insulin and contributes to the metabolic action of insulin.

In the parallel mitogen-activated protein kinase (MAPK) pathway, an activated insulin receptor phosphorylates its intracellular substrate SH2 domain-containing alpha-2 collagen-related protein (Shc), which binds to growth factor receptor-bound protein 2 (Grb2), and results in activation of pre-associated guanosine triphosphate (GTP) exchange factor son of sevenless (Sos). Sos further activates the small GTP binding protein Ras, which then initiates a phosphorylation cascade involving Raf, MAPK/extracellular signal-regulated kinase kinase (MEK), and extracellular signal-regulated kinase (Erk). There are two isoforms of Erk: Erk1 and Erk2 that are ubiquitously expressed. Erk 1/2 are often referred as p42/p44 MAPK (20). Erk1/2 regulates mitogenesis, growth, and differentiation. Apart from Erk1/2, MAPK subfamilies include two other MAPKs; the c-Jun NH2-terminal kinase (JNK) and p38 MAPK. JNK and p38 MAPK are called stress sensitive kinases (21).

JNK protein kinases are encoded by the three genes JNK1, JNK2, and JNK3, which are alternatively spliced to form the JNK isoform (21, 22). The two mitogen-activated protein kinase kinase (MAPKK or MKK) proteins that act as upstream JNK activators are MKK7, which is primarily activated by pro-inflammatory cytokines, and MKK4, which is primarily activated by environmental stress and growth factors. A major JNK target is the transcription factor activator protein-1 (AP-1), which is an important regulator of gene expression (21). JNK protein kinases are involved in the regulation of cell proliferation, differentiation, survival, and migration (22).

The p38 MAPK pathway shares many similarities with the other MAPK pathway, being that it is associated with inflammation, cell proliferation, differentiation, and survival. Four genes are encoded by p38 MAPK, p38 alpha, p38 beta, p38 gamma, and p38 delta. p38 MAPK regulates the expression of many cytokines and is activated in immune cells by inflammatory cytokines. It also has an important role in the activation of immune responses (21, 23). p38 MAPK is also activated by other stimuli including hormones and environmental stress. Moreover, MKK3, MKK6, and MKK4 serve as upstream MAPKs, which are responsible for p38 activation (23).

Emerging evidence suggests that insulin receptor signaling can be enhanced by 5' adenosine monophosphate-activated protein kinase (AMPK), which is a highly conserved Ser/Thr kinase. AMPK is a heterotrimeric complex consisting of a catalytic alpha subunit and regulatory beta and gamma subunits. AMPK is activated under the conditions of hypoxia, glucose deprivation, and ischemia, which deplete cellular adenosine triphosphate (ATP) and increase adenosine monophosphate (AMP) levels, leading to an increase in AMP/ATP ratio (24). It has been reported that exercise, adiponectin and the anti-diabetic drug metformin, can activate AMPK (Figure 1). AMPK activation switches on catabolic pathways that generate ATP, by causing serine phosphorylation of IRS (at amino acid position 789- a positive regulation) (16), endothelial nitric oxide synthase (eNOS) (a positive regulation) (25), and rab-GTPase-activating protein, Akt substrate of 160 kDa (AS160) (a positive regulation) (26); leading to PI3K/Akt activation, nitric oxide (NO) production, and glucose uptake. Whereas, it switches off ATP-consuming anabolic pathways by causing serine phosphorylation of hormone sensitive lipase (HSL) and its inhibition, and threonine phosphorylation of tuberous sclerosis protein 1 and 2 (TSC), leading to anti-lipolysis and inhibition of protein synthesis (27). Due to this background information, AMPK has been suggested to play a role in the pathogenesis of T2DM and thus considered a potential therapeutic target of T2DM. Support for this concept comes from studies using animal models that demonstrate that acute and chronic treatment with the AMPK-activating agent 5-aminoimidazole-4-carboxamide ribofuranoside (AICAR) improves glucose homeostasis and insulin sensitivity (28).

4. INSULIN SIGNALING IN METABOLIC TISSUES

The PI3K/Akt pathway is predominant in the metabolic tissues which controls rapid stimulation of glucose uptake, lipid synthesis, and energy metabolism (Figure 1). Insulin stimulates glucose uptake in muscle and adipocytes via translocation of GLUT4 vesicles to the plasma membrane and induces glycogen synthesis by inhibiting glycogen synthase kinase -3 (GSK-3).

4.1. Pancreas

The islet beta-cells of the pancreas uniquely secrete insulin. Insulin secretion is closely regulated in order to maintain blood glucose levels within a narrow physiological concentration range. Maintenance of the beta-cell mass is a dynamic process which involves an increase (replication/neogenesis) and decrease in the numbers (apoptosis) of beta-cells in order to regulate blood glucose levels within a normal range (29). Numerous pieces of evidence have demonstrated anti-apoptotic role of insulin in the pancreatic beta cells. Insulin activates its own gene (30) and initiates the PI3K/Akt pathway, leading to anti-apoptosis and beta cells survival. Majority of data suggests that beta-cell insulin signaling is essential for insulin exocytosis (31). Study using beta-cell insulin

receptor deficient mice, has demonstrated a marked reduction in islet number, beta-cell mass, and insulin secretion (32). It has been shown that beta-cell damage and IR are partially triggered by inflammatory, oxidative, and endoplasmic reticulum stress-induced pathways (33). It is reported that activation of MAPK pathway (JNK (34, 35), p38 MAPK (36), and Erk (37)) promotes insulin resistance, suppresses insulin production, secretion, and increases apoptosis of beta-cells.

Hence, insulin signaling plays an important role in the pancreatic beta cell and resistance to its actions contributes to beta-cell failure, a pathogenesis of T2DM, which leads to consequent glucotoxicity. This is supported by the observations that elevated glucose concentrations induce beta-cell apoptosis in cultured islets from diabetes-prone Psammomys obesus (38) and from humans (39, 40). In addition to glucotoxicity, lipotoxicity resulted from insulin signaling impairment in adipocytes, can also cause beta-cell dysfunction (41, 42). Lipotoxicity is commonly observed in the obese, IR, and individuals with T2DM (43). Therefore, at the later stage of T2DM, glucotoxicity and lipotoxicity further apply metabolic stress on beta-cells leading to decreased insulin synthesis and beta-cell dysfunction (44).

4.2. Skeletal muscle, adipose tissue, and liver

Following nutrient intake, plasma glucose triggers pancreatic beta cells to release insulin into the blood. This potent anabolic hormone regulates various post-prandial proceedings. Controlling the cellular localization of the GLUT4 (a family of glucose transporters) in muscle and adipose tissue is crucial in the management of blood glucose homeostasis. Normally, in the basal state this transporter resides in an intracellular membrane compartment. Following insulin stimulation, it rapidly populates the plasma membrane. Arrival of GLUT4 at the plasma membrane leads to cellular glucose influx (Figure 1). Moreover, insulin also suppresses production of glucose and very low-density lipoprotein (VLDL) in the liver (2, 45).

Insulin-induced GLUT4 translocation requires PI3K-mediated signal involving upstream insulin receptor, IRS, downstream Akt, and protein kinase C (PKC) target enzymes, which induces phosphorylation of rab-GTPase-activating protein, AS160, leading to glucose uptake. Metabolic actions of insulin in liver, adipose tissue, and skeletal muscle rely on tyrosine phosphorylation of IRS proteins. Results from specific knockout models have shown that different IRS isoform appear to have specific roles in different tissues. IRS-1 proteins seem to be firmly associated with glucose homeostasis in skeletal muscle, adipose tissues, and pancreatic beta cells (12), while IRS-2 is essential for liver metabolism and beta-cell proliferation (46, 47). IRS-3 appears to play a role in the adipose tissue (46).

Normal metabolic processes in skeletal muscle and adipose tissue include stimulating glucose uptake and in liver inducing glycogen synthesis, reducing glucose production, and its release in to the blood. All of which are impaired in the IR condition (4). Although, other functions of insulin can be affected.

In adipose tissue, insulin reduces the release of free fatty acid (FFA) by PI3K/Akt signaling, results in the HSL inhibition, which hydrolyzes triglycerides to release fatty acids (48). IR leads to increased plasma FFA and consequent lipotoxicity. In addition, FFA induces hepatic glucose generation and reduces muscle glucose uptake, all these contribute to elevated blood glucose levels (49, 50). Moreover, it is reported that the level of plasma adiponectin (secreted by adipose tissue) is reduced in T2DM patients (51), which regulates insulin sensitivity with energy metabolism and serves to link obesity with IR. AMPK activation by exercise, metformin or adiponectin, increased insulin-independent and insulin-dependent muscle glucose uptake (52), fatty acid oxidation (51), and inhibited gluconeogenesis (53), lead to an increase in insulin sensitivity.

Impaired insulin signaling in liver may contribute to elevated plasma FFA levels (50). Increased release of FFA from adipose tissue reduces skeletal muscle FFA uptake. The net consequence of this may be an augmented influx of FFA to the liver, which leads to fatty liver and exacerbates IR (54). In accordance with the above views, insulin actions in metabolic tissues play an essential role in regulating glucose homeostasis (46). The consequent hyperglycemia, dyslipidemia, and inflammation underlie the reciprocal relationships between IR derived atherosclerosis.

5. RELEVANCE OF METABOLIC STRESS WITH IR AND ATHEROSCLEROSIS

IR is a hallmark of metabolic disorders including obesity and T2DM. Multiple metabolic risk factors contribute to the development of IR including: hyperglycemia, increase FFA levels, and hyperhomocysteinemia (HHcy). These risk factors also contribute to endothelial dysfunction- an early event in the atherosclerosis development.

5.1. Hyperglycemia

IR is an initiating pathogenic mechanism in T2DM. When beta cells of the pancreas fail to secrete enough insulin to overcome IR, overt hyperglycemia develops (55). However, hyperglycemia is not only a consequence of, but also an important factor in worsening IR. Studies have demonstrated that hyperglycemia impairs insulin sensitivity and insulin induced Akt/PKB phosphorylation/activation in liver, adipose tissue (56), and skeletal muscle (57), thus causing an increase in hepatic glucose production and impair GLUT4 translocation/glucose uptake. All of these factors thus contribute to IR.

Hyperglycemia impairs both metabolic and vascular actions of insulin through multiple biochemical and cellular mechanisms. These IR derived mechanisms are multiply resourced by the following: 1) elevated oxidative stress, 2) increased flux through polyol and hexosamine

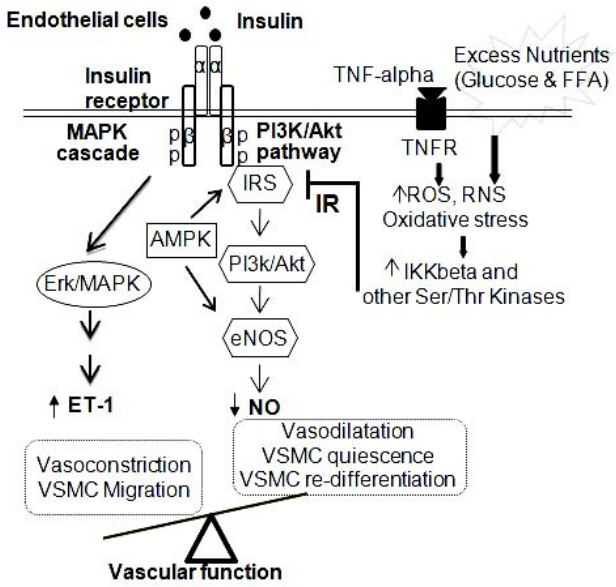

Figure 2. PI3K/Akt pathway impairment is a key feature of IR and vascular dysfunction. Parallel and balanced insulin signaling between PI3K/Akt/eNOS and Ras/MAPK maintains normal vascular tone in healthy conditions. A variety of stimuli, including hyperglycemia, elevated FFA levels, cytokines, and others, increase production of ROS, RNS, and oxidative stress. These leads to activation of IKKbeta and other stress sensitive Ser/Thr kinases, which cause serine phosphorylation of IRS (at amino acid position 307- a negative regulation) and PI3K/Akt inhibition (IR effect). While PI3K/Akt/eNOS signaling is impaired, the Ras/MAPK pathway is less affected or even enhanced due to hyperinsulinemia, leading to reduce NO production and enhance ET-1 secretion, resulting in impaired vascular function. AMPK counteract the stress induced IR by serine phosphorylation of eNOS (a positive regulation) and IRS (at amino acid position 789- a positive regulation), which improves vascular function and IR. IR: insulin resistance, IRS: insulin receptor substrate, PI3K: phosphatidylinositol 3-kinase, eNOS: endothelial nitric oxide synthase, NO: nitric oxide, MAPK: mitogen-activated protein kinase, Erk1/2: extracellular signal-regulated kinase1/2, ET-1: endothelin-1, FFA: free fatty acid, ROS: reactive oxygen species, RNS: reactive nitrogen species, TNF-alpha: tumor necrosis factor-alpha, IKKbeta: inhibitor of nuclear factor kappa B kinase beta subunit, Ser/Thr: Serine/Threonine, VSMCs: vascular smooth muscle cells, AMPK: 5' adenosine monophosphate-activated protein kinase.

biosynthetic pathways, 3) activation of diacylglycerol (DAG) and PKC, 4) formation of advance glycation end-products (AGE). All of these factors then contribute to endothelial dysfunction and accelerate atherosclerotic process (Figure 2). Remarkable evidences have demonstrated an independent relationship between atherosclerosis and glycemic control in patient with T2DM.

Reactive oxygen species (ROS) generated by high glucose impaired, insulin stimulated activation of Akt and eNOS (58). Overexpression of uncoupling protein-1 (UCP-1) or manganese superoxide dismutase (MnSOD) prevents these inhibitory effects of glucose and restores vasodilator actions of insulin (59). Besides impairing insulin signaling pathways, ROS decreases NO bioavailability, reduces cellular tetrahydrobiopterin levels, and promotes generation of superoxide by eNOS (59, 60). In vasculature, increased oxidative stress may impair vessel reactivity, increases vascular smooth muscle cell (VSMC) proliferation, macrophage adhesion, platelet activation, and lipid peroxidation, which ultimately leads to vascular complications (61).

Hyperglycemia may impair metabolic and vascular actions of insulin by increased flux through the polyol and hexosamine biosynthetic pathway (62, 63). Polyol pathway requires an enzyme aldose reductase. Under normal condition, this enzyme has low affinity for glucose (64). Hyperglycemia increases its activity, leads to an increase in reduction of glucose to sorbitol, which is then oxidized to fructose. This reaction consumes a cofactor nicotinamide adenine dinucleotide phosphate (NADPH), which is an essential cofactor for regenerating a critical intracellular antioxidant, reduced glutathione (64). By reducing the amount of reduced glutathione, the polyol pathway increases susceptibility to intracellular oxidative stress (65).

The hexosamine biosynthetic pathway serves as a nutrient sensor, which plays a role in IR and vascular complications by causing reversible O-linked beta-N-acetylglucosamine (O-GlcNAc) modifications at regulatory serine/threonine phosphorylation sites on proteins involved in insulin signaling pathway. For example, increased O-GlcNacylation of IRS-1 may lead to reduced insulin-stimulated translocation of GLUT4 and decreased glucose uptake (66, 67).

Hyperglycemia and elevated FFA increase PKC activity by enhancing de novo synthesis of DAG from glucose (68). This increases oxidative stress through activation of NADPH oxidase. It has been shown that

increased PKC activity promotes vascular occlusion and vascular inflammation by decreasing NO production and increasing endothelin-1 (ET-1) production (69, 70).

One of the most harmful effects of hyperglycemia is the formation of AGE by the non-enzymatic reaction between glucose and proteins/lipids on the vessel wall (71). Formation of AGE is augmented in the presence of elevated circulating glucose (72). Permanent AGE formation disrupts the molecular confirmation and alters the enzymatic activity. For example, human glycated end-products inhibit insulin-stimulated IRS-1 and IRS-2 tyrosine phosphorylation (73). Furthermore, by binding through its receptor (RAGE) and activating of NADPH oxidase, AGE produces ROS (74). AGE reduces NO bioavailability and eNOS expression by increasing eNOS mRNA degradation (75, 76). AGE also enhances ET-1 expression through the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) (77). Hence, AGE alters the balance between NO and ET-1 favoring vasoconstriction and endothelial dysfunction.

Thus, hyperglycemia contributes to IR and endothelial dysfunction, by inhibiting PI3K/Akt signaling pathway and over–activating MAPK signaling pathway.

5.2. Dyslipidemia

Increase in plasma FFA levels contributes to IR, by inhibiting glucose transport and/or phosphorylation with a subsequent reduction in the rates of glucose oxidation and muscle glycogen synthesis (78). Cross-sectional study of young, normal-weight offspring of T2DM patients, have demonstrated an inverse relationship between fasting plasma fatty acid levels and insulin sensitivity, which suggest that altered fatty acid metabolism contributes to IR (79). Excess FFA increases ROS generation, which can also contribute in the pathogenesis of IR (80).

IR has been implicated in promoting dyslipidemia, a well established risk factor of CVD. Dyslipidemia is characterized by hypertriglyceridemia, elevated blood levels of apolipoprotein B, small, dense low-density lipoprotein (LDL) cholesterol, and low high-density lipoprotein (HDL) cholesterol levels. High circulating FFA levels enhances glucose output from the liver and reduces glucose disposal in skeletal muscle, thereby contributing to IR. The role of elevated circulating FFA has been emphasized as a major factor that connects IR and dyslipidemia (1). Moreover, increased FFA stimulates liver to assemble VLDL. Increased plasma levels of VLDL, accelerate VLDL accumulation in the blood vessel wall, followed by higher levels of small, dense LDL. They consequently deliver amounts of cholesterol to the vessel wall, directly bound to proteoglycans within the extracellular matrix and can thereby contribute to plaque formation (49).

Elevated cellular levels of lipid metabolites such as DAG, ceramide, and long-chain fatty acyl CoAs activate PKC and inhibitor of nuclear factor kappa B kinase beta subunit (IKKbeta) leading to serine phosphorylation of IRS-1 at amino acid position 307 (a negative regulation) and inhibit IRS-1 tyrosine phosphorylation in the skeletal muscle (81) and endothelial cell (EC) (82). FFA increases ROS generation and impairs insulin/PI3K/PDK1/Akt/eNOS pathway in EC (Figure 2) (83, 84). FFA infusion intensifies insulin mediated ET-1 release in IR individuals (85). Diminishing forearm lipid oxidation decreases insulin-mediated ET-1 release whereas concurrently increasing NO bioavailability and glucose uptake (86).

5.3. Hyperhomocysteinemia (HHcy)

An elevated plasma level of homocysteine (Hcy) is an independent risk factor for CVD (87). Hcy, a sulfhydryl-containing amino acid, is a metabolite of methionine. Metabolism of Hcy occurs through 2 pathways. Remethylation pathways to methionine, catalyzed by enzymes methionine synthase and methylene tetrahydrofolate reductase (MTHFR) and transsulfuration pathway to cysteine, catalyzed by enzyme cystathionine beta synthase (CBS) - a pyridoxine (vitamin B6) dependent enzyme. Both folic acid and cobalamin (vitamin B12) are important co-factors in remethylation reaction. Hereditary enzyme deficiency, nutritional deficiency of folate, pyridoxine, and cobalamin are associated with elevated blood levels of Hcy and accelerate atherosclerosis (87). It has been shown that HHcy increases oxidant stress, platelet aggregation, enhances activation of coagulation system, VSMC proliferation, and inhibit EC proliferation (88-90). IR has been suggested to arise from similar mechanism and may be possible link between HHcy and atherosclerosis (91).

It has been demonstrated that insulin resistant T2DM patients with CVD have elevated plasma Hcy levels (92). Furthermore, it is reported that acute hyperinsulinemia leads to increased plasma Hcy levels in obese IR patients (93-95). A recent clinical study observed that IR and endothelial function are improved in patients with metabolic syndrome after folate and vitamin B12 therapy (96). This study suggested that prolonged folate administration decreases Hcy and reduces insulin levels, thereby improving IR.

Studies in cultured hepatoma cells indicated that Hcy thiolactone inhibit insulin signaling and it's action by increasing oxidative stress (97, 98). In a separate study using mouse hepatocytes indicated that hyperinsulinemia causes HHcy by decreasing activity of Hcy metabolizing enzymes such as MTHFR and CBS (99, 100). Furthermore, in a study using rats fed with high fat and sucrose diet, has developed obesity which is associated with hyperinsulinemia, IR, and HHcy, along with changes in CBS and MTHFR enzymes in liver (101). These studies suggest that HHcy may cause IR, or vice versa, and that this HHcy and IR connection play important role in the development of CVD. The relationship between Hcy and IR is still ambiguous and complicated, remain to be uncovered.

6. INSULIN SIGNALING IN VASCULAR CELLS (FIGURE 2)

Briefly, atherosclerosis is initiated by endothelial injury and VSMC proliferation, and then injured EC will attract monocytes attending and adhering to endothelium.

Loss of EC will also expose intimae to blood stream and activate platelets. That promotes monocytes differentiation to macrophages and then macrophages engorge with oxidized low-density lipoprotein to generate foam cells, finally build atheroma beneath the endothelial monolayer (102).

6.1. Endothelial cells (ECs)

Insulin receptors were initially identified and characterized by ^{125}I-insulin receptor binding studies in human umbilical vein endothelial cell (HUVEC) (103). In recent years, a complete insulin signaling pathway leading to phosphorylation and activation of eNOS has been elucidated in EC. It has been established that insulin is a vasoactive hormone (6), which stimulates production of NO in EC (6, 104) via IRS/PI3K/Akt pathway (69, 105). This reaction can be blunted in presence of PI3K-inhibitors, wortmannin and LY294002 (69). Down-regulation of Ras, an upstream mediator of MAPK signaling has little effect on insulin-induced NO production in EC (106, 107). Insulin-induced NO production amplifies blood flow and functional capillary recruitment to peripheral tissues. These result in increased delivery of insulin and glucose to skeletal muscle and adipose tissue, which contributes to insulin-mediated glucose uptake. In contrast, impaired insulin signaling inhibits NO production, vascular reactivity, and reduces the delivery of glucose and insulin to the peripheral tissues. It is reported that adiponectin expression is reduced in the aorta of leptin receptor–deficient db/db mice (T2DM mouse model) (108). Adiponectin protects against endothelial dysfunction by activating AMPK and producing NO. Moreover, it has demonstrated that AMPK activation reduces oxidative stress and improves endothelial function in hyperglycemic condition (109).

Insulin can also activate MAPK-dependent signaling pathway, which regulates secretion of the vasoconstrictor ET-1 from endothelium. MAPK pathway regulates biological actions related to growth, mitogenesis, and differentiation (110, 111). Studies in bovine aortic EC (BAEC) and in rat mesenteric arteries have confirmed that insulin acutely stimulates ET-1 secretion via MAPK-dependent signaling pathway (112).

Moreover, insulin stimulates phosphorylation of the alpha subunit of farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase), and activities of both prenyltransferases in EC (113). Activation of FTase and GGTase cause the prenylation of Ras and Rho proteins, which are increased in the aorta and liver of IR humans and animals. Prenylated Ras and Rho proteins are associated with increased mitogenetic responses (114, 115). Collectively, impaired insulin-PI3K and -MAPK signaling in EC contributes to endothelial dysfunction in T2DM.

6.2. Vascular smooth muscle cells (VSMCs)

The presence of insulin receptor was demonstrated by ^{125}I-insulin binding studies in cultured human aortic smooth muscle cells (HASMC) (116). The number of insulin receptors is 10-fold lower in bovine aortic smooth muscle cell (BASMC) compared with BAEC (116). Both insulin and insulin-like growth factor-1 receptors (IGF-1R) are present on rat aortic smooth muscle cell (RASMC), and these receptors are distinct from each other in terms of binding affinity of insulin and IGF-1 (117). Insulin receptors on VSMC are structurally and functionally similar to those in metabolic tissues (8, 118). However, VSMC and metabolic cells use different types of glucose transporter, GLUT1 in VSMC and GLUT4 in skeletal muscle (119).

It has been shown that insulin maintains VSMC quiescence and differentiation via PI3K-dependent pathway. The MAPK-dependent signaling pathway controls proliferation and migration (120, 121). A MEK1/2 inhibitor, PD98059 blocked the mitogenic responses to insulin (121) and PI3K inhibitor; wortmannin reversed quiescent status in VSMC (120). In vasculature, bioavailable NO mainly originates from endothelium. Endothelium-derived NO diffuses into VSMC where it activates guanylate cyclase, which augments cyclic guanosine monophosphate (cGMP) levels that leads to vasorelaxation. Insulin increases NOS activity (eNOS and iNOS) and NO dependent cGMP production in VSMC (122, 123). Both genistein (a tyrosine kinase inhibitor) and wortmannin (a PI3K-inhibitor) block insulin-induced NO production in VSMC, suggesting that the insulin receptor tyrosine kinase and subsequent activation of PI3K are both necessary for insulin-induced eNOS or iNOS activation in VSMC (122).

In general, insulin has anti-atherogenic, anti-inflammatory, and anti-thrombotic effects, and these effects are reversed or blunted in the IR state.

7. RELEVANCE OF IR IN VASCULAR CELLS WITH ATHEROSCLEROSIS

Increasing evidence has suggested an association of hyperinsulinemia with the development of atherosclerosis in diabetics. Endothelial dysfunction, an early event of atherosclerosis, has been documented in the IR states in animals and humans (124, 125). PI3K/Akt pathway is blunted in IR states, leads to eNOS inactivation and reduces NO production in EC (5, 126). Mitogenic MAPK pathway in EC and VSMC remains intact and its associated cell effects may in fact be enhanced (112, 127). The blunted PI3K/Akt signaling and enhanced MAPK signaling contributes to impaired vascular function, leading to further atherogenesis (Figure 2). This is supported by human studies which demonstrate that insulin infusion in healthy individuals stimulates vasodilation and increases blood flow to the peripheral tissues (11, 124, and 128). These effects are blunted in the IR and T2DM individuals (11, 124, and 128). A study using in vitro model of metabolic IR (by blocking PI3K/Akt-dependent signaling) with compensatory hyperinsulinemia (by exposing cells to high insulin concentration) in endothelium, shows decreased eNOS protein expression and NO production (113). While increased prenylation of Ras and Rho proteins via MAPK-dependent signaling, with increased expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM), and E-selection in EC,

and increased rolling interactions of monocytes with EC (8). Impaired insulin receptor autophosphorylation, IRS-1 tyrosine phosphorylation, associated PI3K activity, and Akt activity was observed in blood vessels from homozygous Zucker fatty rats (a model of obesity-related IR) (104). However, MAPK phosphorylation was not changed (104). Reduced eNOS expression and NO production are found in aorta from endothelial cell specific insulin receptor knockout mice and in mice with dysfunctional insulin receptor (Thr1134 substituted with Ala in the kinase domain) (129). Moreover, IR is accompanied with impaired endothelium-dependent vasodilation in IRS-1 deficient mice (130). It has been shown that AMPK counteract the stress induced IR by increasing insulin induced IRS/PI3K/eNOS pathway (Figure 2) (16, 25). These contribute in improving IR and vascular function in T2DM.

Furthermore, in a study using insulin receptor and apolipoprotein E (ApoE) deficient mice indicated that endothelial IR may accelerate atherosclerosis by causing impaired eNOS activation, increased endothelial VCAM expression, and increased leukocyte interaction with EC (131). It is also of interest to determine whether the converse is true, whether endothelial dysfunction inherently confers IR. This is supported by the study using both eNOS and iNOS mouse knockout models (132). Short-term insulin infusion under isoglycemic condition modestly decreases or has no effect on arterial blood pressure. However, hypertension (high blood pressure) occurs in IR, increases fluid volume, arterial stiffness, and even impaired insulin handling, leading to narrowed lumen diameter, which contributes to the development of atherosclerosis (133).

Several mechanisms have been proposed to explain the contribution of impaired insulin signaling in VSMC to atherosclerosis. These include promoting VSMC proliferation and migration, increased extracellular matrix proteins expression, and inducing apoptosis. Hyperglycemia increases PKC activity, NF-kappaB production, and generation of oxygen-derived free radicals in cultured VSMC (134), and heightens the migration of VSMC into nascent atherosclerotic lesions (135). T2DM patients tend to have fewer VSMC in the lesions, which increases the propensity for the plaque rupture (136), and increases vessel wall cytokines, which diminish VSMC, decrease collagen, and increase matrix metalloproteinases, yielding an increased propensity for plaque destabilization and rupture (137).

8. INSULIN SIGNALING IN IMMUNE CELLS

Individuals with T2DM have overly active immune responses, leaving their bodies rife with inflammatory chemicals. On the contrary, IR could also cause inflammation. Inflammatory responses-inflammation is a complex stereotypical reaction of the body, causing damage of its cells and vascularized tissues.

8.1. Monocytes/Macrophages

Monocytes and macrophages are the basic cell types of mononuclear phagocytic system. These are involved in all stages of the immune response. Insulin signaling in macrophages leads to increase in viability, protein synthesis and secretion, and phagocytosis (138).

Insulin activates IRS/PI3K/Akt signaling in monocytes/macrophages, similar to that in the vascular cells and metabolic tissues except that IRS-1 isoform is undetectable in monocytes/macrophages (139, 140). Decreased insulin receptor/IRS-1/2 tyrosine phosphorylation, and reduction in downstream signaling was observed in macrophages from obese, insulin-resistant mice (139) and monocytes from obese subjects (141).

Macrophage apoptosis in atherosclerotic lesions may add further to the monocyte recruitment, by the release of cytokines and may therefore, exacerbate the advancement of vascular lesion (142-144). It has been shown that insulin increases anti-apoptotic gene Bcl-x expression and leads to reduction in apoptosis in macrophages differentiated from monocytic THP-1 cell line (145), which is repressed by PI3K inhibitor (145). This study has demonstrated that in IR state, the defensive function of insulin to reduce macrophage apoptosis may be lost as the PI3K pathway is blunted under these conditions.

8.2. T lymphocytes

Lymphocytes and leukocytes are the cellular components of inflammation, which normally reside in blood and must extravasate the inflamed tissue to aid in inflammation. Chronic inflammation is mediated by mononuclear cells including lymphocytes and monocytes (8).

Unlike monocytes, circulating T lymphocytes do not have insulin receptors. However, T lymphocytes have the atypical capability to express insulin receptors following presentation of an antigen in vivo and an antigen or mitogen in vitro (146), which activate IRS/PI3K/Akt signaling (147) and increase glucose uptake (148).

Similar to monocytes, T lymphocytes adhere and infiltrate across the vascular endothelium into the subendothelial space, where they are immunologically active (149). In patients with T2DM, a product of proinsulin C-peptide, which is increased in insulin-resistant subjects, colocalizes with $CD4^+$ T lymphocytes in atherosclerotic lesions and acts as a chemotactic stimulus for T lymphocytes to adhere and penetrate the vessel wall (150).

9. RELEVANCE OF IR IN IMMUNE CELLS WITH ATHEROSCLEROSIS

Remarkable evidences have demonstrated that atherosclerotic process is regulated by inflammatory mechanisms (151, 152). Vascular endothelium is both affected by and contributes to the inflammatory process. Besides, IR has been progressively associated with inflammatory state (153-155); it may impair endothelial function and contributes to atherosclerosis.

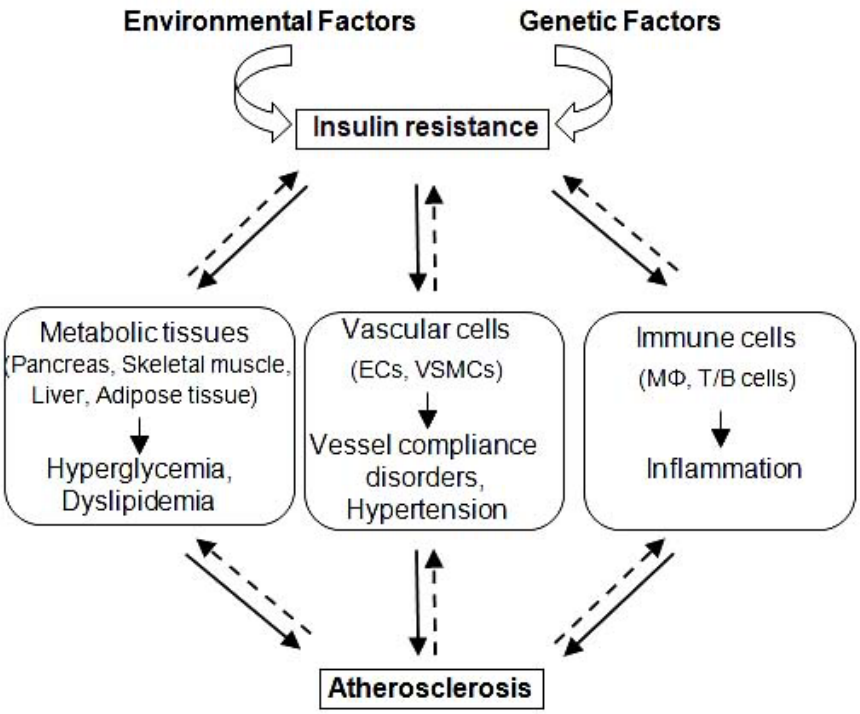

Figure 3. Mechanism of metabolic syndrome: A vicious circle. ECs: endothelial cells, VSMCs: vascular smooth muscle cells, MΦ: macrophages.

In atherosclerosis, macrophage activation/T lymphocytes infiltration secretes pro-inflammatory cytokines and cytotoxic substances (156), which play a role in the initiation of atherosclerotic lesion (157). Epidemiological evidences demonstrated that patients with T2DM or obesity have augmented circulating levels of inflammatory markers, including C-reactive protein (CRP), TNF-alpha, interleukin-6, and ICAM-1 (124, 158). These will predict cardiovascular risk in individuals having diabetes (159). CRP (157) and pro-inflammatory cytokine TNF-alpha (158) have been shown to reduce eNOS activation, increase VCAM and ICAM-1 expression, adhesion of monocytes to the EC, and impair endothelium-dependent vasodilation in both animal and human studies (159). Moreover, TNF-alpha induces EC apoptosis by impairing Akt phosphorylation (160).

It has been demonstrated that FFA, inflammatory cytokines and the RAGE (161, 162) activate transcription factor NF-kappaB, a key regulator of endothelial activation and linked to the pathogeneses of IR (160, 163). NF-kappaB activation involves phosphorylation, ubiquitination, and subsequent degradation of the inhibitory subunit IkB (by IkB kinase). It allows translocation of the regulatory subunits p50 and p65 to the nucleus, where they promote transcriptional expression of inflammatory genes. Genetic suppression or pharmacological inhibition of IKKbeta with salicylates was demonstrated to prevent IR (160, 164). Several in vitro and in vivo studies using animal models and human studies support the relevance between NF-kappaB activation, progression of inflammatory phenotype, IR, and impaired bioactivity of NO (82, 165).

10. SUMMARY

IR is commonly observed in the metabolic syndrome, in which multiple metabolic risk factors are co-existed including abdominal obesity, hyperglycemia, hyperinsulinemia, dyslipidemia, hypertension, and hyperhomocysteinemia. Patients with the metabolic syndrome are at increased risk of developing coronary heart disease, stoke, and T2DM. The prevalence of metabolic syndrome is estimated to be up to 25 percentage of the population in the United States, and increases with age. In this review, insulin receptor is chose to connect these individual clinical manifestations. This is based on its ubiquitous expression in cell/tissue and its function in regulating the metabolisms (glucose, lipid, and insulin) and in modulating basic cellular function (cell proliferation and apoptosis), and in controlling NO production. IR in metabolic tissues leads to metabolic disorders, in vascular cells it results in vessel compliance impairment, and in immune cells it causes inflammation initiation, which all contribute to the development of atherosclerosis (Figure 3). Future studies to identify molecular basis of impaired insulin signaling in different tissues would lead to the discovery of novel therapeutic strategies for IR-related metabolism syndrome to reduce the risk of cardiovascular disease.

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