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Reciprocal Relationships Between Insulin Resistance and Endothelial Dysfunction

Molecular and Pathophysiological Mechanisms

Jeong-a Kim, PhD; Monica Montagnani, MD, PhD; Kwang Kon Koh, MD; Michael J. Quon, MD, PhD

Abstract—Endothelial dysfunction contributes to cardiovascular diseases, including hypertension, atherosclerosis, and coronary artery disease, which are also characterized by insulin resistance. Insulin resistance is a hallmark of metabolic disorders, including type 2 diabetes mellitus and obesity, which are also characterized by endothelial dysfunction. Metabolic actions of insulin to promote glucose disposal are augmented by vascular actions of insulin in endothelium to stimulate production of the vasodilator nitric oxide (NO). Indeed, NO-dependent increases in blood flow to skeletal muscle account for 25% to 40% of the increase in glucose uptake in response to insulin stimulation. Phosphatidylinositol 3-kinase–dependent insulin-signaling pathways in endothelium related to production of NO share striking similarities with metabolic pathways in skeletal muscle that promote glucose uptake. Other distinct nonmetabolic branches of insulin-signaling pathways regulate secretion of the vasoconstrictor endothelin-1 in endothelium. Metabolic insulin resistance is characterized by pathway-specific impairment in phosphatidylinositol 3-kinase–dependent signaling, which in endothelium may cause imbalance between production of NO and secretion of endothelin-1, leading to decreased blood flow, which worsens insulin resistance. Therapeutic interventions in animal models and human studies have demonstrated that improving endothelial function ameliorates insulin resistance, whereas improving insulin sensitivity ameliorates endothelial dysfunction. Taken together, cellular, physiological, clinical, and epidemiological studies strongly support a reciprocal relationship between endothelial dysfunction and insulin resistance that helps to link cardiovascular and metabolic diseases. In the present review, we discuss pathophysiological mechanisms, including inflammatory processes, that couple endothelial dysfunction with insulin resistance and emphasize important therapeutic implications. (*Circulation*. 2006;113:1888-1904.)

Key Words: diabetes mellitus ■ endothelium ■ hypertension ■ insulin

Insulin resistance is typically defined as decreased sensitivity and/or responsiveness to metabolic actions of insulin that promote glucose disposal. This important feature of diabetes, obesity, glucose intolerance, and dyslipidemia is also a prominent component of cardiovascular disorders, including hypertension, coronary artery disease, and atherosclerosis, which are characterized by endothelial dysfunction.¹ Conversely, endothelial dysfunction is present in diabetes, obesity, and dyslipidemias.² Moreover, it is firmly established that these metabolic disorders are major risk factors for cardiovascular diseases.³ In addition to its essential metabolic actions, insulin has important vascular actions that involve stimulation of the production of nitric oxide (NO) from endothelium, leading to vasodilation, increased blood flow, and augmentation of glucose disposal in skeletal muscle.⁴ Elucidation of insulin-signaling pathways regulating endothelial production of NO reveals striking parallels with metabolic insulin-signaling pathways in skeletal muscle and

adipose tissue.^{5,6} Other distinct insulin-signaling pathways in endothelium (unrelated to metabolic actions of insulin) regulate secretion of the vasoconstrictor endothelin-1 (ET-1).⁷ Mechanisms contributing to insulin resistance and endothelial dysfunction include glucotoxicity, lipotoxicity, and inflammation. Molecular and pathophysiological mechanisms underlying reciprocal relationships between insulin resistance and endothelial dysfunction result in a vicious cycle reinforcing the link between metabolic and cardiovascular disorders. Therapeutic interventions that improve endothelial function and/or insulin sensitivity ameliorate metabolic and cardiovascular abnormalities in animal and clinical investigations. Thus, molecular, cellular, physiological, and clinical studies strongly support the hypothesis that reciprocal relationships between insulin resistance and endothelial dysfunction provide a pathophysiological mechanism connecting disorders of metabolic and cardiovascular homeostasis typified by the metabolic syndrome.

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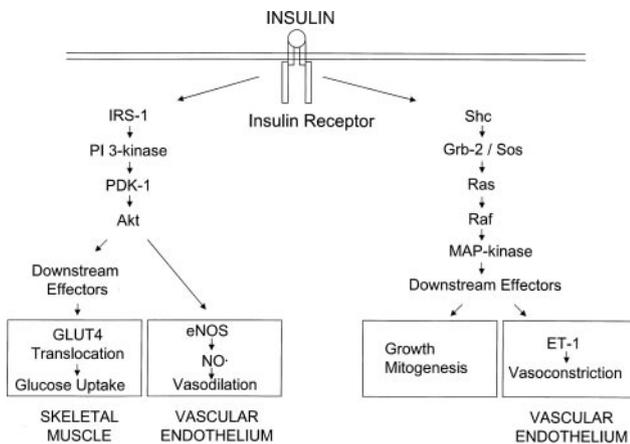


Figure 1. General features of insulin signal transduction pathways. PI 3-kinase branch of insulin signaling regulates GLUT4 translocation and glucose uptake in skeletal muscle and NO production and vasodilation in vascular endothelium. MAP-kinase branch of insulin signaling generally regulates growth and mitogenesis and controls secretion of ET-1 in vascular endothelium.

Insulin Signaling in Vascular Endothelium

General Features of Insulin Signal Transduction Pathways

Figure 1 shows general features of insulin signal transduction pathways.^{8,9} Biological actions of insulin are initiated by the binding of insulin to its cell surface receptor, a ligand-activated tyrosine kinase. Activated insulin receptors phosphorylate intracellular substrates, including insulin receptor substrate (IRS) family members and Shc, which serve as docking proteins for downstream signaling molecules. Tyrosine-phosphorylated motifs on IRSs specifically bind to SH2 domains contained in adaptor proteins, such as the p85 regulatory subunit of phosphatidylinositol (PI) 3-kinase or Grb-2. When SH2 domains of p85 bind to tyrosine-phosphorylated IRS-1, this results in activation of the catalytic p110 subunit of phosphatidylinositol (PI) 3-kinase. Similarly, when the SH2 domain of Grb-2 binds to tyrosine-phosphorylated Shc, this results in activation of the preassociated guanosine triphosphate (GTP) exchange factor Sos. Activation of PI 3-kinase generates lipid products, including PI (3,4,5) P₃. This initiates a cascade of serine kinases where phosphoinositide-dependent kinase-1 (PDK-1) is phosphorylated and activated to phosphorylate and activate Akt and other serine kinases that themselves phosphorylate and activate downstream substrates. This cascade ultimately culminates in the pleiotropic biological actions of insulin. A similar signaling pathway proceeds from Sos, which activates the small GTP binding protein Ras, which then initiates a phosphorylation cascade involving Raf, mitogen-activated protein (MAP)-kinase/extracellular signal-regulated kinase kinase (MEK), and MAP-kinase. Insulin signal transduction pathways constitute a highly complex network that includes multiple feedback loops, cross-talk between major signaling branches, and cross-talk from signaling pathways of heterologous receptors.¹⁰ Nevertheless, it is useful to consider PI 3-kinase-dependent pathways as 1 major branch of insulin signaling that regulates metabolic functions, whereas Ras/MAP-kinase

insulin-signaling pathways are generally not involved in mediating metabolic actions of insulin but rather in promoting mitogenic and growth effects of insulin.

Insulin-Stimulated Production of NO

Insulin-signaling pathways in vascular endothelium leading to the activation of endothelial NO synthase (eNOS) and increased production of NO are completely distinct, separable, and independent from classical calcium-dependent mechanisms used by G-protein-coupled receptors, such as the acetylcholine receptor.¹¹ Recently, a complete biochemical insulin-signaling pathway in endothelium regulating the production of NO has been elucidated. This involves insulin receptor phosphorylation of IRS-1, which then binds and activates PI 3-kinase, leading to phosphorylation and activation of PDK-1, which in turn phosphorylates and activates Akt.^{11–14} Akt directly phosphorylates eNOS at Ser¹¹⁷⁷, resulting in increased eNOS activity and NO production^{11,15} (Figure 1). Activation of PI 3-kinase is necessary for insulin-stimulated production of NO in endothelium.^{12–14} However, it is not sufficient because stimulation of endothelial cells with growth factors such as platelet-derived growth factor activates PI 3-kinase and Akt without leading to phosphorylation or activation of eNOS.^{11,12} In addition to phosphorylation, other posttranslational modifications, including palmitoylation,¹⁶ nitrosylation,^{16,17} and O-GlcNacylation,¹⁸ are important regulatory mechanisms for subcellular targeting and regulation of eNOS activity. All of these regulatory mechanisms may contribute to basal and insulin-stimulated production of NO. The Ras/MAP-kinase branch of insulin-signaling pathways does not contribute to activation of eNOS in response to insulin inasmuch as insulin-stimulated production of NO is not substantially affected by inhibition of these pathways.¹²

Insulin-Stimulated Secretion of ET-1

Little is known about endothelial insulin-signaling pathways regulating secretion of the vasoconstrictor ET-1. However, 1 recent study in bovine aortic endothelial cells (BAECs) has demonstrated that MAP-kinase signaling is required for this process but PI 3-kinase signaling is not⁷ (Figure 2A). Insulin treatment induces a 2-fold increase in ET-1 levels in conditioned media. Pretreatment with wortmannin does not significantly affect insulin-stimulated ET-1 secretion. By contrast, pretreatment with PD98059 completely blocks this effect of insulin. In cell lysates from these experiments, as expected, insulin-stimulated phosphorylation of Akt is completely blocked by wortmannin pretreatment, whereas MAP-kinase phosphorylation is unaffected (Figure 2B, lanes 2 and 3). Furthermore, insulin-stimulated phosphorylation of MAP-kinase is completely blocked by PD98059 pretreatment, whereas Akt phosphorylation is unaffected (Figure 2B, lanes 2 and 4). Taken together, these results directly demonstrate that insulin-stimulated secretion of ET-1 in endothelial cells is mediated by MAP-kinase-dependent signaling pathways independent of PI 3-kinase-dependent signaling.

Insulin-Stimulated Expression of Adhesion Molecules

Another insulin action that regulates vascular function is stimulation of the expression of vascular cell adhesion mol-

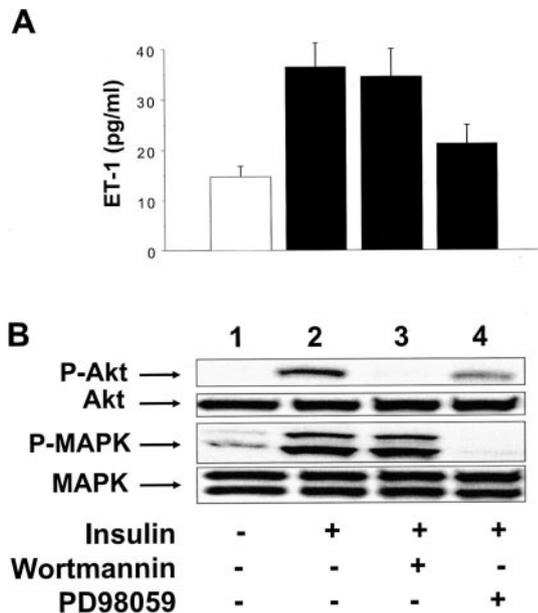


Figure 2. Insulin-stimulated secretion of ET-1 from vascular endothelial cells is mediated by MAP-kinase (MAPK)-dependent pathways but not PI 3-kinase-dependent pathways.⁷ Levels of ET-1 in conditioned media from BAECs were measured under basal conditions and after acute insulin stimulation (100 nmol/L, 5 minutes) without or with pretreatment with wortmannin (PI 3-kinase inhibitor) or PD98059 (MEK inhibitor). P-Akt indicates phospho-Akt; P-MAPK, phospho-MAPK.

ecule (VCAM)-1 and E-selectin on endothelium. MAP-kinase-dependent signaling pathways (but not PI 3-kinase pathways) regulate these functions of insulin.¹⁹

Other Hormones That Mimic Insulin Signaling in Endothelium

In addition to insulin, there is a growing list of hormones involved in the regulation of vascular and metabolic physiology that acutely activate eNOS in vascular endothelium by PI 3-kinase-dependent signaling mechanisms, leading to phosphorylation of eNOS. This includes leptin,²⁰ adiponectin,²¹ high-density lipoprotein (HDL),²² estrogen,²³ glucocorticoids,²⁴ and dehydroepiandrosterone (DHEA).²⁵ Moreover, DHEA has acute nongenomic vascular actions that stimulate the secretion of ET-1 from endothelium by a MAP-kinase-dependent mechanism similar to that used by insulin.²⁵

Hemodynamic Actions of Insulin

Vasodilation leading to increased blood flow is a major physiological consequence of insulin-stimulated production of NO in vascular endothelium. Endothelium-derived NO diffuses into adjacent vascular smooth muscle, where it evokes vasorelaxation. Insulin-mediated vasodilation in skeletal muscle occurs in 2 distinct stages.²⁶ First, dilation of terminal arterioles increases the number of perfused capillaries (capillary recruitment) within a few minutes without concomitant changes in total limb blood flow. Second, relaxation of larger resistance vessels increases overall limb blood flow within 30 minutes after stimulation with physiological concentrations of insulin (maximum flow was reached after 2 hours).²⁷ Overall vasodilator response to insulin is an

integration of both capillary recruitment and increased total blood flow.

Capillary Recruitment in Skeletal Muscle

Insulin-stimulated capillary recruitment was first studied in rat hindlimb by measuring endothelial metabolism of exogenously infused 1-methylxanthine.²⁸ Recently, a more sensitive, specific, and less invasive technique involving ultrasound imaging of skeletal muscle during microbubble contrast infusion has been shown to allow for accurate assessment of capillary recruitment in response to insulin.²⁶ A significant 1.5-fold increase in capillary recruitment has been observed in rat hindlimb 10 minutes after the initiation of insulin infusion during euglycemic glucose clamp (steady-state plasma insulin levels \approx 600 pmol/L). After 30 minutes, capillary recruitment increases to 2-fold over basal, and this is maintained over a 2-hour insulin infusion period.^{26,29} These effects are NO dependent,²⁹ and even very low concentrations of insulin (eg, \approx 300 pmol/L insulin) that do not alter total limb blood flow elicit significant increases in capillary recruitment.^{29,30} Ultrasound imaging of skeletal muscle during microbubble contrast infusion has also been applied in deep flexor muscles of the human forearm, where local intra-arterial insulin infusion (arterial insulin levels \approx 320 pmol/L) causes a 25% increase in muscle capillary blood volume. This is significantly higher than that observed after saline infusion in the same arm or in the contralateral arm that did not receive insulin.³¹

Blood Flow to Skeletal Muscle

Concentration-dependent increases in total limb blood flow in response to intravenous insulin infusion spanning the physiological to pharmacological range is well documented in animals and humans.^{26,27,32–34} However, by contrast with capillary recruitment, the slower time course for insulin-mediated increases in limb blood flow requires several hours for a maximal effect to become evident. Insulin infusion at physiological concentrations under euglycemic glucose-clamp conditions causes a dose-dependent doubling in skeletal muscle blood flow that is NO dependent.^{27,33} However, some controversy exists over the precise time course and whether physiological concentrations of insulin cause significant increases in total limb flow. Some of this controversy may be explained by differential sensitivity and/or technical limitations of various experimental approaches for estimating limb blood flow (eg, plethysmography, thermodilution, positron emission tomography, dye dilution, Doppler ultrasound, and ultrasound measurements of brachial or femoral artery diameter).³⁵ The preponderance of experimental evidence in animals and humans in vivo strongly suggests that insulin signaling in vascular endothelium related to the production of NO has physiological consequences resulting in capillary recruitment and increased blood flow in skeletal muscle, which contributes to glucose disposal.

Opposing Hemodynamic Actions of Insulin

In addition to NO-dependent vasodilator actions, insulin has other biological actions that have an impact on hemodynamic homeostasis. Insulin stimulates secretion of the vasoconstrictic-

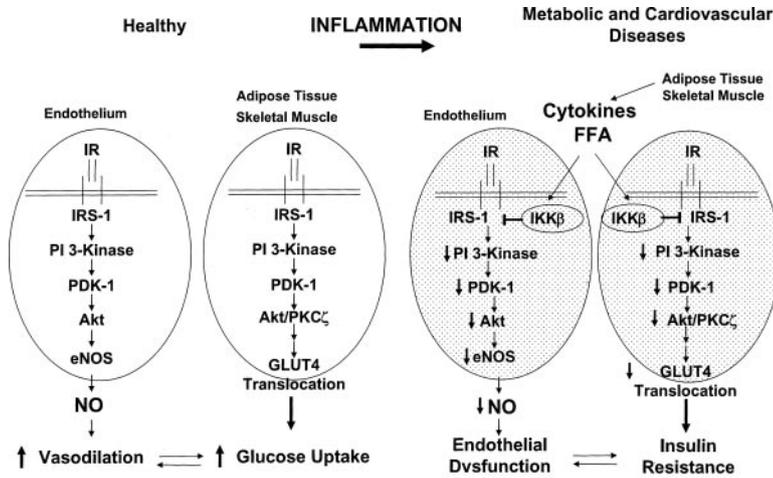


Figure 3. Left, Parallel PI 3-kinase–dependent insulin–signaling pathways in metabolic and vascular tissues synergistically couple metabolic and vascular physiology under healthy conditions. IR indicates insulin receptor. Right, Parallel impairment in insulin–signaling pathways under pathological conditions contributes to synergistic coupling of insulin resistance and endothelial dysfunction.¹⁸³

tor ET-1 from endothelium. Vasodilator actions of insulin in humans are potentiated by ET-1 receptor blockade.³⁶ Consistent with the MAP-kinase dependence of insulin-stimulated secretion of ET-1 in vascular endothelium,⁷ inhibition of MAP-kinase pathways blocks vasoconstrictor effects of insulin in rat skeletal muscle arterioles.³⁷ Stimulation of sympathetic activity by insulin may also contribute to hemodynamic regulation. In healthy lean people, physiological concentrations of insulin increase plasma norepinephrine levels as well as sympathetic nerve activity.³⁸ In patients with regional sympathectomy, NO-dependent vasodilation in response to insulin occurs more quickly in the denervated limb than in the innervated limb.³⁹ Thus, sympathetic activation in response to insulin (presumably α -adrenergic) may oppose vasodilator actions of insulin. However, β -adrenergic activation in response to insulin may cause vasodilation.⁴⁰ Finally, insulin-stimulated renal reabsorption of sodium favors expansion of extracellular fluid volume, which may predispose an individual to hypertension.⁴¹ Thus, insulin has multiple opposing hemodynamic actions with a negligible net effect on blood pressure in normal individuals. A shift in balance between vasoconstrictor and vasodilator actions of insulin may be an important factor in the vascular pathophysiology of insulin resistance.

Insulin Action Couples Hemodynamic and Glucose Homeostasis

Vasodilator actions of insulin play a central physiological role in coupling hemodynamic and metabolic homeostasis under healthy conditions (Figure 3, left). Evidence supporting this hypothesis has emerged from investigations of insulin–signaling pathways in skeletal muscle, adipose tissue, and vascular endothelium as well as from physiological studies manipulating blood flow and glucose metabolism.

Parallel Insulin–Signaling Pathways in Metabolic and Vascular Tissues

Insulin-stimulated glucose uptake in skeletal muscle and adipose tissue is mediated by translocation of the insulin-responsive glucose transporter GLUT4 to the cell surface.^{8,9} This requires PI 3-kinase–dependent signaling pathways that involve the insulin receptor, IRS-1, PI 3-kinase, PDK-1, and

Akt. Ras/MAP kinase pathways do not contribute significantly to insulin-stimulated translocation of GLUT4. Although activation of PI 3-kinase and Akt is necessary, it is not sufficient for GLUT4 translocation because platelet-derived growth factor stimulates activation of PI 3-kinase and Akt without causing translocation of GLUT4 in adipose cells.⁹ As discussed in the previous section on insulin signaling in vascular endothelium, the vascular actions of insulin that stimulate the production of NO require PI 3-kinase–dependent insulin–signaling pathways that bear striking similarities to metabolic insulin–signaling pathways. PI 3-kinase is necessary but not sufficient for insulin-stimulated production of NO, and Ras/MAP-kinase pathways do not participate in the insulin-stimulated production of NO (although they do stimulate the secretion of ET-1 in response to insulin). Activation of specific metabolic insulin–signaling pathways in skeletal muscle results in increased glucose uptake. Activation of highly parallel insulin–signaling pathways in vascular endothelium leads to increased blood flow to skeletal muscle. Thus, shared insulin–signaling pathways in metabolic and vascular target tissues with complementary functions may provide 1 mechanism to couple the regulation of glucose and hemodynamic homeostasis.

Contribution of Blood Flow to Glucose Metabolism

If glucose metabolism is coupled with blood flow, changes in metabolism will induce alterations in blood flow, whereas increasing flow will drive changes in metabolism. It is well established that increased metabolic activity recruits additional blood flow to supply necessary substrates.⁴² Conversely, experiments in rat hindlimb demonstrate that increasing blood flow while maintaining glucose and insulin at constant physiological levels results in flow-dependent increases in glucose disposal.⁴³ Insulin-stimulated increases in capillary recruitment and blood flow enhance the delivery of glucose to skeletal muscle, where mass action promotes glucose transport. Elevations in flow also increase the delivery of insulin to skeletal muscle, where insulin exerts direct effects to promote glucose uptake through stimulating the translocation of GLUT4. Indeed, changes in insulin-mediated capillary recruitment are positively correlated with changes in

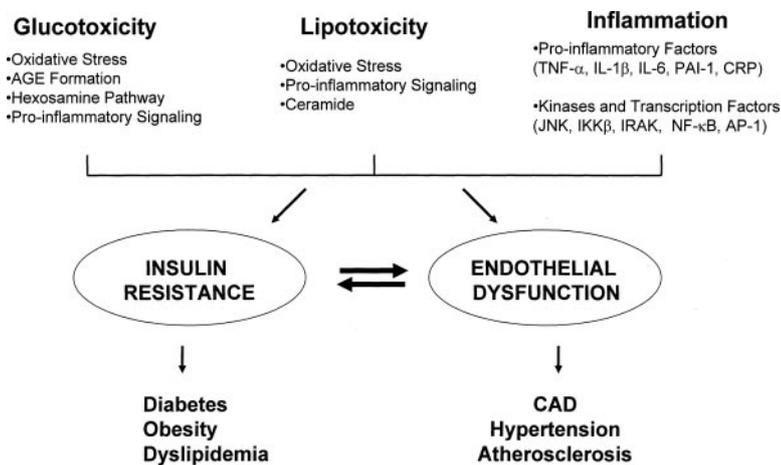


Figure 4. Shared and interacting mechanisms of glucotoxicity, lipotoxicity, and inflammation underlie reciprocal relationships between insulin resistance and endothelial dysfunction that contribute to linkage between metabolic and cardiovascular diseases. CAD indicates coronary artery disease.

insulin-stimulated glucose disposal.²⁹ Of note, physiological concentrations of insulin stimulate the recruitment of capillaries before changes in total blood flow can be detected.²⁶ The time course for insulin-stimulated capillary recruitment approximates the time course for insulin-mediated glucose uptake in skeletal muscle.²⁹ Moreover, inhibitors of NOS that block insulin-mediated capillary recruitment cause a concomitant $\approx 40\%$ reduction in glucose disposal.^{29,32} In human studies, insulin stimulates parallel increases in leg glucose disposal and blood flow in a dose-dependent manner.^{44–46} Although the time course of leg blood flow during physiological hyperinsulinemia is slower than that for glucose uptake, it generally follows leg glucose uptake. Infusion of the competitive NOS inhibitor *N*^G-monomethyl-L-arginine completely blocks the effect of insulin on flow and partially blocks insulin-stimulated leg glucose uptake.^{45,46} Taken together, animal and human studies suggest that skeletal muscle capillary recruitment and blood flow play an important physiological role in augmenting the delivery of insulin and glucose to metabolic insulin target tissues. Insulin-stimulated increases in total limb blood flow, per se, may account for up to 40% of insulin-mediated glucose disposal.⁴⁴ Thus, insulin has direct effects (increasing glucose uptake in skeletal muscle) and substantial indirect effects (promoting glucose disposal by increasing blood flow). This cross-talk between metabolic and vascular tissues is important for coupling glucose homeostasis and endothelial function.

Shared Mechanisms Underlying Insulin Resistance and Endothelial Dysfunction

Genetic and environmental factors contribute to insulin resistance and endothelial dysfunction. Interestingly, some of the same mechanisms underlying acquired insulin resistance also contribute to endothelial dysfunction (Figure 4). In particular, the hyperglycemia of diabetes leads to glucotoxicity, which causes insulin resistance and endothelial dysfunction. Similarly, elevated free fatty acid (FFA) levels in diabetes, obesity, and dyslipidemias lead to lipotoxicity, which underlies other shared mechanisms of insulin resistance and endothelial dysfunction. Proinflammatory states associated with metabolic and cardiovascular diseases represent a third cate-

gory of shared mechanisms between insulin resistance and endothelial dysfunction.

Glucotoxicity and Insulin Resistance

Hyperglycemia associated with diabetes causes insulin resistance by increasing oxidative stress, formation of advanced glycation end products (AGEs), and flux through the hexosamine biosynthetic pathway.

Oxidative Stress

Hyperglycemia increases the production of reactive oxygen species (ROS), although the precise mechanisms remain to be elucidated. Treatment of cells with uncouplers of mitochondrial oxidative phosphorylation or overexpression of uncoupling protein (UCP)-1 or of manganese superoxide dismutase inhibits ROS production in response to hyperglycemia. In addition, these manipulations prevent glucose-induced protein kinase C (PKC) activation, the formation of AGEs, sorbitol accumulation, and the activation of nuclear factor (NF)- κ B.⁴⁷ Increased ROS results in insulin resistance with impaired insulin-stimulated translocation of GLUT4 and glucose uptake. Moreover, antioxidants, including α -lipoic acid, protect against these ROS effects on glucose transport in vitro and in vivo.^{48–50} Increased oxidative stress is associated with the stimulation of various serine/threonine kinases and the activation of transcription factors NF- κ B and activator protein (AP)-1, which lead to insulin resistance. Activation of serine/threonine kinases c-Jun NH₂-terminal kinase (JNK), PKCs, and I κ B kinase complex β (IKK β) leads to serine phosphorylation of IRS-1, which impairs its ability to bind and activate PI 3-kinase. This leads to diminished activation of downstream kinases Akt and PKC- ζ , which results in decreased GLUT4 translocation and glucose transport.^{51–53} In addition, ROS activates proinflammatory signaling, which leads to phosphorylation of IKK β and NF- κ B.⁵⁴ Activation of NF- κ B and AP-1 regulates transcription of proinflammatory genes, including interleukin (IL)-6, IL-1 β , and tumor necrosis factor (TNF)- α . The contribution of inflammatory signaling to insulin resistance and endothelial dysfunction will be discussed in more detail below.

Advanced Glycation End Products

AGE formation is enhanced by hyperglycemia and oxidative stress.⁴⁷ Human glycated end products inhibit insulin-

stimulated tyrosine phosphorylation of IRS-1 and IRS-2, leading to impaired activation of PI 3-kinase and Akt, with a decrease in activity of glycogen synthase in an L6 skeletal muscle cell line.⁵⁵ Moreover, AGE produces ROS and increases oxidative stress by activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase through specific receptors for AGE (RAGEs).⁵⁶ RAGE stimulates proinflammatory signaling, leading to the activation of NF- κ B.⁵⁷

Hexosamine Biosynthetic Pathway

Increased flux through the hexosamine biosynthetic pathway (HSP) is another proposed mechanism by which hyperglycemia causes insulin resistance.⁵⁸ Glutamine:fructose-6-phosphate amidotransferase (GFAT) is the rate-limiting enzyme of this pathway, whose end product is uridine 5'-diphosphate (UDP)-GlcNAc. This, in turn, is a substrate for O-GlcNAc transferase, which mediates posttranslational modification of proteins. Overexpression of GFAT in transgenic mice causes insulin resistance.⁵⁹ HSP may function as a nutrient sensor that plays a role in insulin resistance and vascular complications by causing reversible O-GlcNAc modifications at regulatory serine/threonine phosphorylation sites on proteins involved with insulin signaling.⁶⁰ For example, increased O-GlcNAcylation of IRS-1 may lead to reduced insulin-stimulated translocation of GLUT4 and decreased glucose uptake.^{61,62}

Glucotoxicity and Endothelial Dysfunction

Hyperglycemia induces expression of extracellular matrix and procoagulant proteins, increases apoptosis of endothelial cells, decreases endothelial cell proliferation, and inhibits fibrinolysis, resulting in endothelial dysfunction.⁶³ Many of the molecular mechanisms underlying hyperglycemia-induced insulin resistance also apply to endothelial dysfunction.

Oxidative Stress

Increased superoxide scavenges NO and produces peroxynitrite, which reduces the bioavailability of NO and impairs vasodilation.⁶⁴ In addition, ROS activates PKC- α , PKC- β , and PKC- δ , leading to differential gene expression for eNOS, ET-1, vascular endothelial growth factor (VEGF), transforming growth factor (TGF)- β , and plasminogen activator inhibitor (PAI)-1 and the activation of NF- κ B, which increases proinflammatory gene expression.⁶³ Hyperglycemia induces apoptosis of endothelial cells and enhances the expression of intercellular adhesion molecule (ICAM), VCAM, and E-selectin, as well as production of IL-6 through the production of ROS and activation of PKC.^{65–67} Although PKC- α participates in the activation of eNOS in response to fibroblast growth factor⁶⁸ and VEGF⁶⁹ stimulation, PKC- α also directly phosphorylates eNOS at Thr⁴⁹⁷ (an inhibitory phosphorylation site). Thus, the net role of PKC- α in the modulation of eNOS activity remains to be clarified. Overexpression of UCP-2 inhibits the production of ROS and the activation of NF- κ B, leading to improvement of endothelial function.⁷⁰

Advanced Glycation End Products

Increased intermolecular cross-linking by AGE impairs the function of endothelial proteins.⁷¹ AGE modifications of

extracellular matrix proteins, including collagen and laminin, decrease vessel elasticity and increase fluid filtration.⁷² Moreover, modifications of intracellular and extracellular proteins by AGE affect interactions between endothelial cells and macrophages. Infiltrated macrophages become foam cells that increase vascular inflammation and promote atherosclerosis. RAGE is expressed in endothelial cells, where it contributes to an increase in proinflammatory signaling by activation of NF- κ B. Furthermore, RAGE directly interacts with macrophages, promoting inflammation in the vessel wall.⁷³

Hexosamine Biosynthetic Pathway

Increased flux through the HSP is another proposed mechanism for hyperglycemia-induced vascular complications.⁶³ In endothelial cells, hyperglycemia increases flux through the HSP, which mediates increased expression of TGF- β and PAI-1 relevant to the pathogenesis of vascular complications.⁷⁴ In addition, hyperglycemia increases O-GlcNAcylation of eNOS at the Akt phosphorylation site at Ser¹¹⁷⁹, leading to impairment of eNOS activity. These defects are reversed by decreasing GFAT expression or overexpression of UCP-1 or manganese superoxide dismutase.¹⁸

Lipotoxicity and Insulin Resistance

Elevated levels of FFA observed in insulin-resistant states including diabetes, obesity, and dyslipidemias represent another major factor contributing to acquired insulin resistance.⁷⁵ Infusion of FFA into humans blunts insulin-mediated glucose uptake as well as NO-dependent limb blood flow,⁷⁶ suggesting that elevated FFA levels are another link between insulin resistance and endothelial dysfunction. Like hyperglycemia, elevated FFA levels induce oxidative stress and proinflammatory signaling.

Oxidative Stress

Recent studies using magnetic resonance spectroscopy in humans have revealed that increased FFA levels directly inhibit glucose transport by causing mitochondrial dysfunction.^{77,78} Indeed, increased intramyocellular lipid levels are associated with reduced mitochondrial oxidation in insulin-resistant patients.^{79,80} FFA metabolites including fatty acyl coenzyme A (CoA) and diacylglycerol stimulate novel PKCs, such as PKC- θ , that promote insulin resistance.⁷⁷ PKC- θ directly phosphorylates IRS-1 at Ser¹¹⁰¹ in response to FFA treatment, leading to impaired IRS-1 and Akt function.⁸¹ Consistent with this, PKC- θ -null mice are protected against glucose intolerance caused by lipid infusion.⁸² Mitochondrial dysfunction uncouples oxidative phosphorylation, leading to increased generation of ROS. Moreover, increased expression of NADPH oxidase associated with obesity causes dysregulated production of adipokines, including adiponectin, PAI-1, IL-6, and monocyte chemoattractant protein (MCP)-1, and reduced expression of detoxifying enzymes, such as Cu-Zn superoxide dismutase and peroxisome proliferator-activated receptor γ (PPAR γ). Inhibition of NADPH oxidase with apocynin reduces ROS production, improves glucose metabolism, and attenuates dysregulation of adipokines.⁵¹ Thus, lipotoxicity may increase oxidative stress in adipose tissue, which causes aberrant secretion of adipokines, leading to impaired glucose metabolism in skeletal muscle.

Proinflammatory Signaling

Activation of proinflammatory signaling pathways is a well-established mechanism for FFA to induce insulin resistance. Increased ROS in response to FFA activates NF- κ B, which further stimulates the production of other proinflammatory cytokines, including TNF- α and IL-6.^{83–85} TNF- α activates IKK β and JNK, which play a central role in cross-talk between inflammatory signaling and insulin signaling, leading to insulin resistance by phosphorylating IRS-1/2 on serine residues.^{53,86} Salicylate or aspirin, inhibitors of IKK β , prevent lipid- or obesity-induced insulin resistance.^{87,88} IKK β (+/–) or JNK1 (–/–) knockout mouse models are protected against insulin resistance induced by high-fat feeding.^{53,87,88}

Ceramide

Ceramide, a product derived from long-chain saturated fatty acids inhibits insulin-stimulated activation of Akt and translocation of GLUT4.⁸⁹ Ceramide content in skeletal muscle of obese humans is increased,⁹⁰ and overexpression of acid ceramidase protects against FFA-induced insulin resistance in vitro.⁹¹

Lipotoxicity and Endothelial Dysfunction

Elevated levels of FFA and other features of dyslipidemias directly damage the vascular wall, leading to endothelial dysfunction through many of the same mechanisms involved with FFA-mediated insulin resistance.

Oxidative Stress

Lipid infusion increases ROS production and inflammation in humans, leading to impaired flow-mediated brachial artery dilation.⁹² FFA stimulates NADPH oxidase to produce ROS through a PKC-dependent mechanism in endothelial cells.⁹³ Mitochondrial dysfunction in endothelium increases oxidative stress and uncouples oxidative phosphorylation, which may lead to endothelial dysfunction.⁶³ ROS scavenges NO and produces peroxynitrite, which damages endothelial cells.⁹⁴ Overexpression of UCP-2 attenuates free radical production and oxidative damage mediated by FFA-induced activation of NF- κ B.⁹⁵ UCP-2 overexpression also improves the impaired vascular relaxation and endothelial cell apoptosis induced by FFA.⁷⁰

Proinflammatory Signaling

FFA activates proinflammatory signaling pathways via NF- κ B.⁹⁶ Palmitate stimulates the production of IL-6 in endothelial cells in vitro and raises plasma IL-6 levels in humans.⁹⁷ FFA treatment of endothelial cells impairs insulin-stimulated activation of eNOS and NO production by the activation of IKK β , which leads to impaired insulin signaling in endothelium.⁹⁸

Ceramide

FFA inhibits endothelial cell proliferation and increases apoptosis.⁹⁹ Ceramide activates eNOS and increases NO production.¹⁰⁰ However, ceramide also produces excess ROS, which scavenges NO to produce peroxynitrite.¹⁰¹ Thus, the net effect of ceramide is to reduce the bioavailability of NO, leading to endothelial dysfunction.

Inflammation and Insulin Resistance

Diabetes, obesity, and other chronic metabolic disorders are associated with proinflammatory states characterized by increased circulating markers of inflammation as well as infiltration of adipose tissue with activated macrophages.^{102,103} In particular, the inflammatory marker C-reactive protein (CRP) has been identified as a risk factor for developing type 2 diabetes.¹⁰⁴ There are a number of potential biochemical mechanisms for the contribution of proinflammatory signaling to insulin resistance.¹⁰⁵ The most extensively studied proinflammatory cytokine implicated in insulin resistance is TNF- α . TNF- α activates a variety of serine kinases, including JNK, IKK β , and IL-1 receptor-associated kinase,^{106–109} which directly or indirectly increase serine phosphorylation of IRS-1/2, leading to decreased activity of PI-3 kinase and Akt. In addition, suppressors of cytokine-signaling proteins are induced by treatment of cells with TNF- α , IL-1 β , or IL-6. Increased expression of suppressors of cytokine-signaling proteins interferes with interaction of the insulin receptor and IRS-1 and enhances proteasomal degradation of IRS-1.¹¹⁰ Thus, proinflammatory cytokines may contribute to insulin resistance by modulating insulin signaling and transcription.

Inflammation and Endothelial Dysfunction

Dyslipidemias, coronary heart disease, and atherosclerosis are cardiovascular disorders with endothelial dysfunction that are associated with increased circulating levels of inflammatory markers.¹¹¹ Proinflammatory cytokines, including TNF- α and IL-1 β , signal through their cognate receptors to activate JNK and IKK β , which in turn lead to activation of AP-1 and NF- κ B.¹¹² This inhibits insulin-stimulated activation of eNOS¹¹³ and expression of eNOS.¹¹⁴ NF- κ B also stimulates the expression of adhesion molecules, including ICAM, VCAM, and E-selectin, which contribute to vascular pathology.¹¹⁵ Of importance, NO has anti-inflammatory actions in endothelium to inhibit NF- κ B activity¹¹⁶ and reduce the expression of leukocyte adhesion molecules VCAM, ICAM, and E-selectin.¹¹⁷ Thus, reduced bioavailability of NO under basal conditions caused by insulin resistance may be an additional pathogenic factor in chronic diseases (such as atherosclerosis, hypertension, and diabetes) that have inflammatory components. TNF- α stimulates the expression of other inflammatory proteins, including CRP and IL-6. CRP is an important marker of vascular inflammation whose plasma levels are correlated with a risk of cardiovascular disease.¹¹⁸ CRP may also directly promote cardiovascular disease by modulating the expression of proinflammatory cytokines in endothelium.¹¹⁹ CRP decreases eNOS expression¹²⁰ and up-regulates angiotensin receptor type 1 expression in endothelium.¹²¹ Moreover, CRP increases the expression of endothelial ICAM, VCAM, E-selectin, and MCP-1 and increases the secretion of ET-1.^{122,123} Thus, CRP is an inflammatory marker that may also directly contribute to the pathogenesis of atherosclerosis and endothelial dysfunction.

Insulin Resistance Couples Vascular and Metabolic Pathophysiology

An important consequence of insulin action coupling hemodynamic and glucose homeostasis under healthy conditions is

that in disease states, insulin resistance couples vascular and metabolic pathophysiology. Several distinct mechanisms work together to form a tight reciprocal relationship between insulin resistance and endothelial dysfunction. These include highly parallel insulin-signaling pathways controlling metabolic functions in skeletal muscle and production of NO in endothelium, reciprocal relationships between impaired insulin-stimulated blood flow and glucose uptake, metabolic and vascular consequences of pathway-specific impairment in insulin signaling, pathophysiological cross-talk between metabolic and vascular tissues mediated by hormones including angiotensin II and adiponectin, and shared stressors that individually contribute to insulin resistance and endothelial dysfunction.

Shared Insulin-Signaling Pathways in Metabolic and Vascular Target Tissues

An important determinant of coupling between insulin resistance and endothelial dysfunction is the existence of parallel insulin-signaling pathways in metabolic and vascular tissues with distinct functions. As discussed in a previous section, the insulin-signaling pathway involving the insulin receptor, IRS-1, PI 3-kinase, PDK-1, and Akt regulates GLUT4 translocation and glucose uptake in skeletal muscle and adipose tissue; the same pathway in endothelium regulates the activation of eNOS and production of NO (Figure 3, left). Thus, factors causing impairment in this particular insulin-signaling pathway in metabolic tissues also cause insulin resistance, whereas the same impairment in insulin signaling in endothelium leads to endothelial dysfunction (Figure 3, right).^{5,6,124,125} This reasoning applies to acquired forms of insulin resistance related to previously discussed stressors as well as to genetic causes of insulin resistance. Indeed, mice that are homozygous-null for the IRS-1 gene are not only predictably insulin resistant but also have a hemodynamic phenotype of hypertension with impaired endothelium-dependent vasodilation.¹²⁶ Moreover, patients carrying a point mutation in IRS-1 that has been implicated in insulin resistance also show evidence of genetically based endothelial dysfunction.¹²⁷

Coupling of Impaired Blood Flow With Impaired Glucose Uptake

As mentioned in a previous section, insulin-stimulated increases in blood flow contribute significantly to insulin-stimulated glucose disposal. Thus, impairment in insulin signaling leading to endothelial dysfunction is predicted to cause impaired vasodilation with decreased blood flow that contributes to metabolic insulin resistance in skeletal muscle by reducing delivery of insulin and glucose. This view is supported by evidence from human studies demonstrating positive correlations between insulin resistance with respect to vasodilator actions and metabolic actions of insulin in diabetic and obese subjects.^{45,46} Mice that are homozygous-null for the eNOS gene have an expected hemodynamic phenotype of increased basal blood pressure but also are insulin resistant.¹²⁸ In addition, infusion of TNF- α in rats blunts femoral artery blood flow and inhibits glucose uptake in response to insulin, whereas contractile-induced blood

Hemodynamic Actions of Insulin

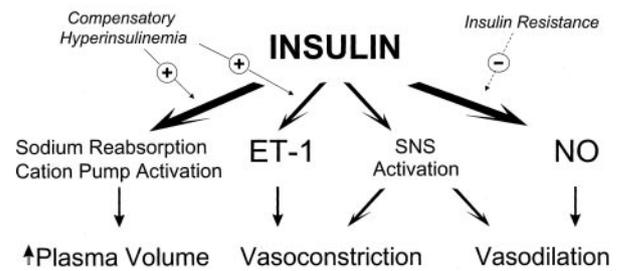


Figure 5. Pathway-specific insulin resistance creates imbalance between prohypertensive and antihypertensive vascular actions of insulin exacerbated by compensatory hyperinsulinemia.⁵ SNS indicates sympathetic nervous system.

flow and glucose uptake are unaffected.¹²⁹ Impairment in insulin-stimulated capillary recruitment (in addition to changes in total blood flow) may also play an important role in insulin resistance.^{130,131} In genetically obese insulin-resistant Zucker fatty rats compared with lean Zucker rats, capillary recruitment and glucose uptake in skeletal muscle are significantly impaired in response to insulin.¹³² Infusion of triglycerides in rats inhibits insulin-stimulated capillary recruitment and glucose uptake in skeletal muscle without affecting total limb blood flow.¹³³

Pathway-Specific Insulin Resistance

A key feature of insulin resistance is that it is characterized by specific impairment in PI 3-kinase–dependent signaling pathways, whereas other insulin-signaling branches, including Ras/MAP-kinase–dependent pathways, are unaffected.^{124,134} This has important pathophysiological implications because metabolic insulin resistance is usually accompanied by compensatory hyperinsulinemia to maintain euglycemia. In the vasculature and elsewhere, hyperinsulinemia will overdrive unaffected MAP-kinase–dependent pathways, leading to an imbalance between PI 3-kinase- and MAP-kinase–dependent functions of insulin. As previously discussed, prohypertensive effects of insulin to promote secretion of ET-1, activate cation pumps, and increase expression of VCAM-1 and other adhesion molecules are under the control of MAP-kinase–signaling pathways (Figure 5). In endothelium, decreased PI 3-kinase signaling and increased MAP-kinase signaling in response to insulin or other hormones including DHEA may lead to decreased production of NO and increased secretion of ET-1 characteristic of endothelial dysfunction (Figure 6). Thus, the antihypertensive effects of insulin to stimulate the production of NO are reduced under conditions of insulin resistance. At the same time, insulin-resistant patients have elevated plasma ET-1 levels, and hyperinsulinemia increases ET-1 secretion in humans.¹³⁵ Pharmacological blockade of ET-1 receptors (ET-A isoform) improves endothelial function in obese and diabetic patients but not in lean insulin-sensitive subjects.^{136,137}

A recent *in vitro* model of metabolic insulin resistance with compensatory hyperinsulinemia provides support for the concept that pathway-specific insulin resistance contributes to the pathophysiology of endothelial dysfunction.¹⁹ Simul-

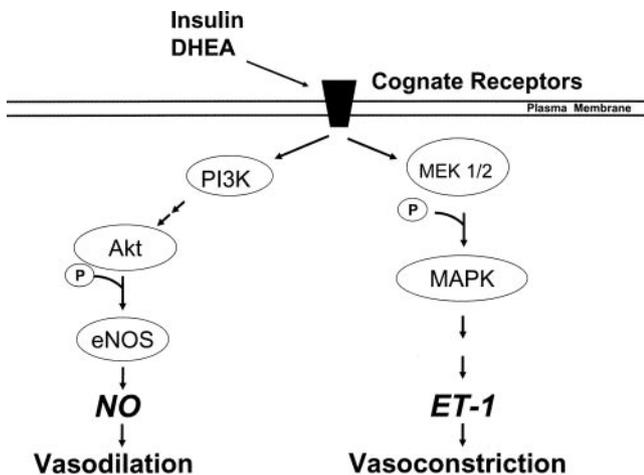


Figure 6. Vasodilator actions of insulin and DHEA mediated by NO are regulated by PI 3-kinase (PI3K)-dependent signaling pathways. Vasoconstrictor actions are regulated by MAPK-dependent signaling pathways.²⁵ P indicates phosphorylation.

taneous treatment of endothelial cells with wortmannin (a PI 3-kinase inhibitor) and high insulin levels blunts PI 3-kinase-dependent effects of insulin, such as induction of eNOS expression and the production of NO. Of note, under these conditions, insulin signaling through Ras/MAP-kinase pathways is substantially enhanced beyond that observed in the absence of wortmannin. This leads to increased prenylation of Ras and Rho proteins via the MAP-kinase pathway and enhanced mitogenic responsiveness of cells to insulin and VEGF, which are known to contribute to the proliferation of vascular smooth muscle cells. In addition, upregulation of endothelial cell adhesion molecules VCAM-1 and E-selectin and increased rolling interactions of monocytes with endothelial cells are observed. Thus, compensatory hyperinsulinemia in the presence of metabolic insulin resistance with pathway-specific impairment of PI 3-kinase in endothelium and vascular smooth muscle cells leads to enhanced mitogenic actions of insulin through MAP-kinase-dependent pathways, which may contribute to key early events in the pathogenesis of hypertension (Figure 7). In addition, decreased production of NO in endothelium mediated by insulin resistance also contributes to accelerated atherosclerosis by multiple mechanisms (Figure 8).

Pathophysiological Cross-Talk Between Metabolic and Vascular Tissues

Adipose tissue secretes a variety of hormones (known collectively as adipokines) that can modulate endothelial function. For example, TNF- α is a proinflammatory cytokine secreted by adipose cells that may cause insulin resistance and endothelial dysfunction.¹¹¹ In addition, components of the renin-angiotensin system (RAS), including angiotensin II, are present in adipose tissue.¹³⁸ Dysregulation of the RAS contributes importantly to hypertension, as demonstrated by the effectiveness of angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor blockers (ARBs) in the treatment of essential hypertension. ACE inhibitors improve insulin sensitivity in humans and decrease the

In Vitro Model of Metabolic Insulin Resistance with Compensatory Hyperinsulinemia in Vascular Endothelium

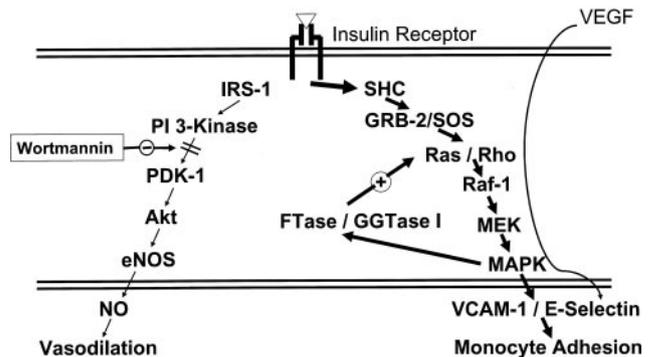


Figure 7. In vitro model of metabolic insulin resistance with compensatory hyperinsulinemia in vascular endothelium. Pathway-specific impairment in PI 3-kinase-dependent signaling decreases expression and activity of eNOS, whereas augmenting MAPK pathways increases expression of VCAM-1 and E-selectin and increases monocyte adhesion to endothelium.¹⁹

incidence of diabetes in patients with cardiovascular disease.¹³⁹ This may be due to inhibition of cross-talk between angiotensin II signaling and insulin signaling in metabolic and vascular tissues.¹⁴⁰ Treatment of endothelial cells with angiotensin II activates JNK and MAP-kinase pathways, leading to increased serine phosphorylation of IRS-1, impaired PI 3-kinase activity, and endothelial dysfunction.¹⁴¹ The endothelial dysfunction mediated by angiotensin II is abolished by ARBs.¹⁴² Inhibition of PI 3-kinase pathways in metabolic tissues would be predicted to cause insulin resistance. In addition to effects on insulin signaling, activation of angiotensin II type 1 receptors by angiotensin II stimulates the production of ROS via NADPH oxidase,¹⁴³ increases expression of ICAM-1,¹⁴⁴ and increases ET-1 release from endothelium.¹⁴⁵ Thus, there are multiple direct and indirect mechanisms for the RAS to contribute to insulin resistance and endothelial dysfunction through the modulation of insulin signaling and other pathways in metabolic and vascular tissues.

Adiponectin is the most abundant adipokine secreted by adipose cells and may couple the regulation of insulin

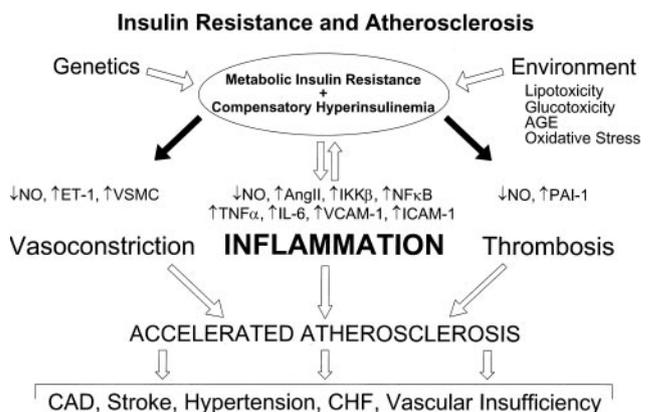


Figure 8. Mechanisms for the contribution of insulin resistance to atherosclerosis. VSMC indicates vascular smooth muscle cell; CHF, congestive heart failure.

sensitivity with energy metabolism. By contrast, with other adipokines, increased levels of adiponectin are associated with increased insulin sensitivity. Decreased plasma adiponectin levels are observed in patients with obesity, type 2 diabetes, hypertension, metabolic syndrome, and coronary artery disease and are significantly associated with low-level chronic inflammatory conditions.^{146,147} Adiponectin has metabolic actions that mimic those of insulin, which promote glucose uptake and inhibit hepatic glucose production.¹⁴⁸ Interestingly, adiponectin also has vascular actions to stimulate the production of NO in endothelium²¹ and has anti-atherogenic and anti-inflammatory properties.^{149–152} Thus, reduced expression of adiponectin may also contribute to cross-talk between metabolic and vascular tissues, leading to insulin resistance and endothelial dysfunction in metabolic and cardiovascular diseases.

Secretion of ET-1 from vascular endothelium may mediate cross-talk with metabolic tissues and thus cause insulin resistance. ET-1 increases serine phosphorylation of IRS-1, leading to decreased PI 3-kinase activity in vascular smooth muscle cells.¹⁵³ This same mechanism may also be operative in metabolic tissues, inasmuch as ET-1 impairs insulin-stimulated translocation of GLUT4 in adipocytes.^{154,155}

Shared Stressors Causing Simultaneous Insulin Resistance and Endothelial Dysfunction

One reason that metabolic and cardiovascular diseases are often associated is that multiple stressors independently cause insulin resistance and endothelial dysfunction. For example, as discussed in the previous section, hyperglycemia leads to increased oxidative stress, increased AGE, inflammation, and increased flux through the HSP, and elevated levels of FFA promote oxidative stress and inflammation. Thus, in diabetes, obesity, metabolic syndrome, dyslipidemias, and cardiovascular diseases, multiple pathogenic stressors simultaneously cause insulin resistance in metabolic tissues and endothelial dysfunction in vascular tissues.

Insights From Animal Models of the Metabolic Syndrome

The spontaneously hypertensive rat (SHR) is a genetic model of hypertension that is also insulin resistant.¹⁵⁶ Defects in vascular responses to insulin can be detected in SHRs before the onset of hypertension, suggesting that elevated blood pressure per se does not determine insulin resistance in this model.¹⁵⁷ Compared with age-matched normotensive Wistar-Kyoto (WKY) control rats, SHRs at 12 weeks of age are overweight, hypertensive, hyperinsulinemic, and insulin resistant, with normal fasting glucose.⁷ Thus, SHRs may be a useful model of the human metabolic syndrome, in which concepts such as coupling between insulin resistance and endothelial dysfunction and the role of pathway-specific insulin resistance may be evaluated.

In the mesenteric vascular bed (MVB) of SHRs *ex vivo* at 12 weeks of age, the vasodilator response to acetylcholine is comparable to that in WKY control rats. Thus, endothelial function with respect to acetylcholine appears normal. However, NO-dependent vasodilator response to insulin is significantly impaired, consistent with the concept that impaired

insulin signaling leading to insulin resistance in metabolic tissues also causes endothelial dysfunction with respect to vasodilator actions of insulin. Interestingly, inhibition of PI 3-kinase pathways with wortmannin significantly reduces insulin-mediated vasodilation in the MVB of WKY rats but has no effect on SHRs. This suggests that PI 3-kinase-dependent pathways are blunted in endothelium of SHRs, consistent with insulin resistance. Moreover, treatment with PD98059 (an inhibitor of MAP-kinase-dependent pathways) unmasks vasodilator actions of insulin in the MVB of SHRs but has no detectable effect on WKY rats. Similar findings are evident after treatment of MVB with the ET-1 receptor antagonists BQ788 and BQ123. Taken together, these data suggest that in SHRs impaired PI 3-kinase pathways lead to decreased production of NO and that increased insulin signaling through MAP-kinase pathways leads to elevated secretion of ET-1. This pathway-specific insulin resistance causing imbalance in vasodilator and vasoconstrictor actions of insulin may be exacerbated by compensatory hyperinsulinemia present in SHRs. Moreover, these defects in endothelial insulin signaling in SHRs provide an explanation for the decreased bioavailability of NO as well as the increased secretion of ET-1, which may conspire to elevate peripheral vascular resistance and contribute to hypertension and atherosclerosis. Thus, SHRs as a model of the metabolic syndrome exemplify the concepts of parallel insulin-signaling pathways in metabolic and vascular tissues helping to couple blood flow and metabolism as well as pathway-specific insulin resistance leading to vascular pathophysiology. Further evidence to support the concept of a reciprocal relationship between insulin resistance and endothelial dysfunction comes from therapeutic interventions in SHRs with the insulin-sensitizer rosiglitazone.¹⁵⁸ Treatment of SHRs with this drug for 3 weeks results in lowering blood pressure, improved insulin sensitivity, decreased insulin levels, and decreased ET-1 levels as well as improvement in endothelial function with normalization of vasodilator responses to insulin in MVB. Thus, insulin sensitizers may rebalance insulin signaling through PI 3-kinase- and MAP-kinase-dependent pathways in metabolic and vascular tissues, resulting in improvement in metabolic and hemodynamic phenotypes (Figure 9).

Insights From Therapeutic Interventions in Humans

Insulin resistance and low plasma adiponectin levels characteristic of metabolic disorders including diabetes and obesity may play an important role in the pathogenesis of cardiovascular diseases characterized by endothelial dysfunction (eg, atherosclerosis, hypertension, and coronary heart disease). Conversely, endothelial dysfunction may contribute significantly to insulin resistance, as described in previous sections. Thus, improving insulin resistance and raising plasma adiponectin levels may be beneficial for the treatment of cardiovascular diseases, and improving endothelial dysfunction may be beneficial for the treatment of metabolic disorders. Results from therapeutic interventions using pharmacological or nonpharmacological therapies aimed at improving insulin sensitivity and endothelial function in metabolic and

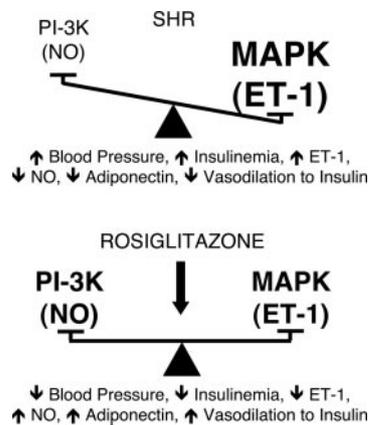


Figure 9. SHRs serve as a model of the metabolic syndrome with hypertension, hyperinsulinemia, insulin resistance, overweight, elevated ET-1 levels, and decreased adiponectin levels, which are associated with decreased vasodilator response to insulin due to decreased PI 3-kinase tone and increased MAPK tone.⁷ After treatment of SHRs with rosiglitazone for 3 weeks, blood pressure, insulin levels, and ET-1 levels were lower, and adiponectin levels and insulin sensitivity were increased, with increased vasodilator response to insulin, consistent with rebalancing between PI 3-kinase and MAPK branches of insulin signaling.¹⁵⁸

cardiovascular diseases support a reciprocal relationship between insulin resistance and endothelial dysfunction that is particularly relevant to treatment of the metabolic syndrome.

Pharmacological Therapies Targeting Insulin Resistance

Thiazolidinediones (synthetic PPAR- γ ligands) are insulin sensitizers that also increase forearm blood flow in humans.¹⁵⁹ Metformin, another agent that improves insulin sensitivity, also improves endothelium-dependent vasodilation in patients with type 2 diabetes.¹⁶⁰ Thiazolidinediones have antiatherogenic properties resulting in inhibition of vascular smooth muscle cell proliferation and decreased accumulation of lipids by macrophages, which may be mediated by anti-inflammatory mechanisms.^{161,162} Moreover, administration of thiazolidinediones significantly increases adiponectin expression and plasma levels in patients with insulin resistance or type 2 diabetes without affecting body weight.^{147,163,164} Adiponectin directly stimulates the production of NO from vascular endothelium using a PI 3-kinase-dependent signaling mechanism similar to that of insulin, which may explain its effects, to oppose atherogenesis and improve endothelial function.²¹ Taken together, these studies suggest that drugs that improve insulin sensitivity may have direct and indirect effects: improving endothelial function and opposing atherogenesis through mechanisms that include enhancing PI 3-kinase-dependent signaling in vascular endothelium, increasing the expression and plasma concentrations of adiponectin, and anti-inflammatory actions. Thus, pharmacological therapies targeting insulin resistance may be beneficial in the treatment of cardiovascular disorders associated with insulin resistance. Indeed, therapy with thiazolidinediones or metformin lowers blood pressure in insulin-resistant patients who are also hypertensive^{165,166} and reduces cardiovascular events in randomized clinical trials.¹⁶⁷

Pharmacological Therapies Targeting Endothelial Dysfunction

Some drugs used for the treatment of hypertension also have beneficial metabolic effects. ACE inhibitors reduce circulating angiotensin II levels, and ARBs block the actions of angiotensin II. These effects lower blood pressure, improve endothelial function, and reduce circulating markers of inflammation. In addition, treatment of patients with ACE inhibitors or ARBs results in significant increases in adiponectin levels and improvement in insulin sensitivity without changing body mass index.^{168–170} These beneficial metabolic effects may be mediated, in part, by blocking inhibitory cross-talk between angiotensin II receptor signaling and insulin receptor signaling at the level of IRS-1 and PI 3-kinase.¹⁴⁰ ACE inhibitors and ARBs may also have direct effects (eg, inducing PPAR- γ activity) that augment insulin-stimulated glucose uptake and promote differentiation of adipocytes.^{171,172} Losartan (ARB) therapy significantly increases plasma adiponectin levels and insulin sensitivity relative to baseline measurements in hypercholesterolemic hypertensive patients (Figure 10, panels A and B).¹⁶⁹ Of note, these findings are significantly correlated with improvements in endothelial function and inflammatory markers (Figure 10, panels C and D). Similar findings are observed with ramipril (ACE inhibitor) therapy in patients with type 2 diabetes.¹⁷⁰ However, in both of these studies, treatment with simvastatin (3-hydroxy-3-methylglutaryl [HMG]-CoA reductase inhibitor) does not increase adiponectin levels or improve insulin sensitivity. Nevertheless, simvastatin does improve endothelial function and inflammatory markers in an additive manner when combined with losartan or ramipril. This suggests that only some mechanisms for improving endothelial function have a beneficial effect on insulin sensitivity and adiponectin levels. Recent clinical trials have demonstrated that using ACE inhibitors or ARBs to treat patients with cardiovascular disease significantly lowers the risk of developing type 2 diabetes.^{139,173} Conversely, using ACE inhibitors to treat patients with type 2 diabetes significantly improves cardiovascular outcomes.¹⁷⁴ Thus, therapeutic interventions with ACE inhibitors and ARBs support the existence of reciprocal relationships between endothelial dysfunction and insulin resistance.

Fibrates are synthetic PPAR- α ligands that improve the circulating lipoprotein profile, resulting in improved endothelial function, reduced vascular inflammation, and reduction in cardiovascular events in randomized clinical trials.^{175,176} In one recent study, fenofibrate therapy (2 months) significantly increased plasma adiponectin levels and insulin sensitivity without changing body weight in patients with primary hypertriglyceridemia.¹⁷⁷ These findings are significantly correlated with improvements in endothelial function and inflammatory markers. The fact that body weight did not change raises the possibility that fenofibrate is directly altering adiponectin levels independent of adiposity. Thus, increased adiponectin levels may contribute to an improvement in insulin sensitivity and endothelial function rather than simply reflecting a change in adiposity. In another study, fenofibrate therapy significantly increased plasma adiponectin levels and insulin sensitivity without changing body

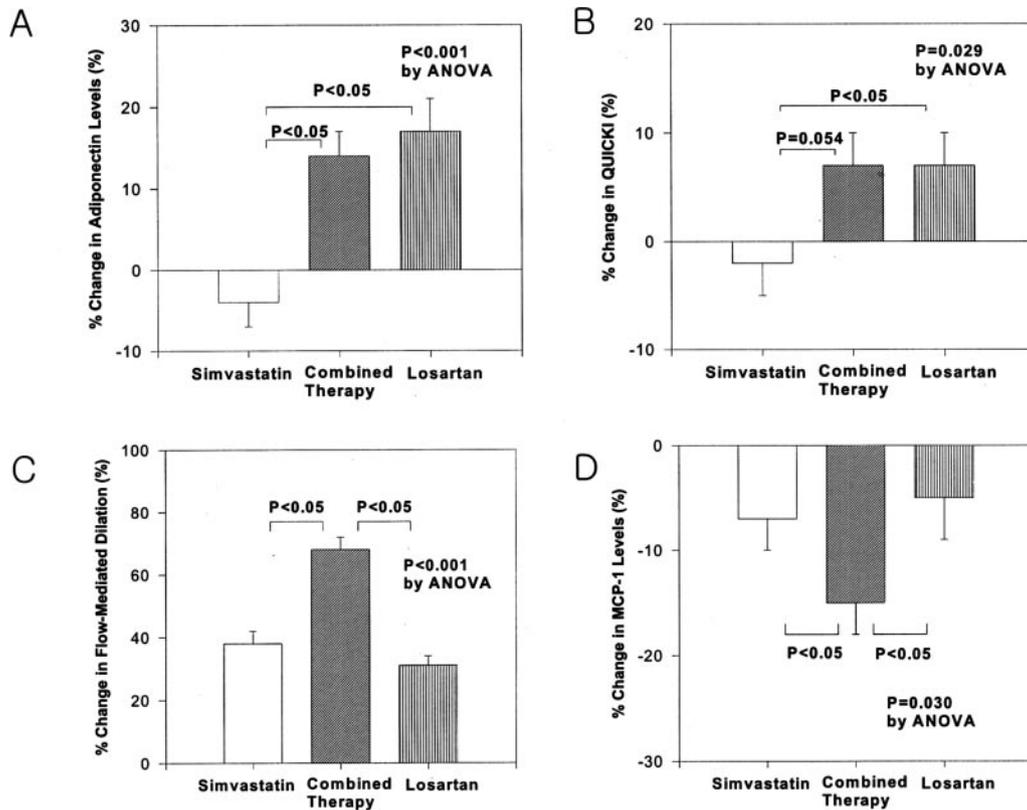


Figure 10. Losartan therapy simultaneously improves adiponectin levels (A), insulin resistance (B), endothelial dysfunction (C), and inflammatory markers (D) in subjects with hypercholesterolemia and hypertension.¹⁶⁹ QUICKI indicates Quantitative Insulin-Sensitivity Check Index.

weight in patients with combined hyperlipidemia.¹⁷⁸ Again, these findings are significantly correlated with improvements in endothelial function and inflammatory markers. However, in that study,¹⁷⁸ treatment with atorvastatin (HMG-CoA reductase inhibitor) did not increase adiponectin levels or improve insulin sensitivity. Nevertheless, atorvastatin did improve endothelial function and inflammatory markers in an additive manner when combined with fenofibrate. Thus, in that study,¹⁷⁸ beneficial effects of statins on endothelial function and inflammatory markers did not translate to improvements in metabolic parameters. Nevertheless, as with ACE inhibitors and ARBs, therapeutic interventions with fibrates suggest that some mechanisms for improving endothelial function result in improved insulin sensitivity.

Nonpharmacological Lifestyle Interventions

Lifestyle modifications including diet, weight loss, and physical exercise decrease insulin resistance, increase adiponectin levels, and improve endothelial dysfunction. Significant increases in adiponectin levels and reduction in insulin resistance have been observed in diabetic and nondiabetic patients after 2 months of diet-induced weight loss.¹⁷⁹ Combining diet control and physical exercise also increases plasma levels of adiponectin. In obese insulin-resistant individuals stratified by glucose tolerance after weight loss in response to a combined hypocaloric diet and moderate physical activity, adiponectin levels and insulin sensitivity increase significantly, especially among diabetic subjects.¹⁸⁰ With respect to

vascular markers of inflammation and endothelial dysfunction, compared with matched subjects consuming a control diet, patients with metabolic syndrome consuming a Mediterranean-style diet have significantly reduced serum concentrations of inflammatory markers as well as decreased insulin resistance and improved endothelial function.¹⁸¹ Similarly, in a cohort of obese women, a 2-year lifestyle intervention consisting of weight loss, physical exercise, and Mediterranean-style diet resulted in decreased body mass index, decreased inflammatory markers, and increased adiponectin levels compared with results in matched control subjects in a nonintervention group.¹⁸² These studies suggest that nonpharmacological lifestyle interventions that improve metabolic and cardiovascular health may be efficacious, in part, because of coupling between metabolic and vascular physiology.

Conclusions

Many varieties of interacting and distinct molecular, cellular, and physiological mechanisms in metabolic and vascular tissues contribute to important reciprocal relationships between insulin resistance and endothelial dysfunction that explain epidemiological data supporting associations and increased risks linking metabolic and cardiovascular disorders. Among these mechanisms, parallel insulin-signaling pathways in metabolic and vascular tissues, pathway-specific insulin resistance, cross-talk between inflammatory signaling and insulin signaling, coupling of blood flow with glucose

metabolism, pathophysiological cross-talk between metabolic and vascular tissues, and shared stressors contributing to insulin resistance and endothelial dysfunction are particularly important. Thus, a combination of therapeutic approaches that target multiple mechanisms is likely to have beneficial effects on metabolic and cardiovascular health that go beyond monotherapy with single agents.

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Disclosures

None.

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