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# Cardiovascular Actions of Insulin

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**Insulin has important vascular actions to stimulate production of nitric oxide from endothelium. This leads to capillary recruitment, vasodilation, increased blood flow, and subsequent augmentation of glucose disposal in classical insulin target tissues (*e.g.*, skeletal muscle). Phosphatidylinositol 3-kinase-dependent insulin-signaling pathways regulating endothelial production of nitric oxide share striking parallels with metabolic insulin-signaling pathways. Distinct MAPK-dependent insulin-signaling pathways (largely unrelated to metabolic actions of insulin) regulate secretion of the vasoconstrictor endothelin-1 from endothelium. These and other cardiovascular actions of insulin contribute to coupling metabolic and hemodynamic homeostasis under healthy conditions. Cardiovascular diseases are the leading cause of morbidity and mortality in insulin-resistant individuals. Insulin resistance is typically defined as decreased sensitivity and/or responsiveness to metabolic actions of insulin. This cardinal feature of diabetes, obesity, and dyslipidemia is also a prominent component of hypertension, coronary heart disease, and atherosclerosis that are all characterized by endothelial dysfunction. Conversely, endothelial dysfunction is often present in metabolic diseases. Insulin resistance is characterized by pathway-specific impairment in phosphatidylinositol 3-kinase-dependent signaling that in vascular endothelium contributes to a reciprocal relationship between insulin resistance and endothelial dysfunction. The clinical relevance of this coupling is highlighted by the findings that specific therapeutic interventions targeting insulin resistance often also ameliorate endothelial dysfunction (and vice versa). In this review, we discuss molecular mechanisms underlying cardiovascular actions of insulin, the reciprocal relationships between insulin resistance and endothelial dysfunction, and implications for developing beneficial therapeutic strategies that simultaneously target metabolic and cardiovascular diseases. (*Endocrine Reviews* 28: 463–491, 2007)**

I. Introduction
II. Insulin-Signaling Pathways Regulating Cardiovascular Physiology
  A. General features of insulin signal transduction pathways
  B. Insulin signaling in vascular endothelium regulating production of NO
  C. Insulin signaling in vascular endothelium regulating production of ET-1 and adhesion molecules
  D. Insulin signaling in vascular smooth muscle
  E. Insulin signaling in heart
III. Cardiovascular Actions of Insulin
  A. Insulin-stimulated capillary recruitment and blood flow
  B. Vasoconstrictor actions of insulin
  C. Effects of insulin on blood pressure
  D. Regulation of cardiac function by insulin
  E. Role of insulin to couple hemodynamic and metabolic physiology
IV. Reciprocal Relationships between Insulin Resistance and Endothelial Dysfunction
  A. Pathway-selective insulin resistance
  B. Lipotoxicity
  C. Glucotoxicity
  D. Proinflammatory signaling and adipocytokines
  E. Effects of compensatory hyperinsulinemia on blood pressure
V. Insights from Genetics and Therapeutic Interventions
  A. Animal models
  B. Human studies
VI. Summary and Conclusions

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Abbreviations: AGE, Advanced glycation end-product; ARB, angiotensin-II receptor blocker; BAEC, bovine aortic endothelial cells; cGKI, cGMP-dependent protein kinase 1; CoA, coenzyme A; CRP, C-reactive protein; EGCG, epigallocatechin gallate; eNOS, endothelial NOS; ET-1, endothelin-1; FFA, free fatty acids; GLUT, glucose transporter; GFAT, glutamine:fructose-6-phosphate amidotransferase; HSP, heat shock protein; HUVEC, human umbilical vein endothelial cells; IGF-IR, IGF-I receptor; IKK$\beta$, inhibitory kB kinase; iNOS, inducible NOS; IR, insulin receptor; IRS, IR substrate; JNK, Jun N-terminal kinase; MBF, myocardial blood flow; MLC, myosin light chain; NADPH, nicotinamide adenine dinucleotide phosphate; NF-$\kappa$B, nuclear factor-$\kappa$B; NO, nitric oxide; NOS, NO synthase; PDGF, platelet-derived growth factor; PDK1, phosphoinositide-dependent protein kinase-1; $PGI_2$, prostacyclin; PI3K, phosphatidylinositol 3-kinase; $PI(3,4,5)P_3$, phosphatidylinositol 3,4,5-trisphosphate; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; RAS, renin-angiotensin system; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase; SGK1, serum- and glucocorticoid-inducible kinase-1; SH2, Src homology 2; SHR, spontaneously hypertensive rat; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VENIRKO, vascular endothelium IR knockout; VSMC, vascular smooth muscle cells.

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## I. Introduction

THE ESSENTIAL ROLE of insulin in regulating glucose homeostasis led to its discovery approximately 85 yr ago (1). However, it was not until 1949 that the ability of insulin to promote glucose uptake was experimentally dem-

onstrated (2). The biological actions of insulin are mediated by specific cell surface receptors that were first described in 1971 (3). Insulin receptors are expressed on nearly every cell in the body, and the molecular cloning of the insulin receptor in 1985 (4, 5) allowed for investigations into the signal transduction mechanisms underlying insulin action in both cellular and physiological contexts. Over the last 20 yr, great progress has been made in understanding the signal transduction pathways controlling classical metabolic actions of insulin to promote glucose uptake in skeletal muscle and adipose tissue through translocation of the insulin-responsive glucose transporter (GLUT) 4 (6). These studies have informed more recent investigations into nonclassical cardiovascular actions of insulin that play an important role in coupling metabolic and cardiovascular physiology (7). In insulin-resistant conditions, impairment of shared insulin-signaling pathways in metabolic and cardiovascular tissues contributes to reciprocal relationships between insulin resistance and endothelial dysfunction. Multiple independent and interdependent mechanisms involving glucotoxicity, lipotoxicity, and inflammation contribute to a vicious synergy between insulin resistance and endothelial dysfunction that helps to explain frequent associations between metabolic and cardiovascular diseases exemplified by the metabolic syndrome. A thorough understanding of the cardiovascular actions of insulin in health and disease has important implications for developing novel therapeutic strategies to improve metabolic and cardiovascular health simultaneously.

## II. Insulin-Signaling Pathways Regulating Cardiovascular Physiology

### *A. General features of insulin signal transduction pathways*

The specific binding of insulin to its cognate cell-surface receptor initiates activation of complex signal transduction networks that regulate diverse cellular functions (8, 9). The insulin receptor (IR), a ligand-activated tyrosine kinase, phosphorylates intracellular substrates including IR substrate (IRS) family members and Shc that serve as docking proteins for downstream signaling molecules (10). Tyrosine phosphorylation of IRS family members at multiple sites creates Src homology 2 (SH2)-domain binding motifs for numerous SH2-domain-containing effectors including phosphatidylinositol 3-kinase (PI3K) and Grb-2. PI3K is a heterodimer composed of a regulatory p85 subunit and a catalytic p110 subunit. PI3Ks have been divided into three classes. The heterodimeric class IA PI3Ks signal downstream of tyrosine kinases such as the IR. Multiple isoforms of p85 and p110 exist and consist of p110$\alpha$, p110$\beta$, and p110$\delta$ catalytic subunit isoforms that bind to the major regulator p85 isoforms, p85$\alpha$, p55$\alpha$, and p50$\alpha$ (11). When SH2 domains of p85 subunit bind to tyrosine-phosphorylated motifs on IRS-1, this allosterically activates the preassociated p110 catalytic subunit to generate the lipid product phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)$P_3$] from the substrate phosphatidylinositol 4,5-bisphosphate (12). PI(3,4,5)$P_3$ binds to the pleckstrin-homology domain in 3-phosphoinositide-dependent protein kinase-1 (PDK-1), resulting in its phosphorylation and activation to subsequently phosphorylate and activate other downstream serine-threonine kinases including Akt and atypical protein kinase C (PKC) isoforms (13, 14). A phosphorylation cascade of serine-threonine kinases downstream from PDK-1 in this PI3K-dependent branch of the insulin-signaling pathway culminates in many of the metabolic actions of insulin (Fig. 1). In addition to PI3K-dependent insulin signaling, another major insulin signaling branch involves tyrosine-phosphorylated IRS-1 or Shc binding to the SH2 domain of Grb-2 that results in activation of the preassociated GTP exchange factor Sos (8, 15). This activates the small GTP binding protein Ras, which then initiates a kinase phosphorylation cascade involving Raf, MAPK/extracellular signal-regulated kinase kinase, and MAPK (8, 16). This MAPK-dependent branch of insulin-signaling pathways generally regulates biological actions related to growth, mitogenesis, and differentiation (Fig. 1). Protein tyrosine phosphatases (*e.g.*, PTP1B) that dephosphorylate the IR and IRS-1 and lipid phosphatases (*e.g.*, SHIP-2 and PTEN) that dephosphorylate PI(3,4,5)$P_3$ play important roles in negative regulation of insulin-signaling pathways (17). Insulin signal transduction pathways are arranged in highly complex networks that include multiple feedback loops, cross-talk between major signaling branches, and cross-talk from signaling pathways of heterologous receptors (18). All of these complexities contribute to the specificity of insulin signaling and insulin action. In addition, the multifunctional nature of insulin responses is context dependent as reflected by the distinct role of insulin-stimulated PI3K/Akt pathway in promoting normal physiological, but not pathophysiological cardiac growth (19–21). These studies suggest that the growth-promoting actions of insulin are not exclusively mediated by the MAPK pathway. Nevertheless, one useful conceptual oversimplification is to consider two major signaling branches: PI3K-dependent pathways that mediate metabolic actions of insulin and MAPK-kinase-dependent pathways that mediate nonmetabolic mitogenic and growth effects of insulin (Fig. 1) (7). As described below, these two major branches of insulin-signaling pathways also regulate distinct biological functions related to regulation of cardiovascular homeostasis.

### *B. Insulin signaling in vascular endothelium regulating production of NO*

Among the most important cardiovascular actions of insulin is the stimulation of increased production of the potent vasodilator nitric oxide (NO) from vascular endothelium (22). In endothelial cells, endothelial NO synthase (eNOS) catalyzes the conversion of the substrate L-arginine to the products NO and L-citrulline (23). Classical vasodilators including acetylcholine stimulate an increase in intracellular calcium that promotes the binding of calcium/calmodulin to eNOS. In the presence of a variety of cofactors, this results in dissociation of eNOS from caveolin-1 with subsequent dimerization and activation of eNOS (23, 24). The insulin-signaling pathway in vascular endothelium that regulates activation of eNOS employs a phosphorylation-dependent mechanism that is completely distinct, separable, and independent from classical calcium-dependent mechanisms used by G protein-coupled receptors such as the acetylcholine

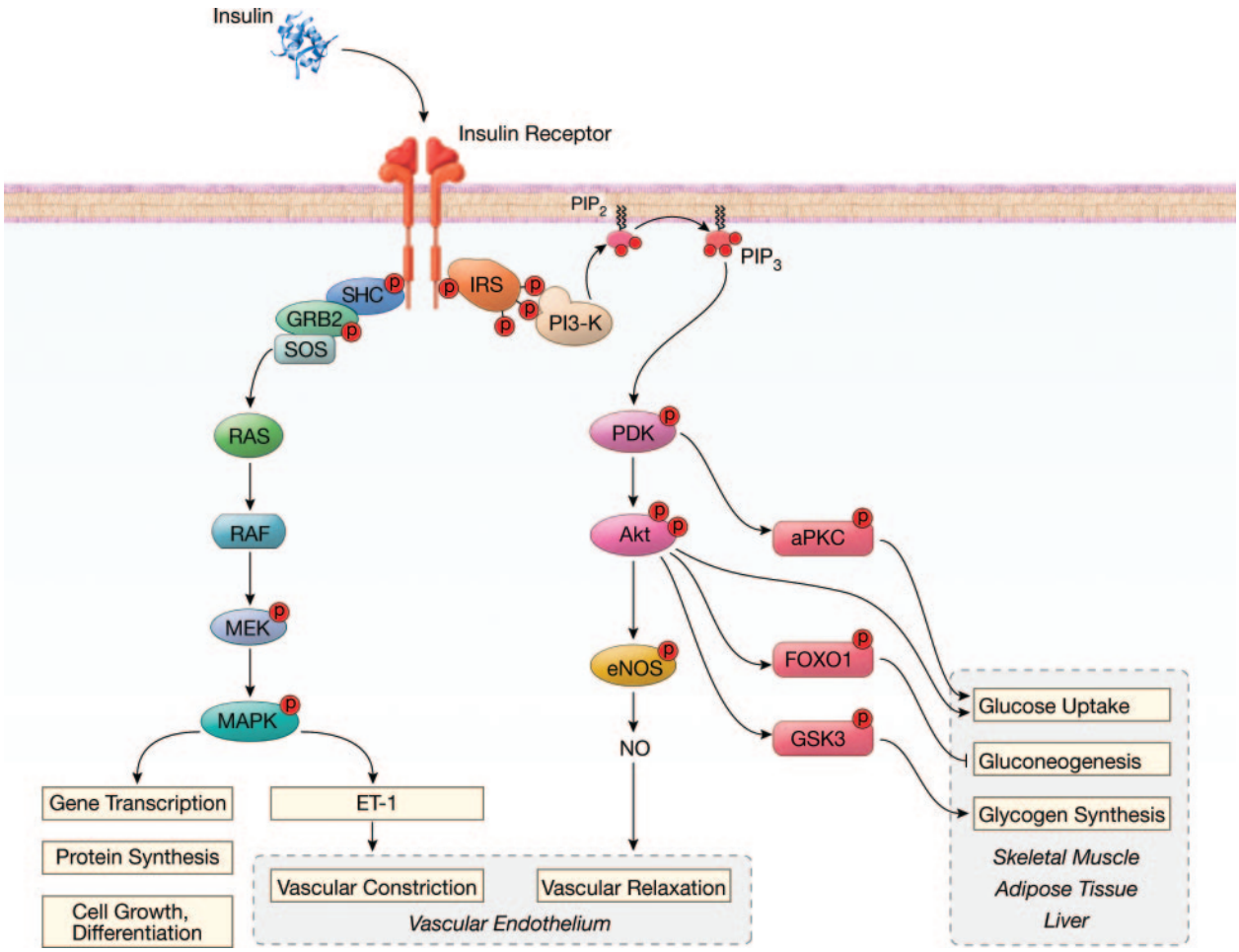


FIG. 1. General features of insulin signal transduction pathways. PI3K branch of insulin signaling regulates glucose metabolism in skeletal muscle, adipose tissue, and liver while stimulating NO production and vasodilation in vascular endothelium. MAPK branch of insulin-signaling pathways generally regulates growth and mitogenesis and controls secretion of ET-1 in vascular endothelium.

receptor (24–27). In recent years, a complete biochemical signaling pathway leading from the IR to phosphorylation and activation of eNOS has been elucidated in vascular endothelial cells in primary culture. This pathway requires activation of the IR tyrosine kinase, which then phosphorylates IRS-1 leading to binding and activation of PI3K; and subsequent activation of PDK-1, which then phosphorylates and activates Akt, which directly phosphorylates and activates eNOS, leading to increased production of NO within a matter of minutes (Fig. 1) (22, 25, 28, 29).

*1. IR tyrosine kinase.* In human endothelial cells, IRs are expressed on the cell surface at a level approximately 10-fold less than the related IGF-I receptor (IGF-IR) (~40,000 IR and ~400,000 IGF-IR per cell) (22). IGF-IRs and hybrid receptors (IR/IGF-IR) composed of heterodimers containing an $\alpha\beta$-chain of the IR associated with an $\alpha\beta$-chain of the IGF-IR have low affinity for insulin (30). Physiological concentrations of insulin (100–500 pM) selectively activate IR and downstream signaling pathways leading to activation of eNOS, whereas supraphysiological concentrations of insulin activate IGF-IR and hybrid receptors (22, 31). The first study directly demonstrating that insulin stimulates production of NO from endothelial cells used an NO-selective electrode to show that levels of NO produced in human umbilical vein endothelial cells (HUVEC) in response to a maximally stimulating concentration of insulin are approximately twice those that can be elicited by IGF-I stimulation (22). Overexpression of wild-type IRs in HUVEC causes a 3-fold increase in insulin-stimulated production of NO that is not seen in HUVEC transfected with mutant kinase-deficient IRs (28). These data suggest an essential role for IR tyrosine kinase activity in activation of eNOS in response to insulin.

*2. IRSs.* IRS-1 is a major substrate for the IR tyrosine kinase. Overexpression of wild-type IRS-1 in bovine aortic endothelial cells (BAEC) results in a 3-fold increase in NO production in response to a maximally stimulating concentration of insulin (determined using the NO-specific fluorescent dye DAF-2) (29). These results suggest that IRS-1 is capable of mediating insulin-stimulated activation of eNOS. The relative contribution of IRS-2 in insulin-mediated NO production in endothelial cells is not known. However, overexpression of IRS-2 in rat fibroblasts cotransfected with eNOS significantly increases insulin-stimulated NO production (29). Expression of a mutant IRS-1 (IRS1-F6) that is unable to bind to the p85 subunit of PI3K inhibits both insulin-stimulated PI3K activity and NO production in endothelial cells

(29). Introduction of an IRS-1 antisense ribozyme into endothelial cells substantially reduces insulin-stimulated production of NO (29). Taken together, these results suggest that IRS-1 is a necessary component (and the predominant IRS family member) of the insulin-signaling pathway leading to activation of PI3K that then enhances activation of eNOS.

*3. PI3K.* Downstream from IRS-1, the essential role of PI3K in mediating insulin-stimulated production of NO is demonstrated in studies where preincubation of HUVEC with wortmannin (PI3K inhibitor) blocks NO production in response to insulin (22, 27). More importantly, overexpression of a dominant inhibitory mutant of the p85 regulatory subunit of PI3K significantly and substantially inhibits insulin-mediated production of NO in transfected HUVEC (28). In addition, PI3K mediates effects of insulin to stimulate increased expression of eNOS in endothelial cells (32, 33). Interestingly, inhibition of MAPK-dependent insulin-signaling pathways may enhance the PI3K-dependent vascular actions of insulin on eNOS (33, 34).

*4. PDK-1.* PDK-1 is immediately downstream from PI3K. Overexpression of wild-type PDK-1 in BAEC results in a 2-fold increase in insulin responsiveness with respect to production of NO. Expression of a kinase-deficient PDK-1 mutant significantly blocks insulin-mediated production of NO (29). These data suggest that PDK-1 is an essential component of the insulin-signaling pathway leading to production of NO in vascular endothelial cells.

*5. Akt.* Akt is activated in response to phosphorylation by PDK-1. Akt directly phosphorylates human eNOS at $Ser^{1177}$ (equivalent to $Ser^{1179}$ in bovine eNOS), resulting in enhanced eNOS activity (35). With respect to insulin signaling, the Akt phosphorylation site on eNOS is absolutely essential for activation of eNOS because cells expressing a mutant eNOS with a disrupted Akt phosphorylation site (alanine substituted for serine at position 1179) are unable to produce NO in response to insulin (25). Overexpression of dominant inhibitory mutant Akt proteins in HUVEC nearly completely inhibits production of NO in response to insulin (28). Akt1 is the predominant isoform in the vasculature, and endothelial cells from Akt1 knockout mice have significantly low levels of active eNOS (36). Therefore, it is most likely that Akt1 isoform mediates insulin-induced activation of eNOS. Moreover, pretreatment of cells with the calcium chelator BAPTA does not inhibit the ability of insulin to stimulate phosphorylation of eNOS at $Ser^{1179}$ or enhance eNOS activity (25). In addition, insulin treatment does not alter intracellular calcium levels in endothelial cells (27). This suggests that insulin-stimulated production of NO is calcium-independent and mediated by activation of Akt1.

*6. Role of HSP90.* Association of heat shock protein (HSP) 90 with eNOS is critically important for eNOS-mediated NO production (24). Although insulin-induced eNOS activation is calcium-independent, insulin stimulates calmodulin binding to eNOS (26). This requires HSP90 binding to eNOS, which facilitates insulin-stimulated activation of eNOS mediated by phosphorylation of eNOS at $Ser^{1177}$ by Akt.

Akt is a necessary signaling molecule for insulin-stimulated activation of eNOS. However, activation of Akt *per se* is not sufficient for activation of eNOS. For example, treatment of endothelial cells with either insulin or platelet-derived growth factor (PDGF) results in comparable phosphorylation and activation of endogenous Akt. Nevertheless, only insulin (but not PDGF) treatment results in phosphorylation and activation of eNOS at the Akt phosphorylation site $Ser^{1179}$ with consequent production of NO (22, 25, 26). One potential mechanism underlying this specificity may be that insulin (but not PDGF) elicits the formation of a ternary eNOS-HSP90-Akt complex (26).

*7. Role of protein phosphatases.* eNOS activity is enhanced by phosphorylation at $Ser^{1177}$ and decreased by phosphorylation at $Thr^{495}$ (37). The temporal dynamics of phosphorylation at these regulatory sites involves both kinases and Ser/Thr phosphatases. For example, PP-2A may specifically dephosphorylate eNOS at $Ser^{1177}$, whereas PP-1 has been implicated in dephosphorylation of eNOS at $Thr^{495}$ (24, 38). In endothelial cells, insulin stimulates an acute decrease in phosphorylation of eNOS at $Thr^{495}$ while simultaneously increasing phosphorylation of eNOS at $Ser^{1177}$ (39). It is possible that insulin is activating a phosphatase targeting $Thr^{495}$ while inhibiting a phosphatase targeting $Ser^{1177}$.

*8. Other vasodilators.* Prostacyclin ($PGI_2$), a metabolite of arachidonic acid produced by cyclooxygenase-1 in endothelial cells, is another endothelial-derived vasodilator (40). Insulin acutely stimulates production of $PGI_2$ from vascular endothelium (41, 42). NO can directly suppress activity of cyclooxygenase-1 and decrease both basal and stimulated release of $PGI_2$ (40, 43). However, inhibition of insulin-stimulated NO production using N (G)-nitro-L-arginine methyl ester does not prevent a $PGI_2$ production in endothelial cells (41). This suggests that insulin has direct actions to stimulate $PGI_2$ production in an NO-independent fashion. Insulin-signaling pathways regulating $PGI_2$ production are yet to be elucidated.

### *C. Insulin signaling in vascular endothelium regulating production of ET-1 and adhesion molecules*

Endothelin-1 (ET-1) is a vasoconstrictor secreted by endothelial cells that opposes vasodilator actions of NO (44). Recent studies in BAEC and in mesenteric vascular beds have demonstrated that insulin and other hormones acutely stimulate the secretion of ET-1 using MAPK-dependent (but not PI3K-dependent) signaling pathways (45–47).

Endothelial expression of cellular adhesion molecules including intercellular adhesion molecule-1, vascular cell adhesion molecule (VCAM-1), and E-selectin is critical in modulating cell-cell interactions between circulating inflammatory cells and vascular endothelium. Insulin stimulates increased expression of VCAM-1 and E-selectin on endothelium using MAPK-dependent, but not PI3K-dependent, signaling pathways (33). Blockade of PI3K-dependent pathways enhances the effects of insulin or vascular endothelial growth factor (VEGF) to increase expression of these adhesion molecules (33).

### *D. Insulin signaling in vascular smooth muscle*

*1. Production of NO in vascular smooth muscle cells (VSMC).* In the vasculature, bioavailable NO originates mostly from the endothelium. Endothelial-derived NO diffuses into VSMC where it activates guanylate cyclase to increase cGMP levels that evoke vasorelaxation. However, expression of eNOS, inducible NOS (iNOS), and neuronal NOS mRNA and protein has been detected in VSMCs in certain contexts (48–55). This raises the possibility that NO production in VSMCs may act in an autocrine fashion to regulate vasodilator functions. In VSMC, insulin increases NOS (eNOS and iNOS) activity and NO-dependent GMP production (48, 51, 56, 57). VSMCs express both IR and IGF-IR (58). Physiological concentrations of insulin stimulate IR autophosphorylation in VSMC and result in a rapid increase in cGMP levels by activating eNOS in human VSMCs (51, 56, 59, 60). Likewise, stimulation of IR/IGF-IR evokes a rapid release of NO in VSMC (as assessed using an NO-selective electrode) (61). Genistein (tyrosine kinase inhibitor) and wortmannin (PI3K inhibitor) both block effects of insulin to stimulate activation of NO in VSMC, suggesting that the IR tyrosine kinase and subsequent activation of PI3K are both necessary for regulation of eNOS or iNOS by insulin in VSMC (48, 56).

*2. VSMC contractility.* Insulin attenuates VSMC contractility by regulating agonist-induced increases in cytosolic calcium through voltage-sensitive calcium channels and altering the activity of myosin light chain phosphatases (62–64). This may be mediated by signaling molecules including Rho kinase and PKC (65–67). RhoA, a small GTP binding protein, plays a key role in agonist-induced VSMC contraction (68). Rho activation and membrane localization are regulated by geranylgeranylation and phosphorylation of Rho (68). Active RhoA recruits and stimulates ROK-$\alpha$, which then phosphorylates and inhibits myosin light chain (MLC) phosphatase leading to an increase in levels of phosphorylated MLC and heightened vascular tone (69, 70). In VSMCs, insulin acutely inhibits geranyl-geranyl transferase and decreases membrane levels of RhoA (66). In addition, insulin stimulates phosphorylation of RhoA at $Ser^{188}$, which prevents it from binding to and activating ROK-$\alpha$ (66). These inhibitory actions of insulin are NO/cGMP-dependent, and may be mediated by cGMP-dependent protein kinase 1 (cGKI) $\alpha$ (66, 71). Moreover, insulin-activated cGKI$\alpha$ interacts with and activates MLC phosphatase (72). In VSMC, wortmannin (PI3K inhibitor) (48) and small interfering RNA against Akt (73) abrogates the effects of insulin on the RhoA/ROK/MLC phosphatase pathway, whereas expression of constitutively active Akt up-regulates cGKI$\alpha$, ROK-$\alpha$, and MLC phosphatase activities (73). Thus, the PI3K/Akt insulin-signaling pathway in VSMC is likely to mediate decreased contractility.

*3. Calcium flux in VSMC.* Treatment of VSMC with insulin impairs agonist-evoked increases in intracellular calcium and accelerates the rate of calcium decline by inhibiting calcium influx and stimulating calcium efflux (61, 62, 74–79). Insulin treatment results in hyperpolarization of membrane potential (80), stimulation of sodium pumps (81), and activation of $Ca^{2+}$-dependent $K^+$ channels (82), which all tend to reduce $Ca^{2+}$ influx via voltage-operated channels (74). Insulin also activates $Ca^{2+}$-pumps at the plasma membrane and sarcoplasmic reticulum to enhance cytoplasmic $Ca^{2+}$ efflux (79). Treatment of VSMC with genistein and tyrophostin A-23 (tyrosine kinase inhibitors) attenuates these effects, suggesting that these actions of insulin are mediated by the IR (83). Inhibition of NOS also inhibits these effects of insulin on intracellular calcium (60). NO/cGMP is known to increase activity of sodium pumps (60) and activate $Ca^{2+}$-dependent $K^+$ channels (84). Moreover, IR/IGF-IR regulates sodium pumps through signaling by PI3K and atypical PKC-$\zeta$ (85). Thus, insulin-stimulated pathways involving PI3K/Akt/NO may help to regulate decreases in intracellular calcium in VSMC that result in decreased vasoconstrictor tone.

### *E. Insulin signaling in heart*

Insulin regulates metabolism in the heart by modulating glucose transport, glycolysis, glycogen synthesis, lipid metabolism, protein synthesis, growth, contractility, and apoptosis in cardiomyocytes (86–88). In addition, vasodilator actions of insulin in coronary vasculature augment myocardial perfusion (89). IRs are expressed at levels of about 10,000 to 100,000 receptors per cardiomyocyte. Oxidation of fatty acids supplies approximately 70% of the heart's energy needs, however glucose and lactate may account for up to 30% of total ATP production. Insulin-stimulated glucose uptake in cardiomyocytes is mediated primarily by the insulin-responsive GLUT4. However, in addition to the basal cardiac glucose uptake mediated by GLUT1, contraction-mediated GLUT4 translocation to the sarcolemma may contribute significantly to myocardial glucose uptake (90). As in other insulin-sensitive tissues, insulin signaling via PI3K/Akt pathways plays a key role in cardiac glucose uptake. Insulin-stimulated activation of Akt also promotes cardiac glycogen accumulation by simultaneously inhibiting activity of both glycogen synthase kinase 3 and AMP-activated protein kinase (86, 91). Moreover, in the heart, insulin-stimulated Akt phosphorylates the transcription factor FOXO-1, which is known to affect glucose and lipid metabolism (92).

*1. Cardiac contractility.* Insulin enhances cardiac contractility *in vivo* in humans as well as in isolated cardiac muscle (86, 87, 93–95). Myocardial excitation is associated with *trans*-membrane movement of extracellular $Ca^{2+}$ into cardiac myocytes through activated $Ca^{2+}$ channels and reverse $Na^{2+}$/$Ca^{2+}$ exchange. This influx of $Ca^{2+}$ stimulates additional release of $Ca^{2+}$ from the sarcoplasmic reticulum via ryanodine receptors, which results in myofilament activation and contraction. Studies in isolated human cardiac myocytes suggest that insulin enhances $Ca^{2+}$ influx through activation of L-type $Ca^{2+}$ channels and reverse-mode $Na^{2+}/Ca^{2+}$ exchange (94, 95). The PI3K inhibitors wortmannin or LY294002 inhibit the inotropic actions of insulin (94–96). The role of Akt in inotropic actions of insulin has not been directly assessed. Overexpressing Akt in cardiac myocytes is associated with increased cytoplasmic $Ca^{2+}$ due to enhanced influx through L-type $Ca^{2+}$ channels and release from sarcoplasmic reticulum (20, 97). Insulin also enhances myofilament $Ca^{2+}$ sen-

sitivity (95). Moreover, insulin increases cardiac NO production through the PI3K/Akt/eNOS pathway (98), and this may contribute to inotropic effects of insulin (99). Chronic overexpression of myocardial Akt (~15-fold) leads to cardiac dysfunction and heart failure (100), whereas a smaller (~2-fold) increase in Akt activity/expression associated with exercise (for 4 wk) is not associated with impaired contractility (101). These studies suggest that the effects of Akt activation on cardiac contractility may be dependent on the magnitude and duration of Akt activation (102).

*2. Cardiac growth.* The PI3K/PDK-1/Akt branch of insulin-signaling pathways also plays an important role in developmental and physiological growth of the heart (19, 87, 88, 103). Downstream from Akt, activation of mammalian target of rapamycin promotes cardiac growth, whereas suppression of GSK3$\beta$ and FOXO helps to regulate cardiomyocyte size (104, 105). Constitutive overexpression of Akt leads to cardiac hypertrophy and dysfunction (20, 105). Thus, beneficial effects of Akt on cardiac growth may depend on temporal patterns of Akt activation as well as subcellular localization of Akt (20, 97, 104, 105). Pathological cardiomyocyte hypertrophy may be regulated by a distinct subset of the insulin-signaling pathways involving MAPK, p38 MAPK, and small G proteins Rho and Ras in addition to other signaling pathways. including PI3K/Akt pathway, calcineurin-nuclear factor of activated T cell pathway, kinases regulating histone deacetylases, cyclin-dependent kinase-7 and -9, PKC, and calmodulin kinase (104, 106).

## III. Cardiovascular Actions of Insulin

PI3K-dependent insulin-signaling pathways in vascular endothelium described above regulate vasodilator actions of insulin, whereas MAPK-dependent insulin-signaling pathways tend to promote prohypertensive actions of insulin in various tissues. Under healthy conditions, the various cardiovascular actions of insulin exist in a balance that contributes to cardiovascular homeostasis (Fig. 2).

### *A. Insulin-stimulated capillary recruitment and blood flow*

In humans, iv insulin infusion stimulates vasodilation and increased blood flow in an NO-dependent fashion (107, 108). Increases in insulin levels that accompany ingestion of a mixed meal or a glucose load also increase limb blood flow, decrease vascular resistance, and increase sympathetic activity in some (109–112), but not in other studies (113, 114). These effects occur at physiological concentrations of insulin and in a relatively short time (30–60 min). Vasodilator actions of insulin comprise a spatially and temporally heterogeneous process that occurs in distinct stages (115). 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. This is followed by relaxation of larger resistance vessels, which increases overall limb blood flow (maximum flow reached after 2 h) (116). The overall vasodilator response to insulin is an integration of enhanced capillary recruitment and elevated total blood flow.

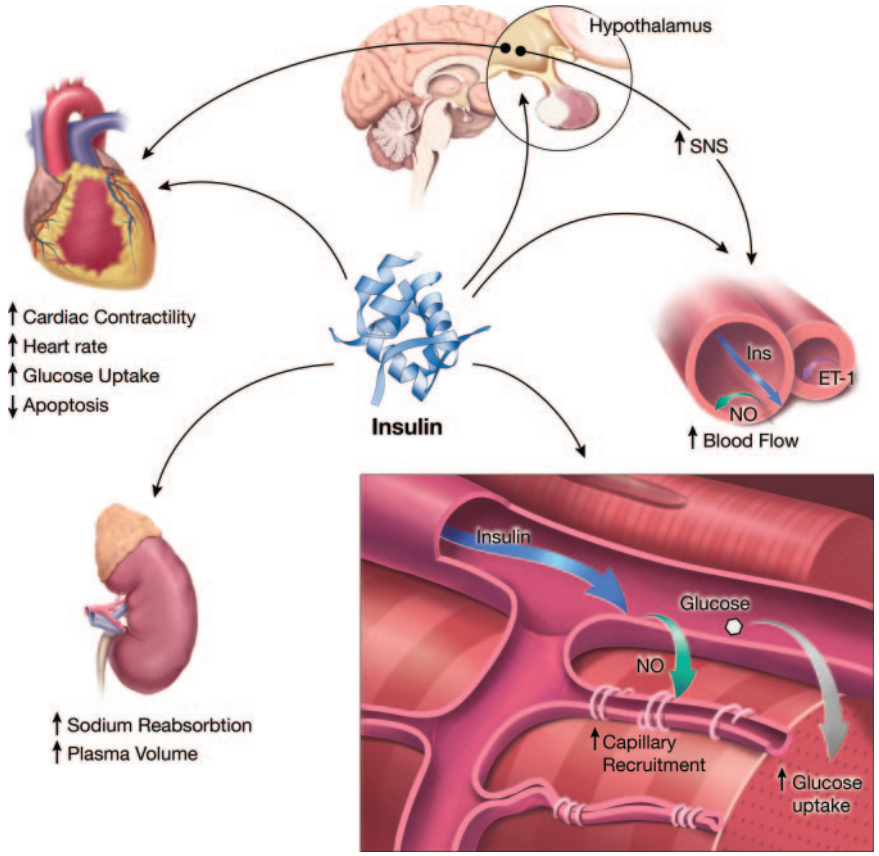


FIG. 2. Cardiovascular targets and actions of insulin.

*1. Skeletal muscle capillary recruitment.* The microvascular unit, a group of capillaries fed by a single terminal arteriole, is the smallest functional unit for control of blood flow in skeletal muscle (117). Dilation of terminal arterioles can produce "maximal" opening (*i.e.*, recruitment) of downstream dependent capillaries without initially changing total blood flow (118). Animal studies using isolated hindlimb preparations and human studies using limb or tissue balance techniques support the concept that insulin significantly modulates microvascular perfusion through capillary recruitment (93, 111, 115, 119–133). Insulin-stimulated capillary recruitment was first studied by Rattigan *et al.* (122) in rat hindlimb by measuring endothelial metabolism of exogenously infused 1-methylxanthine. Recently, a highly sensitive and specific, noninvasive technique using ultrasound imaging of skeletal muscle during microbubble contrast infusion has allowed for real-time assessment of capillary recruitment in response to insulin (111, 115, 127, 128, 130). In the hindlimb of anesthetized rats, insulin infusion (steady-state plasma insulin levels of ~600 pM) increases microvascular volume by 1.5-fold after 5–10 min and is maximal (2.5-fold) after 20 min of insulin infusion (130). Insulin-stimulated capillary recruitment occurs well before changes in total limb blood flow that peak after 2 h of insulin infusion (130). Upon cessation of insulin infusion, increases in microvascular volume persist for 15–30 min after insulin concentrations return to basal levels (131). Pretreatment with N (G)-nitro-L-arginine methyl ester (NOS inhibitor) attenuates insulin-enhanced capillary volume by 50 to 70%, suggesting that these effects are partially NO-dependent (129, 130). In addition to effects on endothelium-derived NO, direct actions of insulin on VSMC, release of other vasoactive factors, changes in muscle metabolism, and alterations in sympathetic activity may contribute to insulin-stimulated capillary recruitment. Consistent with studies in rats, in deep flexor muscles of the human forearm, local intraarterial infusion of insulin (arterial plasma levels of ~300 pM) results in a 25% increase in muscle capillary blood volume (127). Similarly, 1 h after a mixed meal, microvascular volume in human forearm increases by approximately 45% (111). Thus, physiological concentrations of insulin in both animals and humans rapidly enhance skeletal muscle capillary recruitment.

*2. Limb blood flow.* Intravenous infusion of insulin increases total limb blood flow in a majority (93, 108, 121, 127, 132–150) but not all (151–156) studies in humans. Increases in bulk muscle blood flow have been demonstrated at both physiological (108, 132, 134, 135, 137, 140, 143) and supraphysiological (123, 134, 140, 142, 150, 157) concentrations of insulin. Some controversy exists over whether physiological concentrations of insulin cause significant increases in total limb flow (147, 158). This may be the result of differences in subject selection as well as differences in physical fitness, muscularity, endothelial function, and capillary density of study subjects. Technical limitations or differences in sensitivity of various experimental approaches for estimating limb blood flow (*e.g.*, plethysmography, thermodilution, positron emission tomography, dye dilution, Doppler ultrasound, and ultrasound measurements of brachial or femoral artery diameter) may also contribute to conflicting reports (140, 159, 160). Nevertheless, the preponderance of experimental evidence in humans suggests that physiological concentrations of insulin increase total limb blood flow, albeit with a slower time course than capillary recruitment. It remains unclear whether changes in capillary recruitment and total blood flow are independent or functionally coupled.

Insulin-induced vasodilation does not depend on concomitant changes in carbohydrate metabolism (108). Scherrer *et al.* (139) and Steinberg *et al.* (107) were among the first to provide compelling evidence that NO mediates the vasodilator actions of insulin. Coinfusion of L-NMMA (NOS inhibitor) during steady-state hyperinsulinemia under euglycemic conditions abrogates insulin-induced increases in blood flow in the leg (107) and forearm (139). Insulin also increases NOx (nitrate/nitrite, stable oxidative end-products of NO) release from the leg (161). Furthermore, a significant 2-fold increase in NOS activity (without significant changes in NOS protein content) is observed in human skeletal muscle in response to insulin stimulation (determined by biopsy of vastus lateralis) (162).

### *B. Vasoconstrictor actions of insulin*

In addition to vasodilator actions of insulin discussed above, opposing hemodynamic actions of insulin include activation of the sympathetic nervous system and stimulation of secretion of the vasoconstrictor ET-1 from vascular endothelium.

*1. Role of sympathetic nervous system.* In humans, the role of the sympathetic nervous system to mediate vasoconstriction in the integrated hemodynamic response to insulin has been reviewed in detail previously (163). In healthy lean individuals, physiological concentrations of insulin increase venous catecholamine levels and sympathetic nerve activity (135, 156, 164). In addition, insulin infusion augments centrally mediated sympathetic outflow to skeletal muscle in humans and rats (163, 165). The classical observation that insulin decreases arterial pressure in patients with autonomic failure highlights the role of insulin-induced sympathetic vasoconstriction in normal individuals (166). Indeed, in people who have undergone regional sympathectomy, NO-dependent vasodilation in response to insulin in the denervated limb occurs more quickly than in the innervated limb (167). This suggests that heightened sympathetic vasoconstrictor tone stimulated by insulin opposes the vasodilator actions of insulin mediated by NO. In animal models, the cholinergic system may also be involved in mediating vasoactive actions of insulin (168). However, in humans, neither cholinergic nor $\beta$-adrenergic pathways seem to be involved in modulating vasodilator actions of insulin (138). Under conditions of the euglycemic hyperinsulinemic glucose clamp, the rise and fall in peak muscle sympathetic activity temporally lags behind the rise in plasma insulin concentrations. This may reflect the time required for insulin to redistribute and cross the blood-brain barrier as well as the activation/inactivation kinetics of cellular events that trigger an increase in nerve activity (135). Of note, in rats, differential hypothalamic activation of PI3K and MAPK has been demonstrated in the regional sympathetic responses to insulin (165). Insulin-induced release of

NO may oppose sympathetically mediated vasoconstriction at the level of the myocyte as well as in the central nervous system. In proximal and distal arterioles regulating total blood flow and capillary recruitment, respectively, smaller vessels are associated with increased sensitivity to insulin-mediated vasodilation. However, in the face of elevated sympathetic nerve activity, distal arterioles vasodilate in response to insulin, whereas proximal arterioles undergo sustained vasoconstriction (169). Thus, various parts of the vascular tree have a differential response to insulin (149, 170) and sympathetic nerve activity (169).

*2. Role of ET-1.* In addition to production of NO, insulin regulates synthesis and secretion of ET-1 from vascular endothelium. Consequently, in mice with targeted deletion of the IR in vascular endothelium [vascular endothelium IR knockout (VENIRKO) mice], expression of both eNOS and ET-1 is significantly diminished (171). In humans, the effects of insulin to change circulating levels of ET-1 are unclear (172–174). However, because ET-1 is a paracrine factor, plasma concentrations are less relevant than local concentrations and do not predict ET-1 activity in the vascular milieu (44). Supporting this concept, vasodilator actions of insulin are potentiated by ET-1 receptor blockade in animals (175) and humans (176). Consistent with the MAPK dependence of insulin-stimulated secretion of ET-1 in vascular endothelium, inhibition of MAPK blocks vasoconstrictor effects of insulin in rat skeletal muscle arterioles (177). A shift in balance between vasoconstrictor and vasodilator actions of insulin mediated by pathway-specific impairment in PI3K signaling may be an important factor in the vascular pathophysiology of insulin resistance and endothelial dysfunction.

### *C. Effects of insulin on blood pressure*

As discussed above, insulin has opposing vasodilator and vasoconstrictor actions such that the net hemodynamic effect of insulin on blood pressure is minimal in healthy humans. Indeed, short-term insulin infusion under isoglycemic conditions modestly decreases (157) or has no effect on arterial blood pressure (108, 119, 135, 178–180). In these studies, iv insulin infusion significantly increases heart rate and cardiac output and decreases total peripheral resistance. However, the fall in systemic vascular resistance is modest (~15%) when compared with the reduction in leg vascular resistance (~40%), suggesting a differential and specific effect of insulin to dilate skeletal muscle vasculature (119). By contrast, with iv insulin infusion, intraarterial infusion of insulin does not change (150, 155, 176, 181–183) or minimally increases limb blood flow (123, 146, 184). This is most likely because insulin simultaneously stimulates both NO production and ET-1 secretion. In the presence of ET-1 receptor blockade, intraarterial insulin infusion causes measurable vasodilation (176).

Acute infusion of insulin also promotes sodium retention by enhancing distal tubular sodium reabsorption in normal and insulin-resistant individuals (185, 186). However, prolonged hyperinsulinemia during euglycemic glucose clamp conditions results in compensatory natriuresis due to diminished proximal tubular sodium reabsorption and increases in renal plasma flow and glomerular filtration rate in healthy individuals (187, 188). Due to the small effects on sodium retention and compensatory natriuresis, it is unlikely that renal actions of insulin play an important role in modulating blood pressure acutely.

### *D. Regulation of cardiac function by insulin*

Due to high basal oxygen extraction, cardiac oxygen demand is a dominant determinant of myocardial blood flow (MBF). The coronary microcirculation is a major contributor to coronary vascular resistance and MBF. In the setting of increased myocardial oxygen consumption, myocardial hyperemia is initially associated with increased capillary blood flow velocity and followed by capillary recruitment (189). As previously discussed, insulin action in the endothelium and the vascular wall modulates hemodynamics through changes in both flow and capillary recruitment. Consistent with this, accumulating evidence suggests that insulin enhances MBF in the heart (89, 190–195). In addition, insulin increases cardiac contractility resulting in increased myocardial work and oxygen consumption (93). Because MBF and myocardial oxygen consumption are tightly coupled and regulated, it is difficult to evaluate direct actions of insulin on the coronary vasculature *in vivo*. One approach used to address this issue is to evaluate effects of insulin under conditions in which MBF and oxygen consumption are uncoupled by simultaneous infusion of adenosine. Under these circumstances, physiological concentrations of insulin enhance adenosine-stimulated MBF and coronary flow reserve in humans (89, 190, 191). Similarly, physiological hyperinsulinemia in healthy subjects increases MBF, specifically in areas of the myocardium associated with high rates of glucose uptake (192). This suggests coupling between metabolic and vascular actions of insulin in the heart. As observed in human skeletal muscle, ingestion of a mixed meal enhances capillary recruitment in the heart (196). However, meals evoke a complex neuroendocrine response in addition to changes in plasma insulin that may independently influence cardiac hemodynamics. In fact, ingestion of a mixed meal is associated with an increase in left ventricular ejection fraction and contractility that may augment MBF independent from the effects of insulin (197). Therefore, meal-induced increases in MBF cannot be solely attributed to effects of insulin. Nevertheless, concomitant infusion of insulin along with a meal further enhances myocardial capillary recruitment, suggesting that insulin does have some direct effects to increase flow in the myocardial capillary bed (194).

### *E. Role of insulin to couple hemodynamic and metabolic physiology*

Studies in animals and humans suggest that insulin-stimulated increases in skeletal muscle capillary recruitment and blood flow play an important physiological role in augmenting the delivery of insulin and glucose to skeletal muscle. Glucose delivery to skeletal muscle is dependent on muscle blood flow and vascular capillary surface area and permeability. When capillary surface area and permeability are small, increasing blood flow *per se* has a minimal effect on net glucose uptake (198, 199). However, after a mixed meal or an

oral glucose load, recruitment of capillaries expands the capillary surface area and increases muscle blood flow, which together substantially increase glucose and insulin delivery (111, 148). Changes in insulin-mediated capillary recruitment are positively correlated with changes in insulin-stimulated glucose disposal (130). The time course for insulin-stimulated capillary recruitment approximates the time course for insulin-mediated glucose uptake in skeletal muscle (130). Moreover, inhibitors of NOS that block insulin-mediated capillary recruitment cause a concomitant 40% reduction in glucose disposal (129, 130). In human studies, under conditions of high glucose extraction, insulin stimulates parallel increases in leg glucose disposal and blood flow in a dose-dependent manner (109, 136, 200, 201). Although the time course of increases in 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)-nitro-L-arginine methyl ester, completely blocks the effect of insulin to increase flow and partially blocks insulin-stimulated leg glucose uptake (134, 136). Thus, in addition to direct PI3K-dependent metabolic actions of insulin to promote glucose uptake in skeletal muscle through stimulating translocation of insulin-responsive GLUTs, the PI3K-dependent vascular actions of insulin to increase blood flow and capillary recruitment substantially contribute to promoting glucose disposal under healthy conditions and help to couple metabolic and hemodynamic homeostasis (Figs. 1 and 2).

## IV. Reciprocal Relationships between Insulin Resistance and Endothelial Dysfunction

### *A. Pathway-selective insulin resistance*

A key feature of insulin resistance is that it is characterized by specific impairment in PI3K-dependent signaling pathways, whereas other insulin-signaling branches including Ras/MAPK-dependent pathways are unaffected (202, 203). This has important pathophysiological implications because metabolic insulin resistance is usually accompanied by compensatory hyperinsulinemia to maintain euglycemia (Fig. 3). In the vasculature and elsewhere, hyperinsulinemia will overdrive unaffected MAPK-dependent pathways leading to an imbalance between PI3K- and MAPK-dependent functions of insulin (45). 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 MAPK-signaling pathways. In endothelium, decreased PI3K signaling and increased MAPK signaling in response to insulin may lead to decreased production of NO and increased secretion of ET-1 characteristic of endothelial dysfunction. Thus, antihypertensive effects of insulin to stimulate 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 (173). Pharmacological blockade of ET-1 receptors (ET-A isoform) improves endothelial function in obese and diabetic patients but not in lean insulin-sensitive subjects (204, 205).

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 (33). Simultaneous treatment of endothelial cells with wortmannin (PI3K inhibitor) and high insulin levels blunts PI3K-dependent effects of insulin such as induction of eNOS expression and production of NO. Of note, under these conditions, insulin signaling through Ras/MAPK pathways is substantially enhanced beyond that observed in the absence of wortmannin. This leads to increased prenylation of Ras and Rho proteins via the MAPK pathway and enhanced mitogenic responsiveness of cells to insulin and VEGF that are known to contribute to proliferation of vascular smooth muscle cells. In addition, up-regulation of endothelial cellular adhesion molecules VCAM-1 and E-selectin and increased rolling interactions of monocytes with endothelial cells is observed. Thus, compensatory hyperinsulinemia in the presence of metabolic insulin resistance with pathway-specific impairment of PI3K in endothelium and vascular smooth muscle cells leads to enhanced mitogenic actions of insulin through MAPK-dependent pathways that may contribute to key early events in the pathogenesis of hypertension. As discussed below, some mechanisms underlying insulin resistance also contribute independently to endothelial dysfunction. Proinflammatory signaling stimulated by glucotoxicity and lipotoxicity in dysmetabolic states contributes to shared mech-

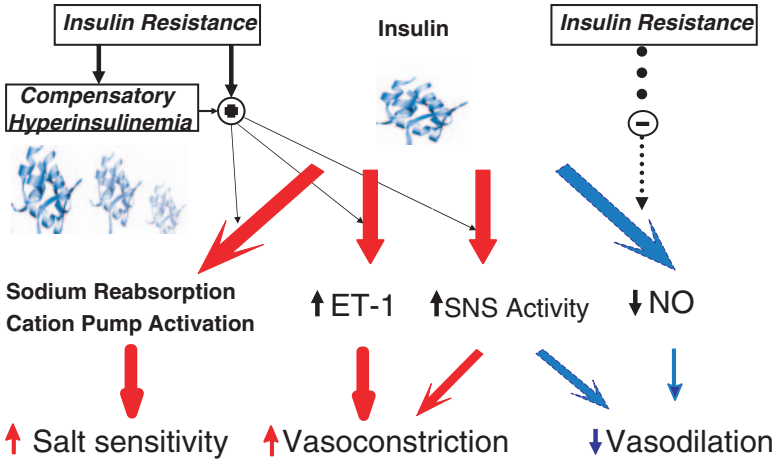


FIG. 3. Pathway-selective insulin resistance in PI3K signaling creates imbalance between prohypertensive and antihypertensive vascular actions of insulin exacerbated by compensatory hyperinsulinemia. SNS, Sympathetic nervous system.

anisms of insulin resistance and endothelial dysfunction. The molecular and cellular mechanisms that mediate insulin resistance and endothelial dysfunction are multiple and reflect complex interactions between inflammatory and metabolic pathways (Fig. 4).

### *B. Lipotoxicity*

Insulin-resistant states are characterized by inappropriately high circulating levels of free fatty acids (FFA). Insulin resistance in adipocytes leads to increased activity of hormone-sensitive lipase resulting in breakdown of triglycerides and release of FFAs that contribute to metabolic insulin resistance (206–208). Magnetic resonance spectroscopy studies demonstrate that mitochondrial dysfunction associated with accumulation of intramyocellular lipids may contribute to the accompanying insulin resistance in skeletal muscle (206, 209). Exposure of the vasculature, myocardium, and skeletal muscle to high levels of FFA initiates multiple cellular processes including impaired insulin signaling (210, 211), oxidative stress (212, 213), alterations in local renin-angiotensin system (RAS) (214), and enhanced VSMC adrenergic sensitivity (215). All of these factors contribute to cardiac, vascular, and metabolic insulin resistance (208).

*1. Impaired insulin signaling.* Treatment of vascular endothelial cells with FFA reduces basal and insulin-stimulated eNOS activity and NO production (211). Moreover, FFA treatment impairs insulin-stimulated activation of PI3K, PDK1, Akt, and eNOS (211). These effects of FFA are specific to insulin because FFA treatment does not alter the ability of VEGF to stimulate the PI3K/Akt/eNOS pathway. This impairment in insulin signaling in the endothelium caused by FFA treatment is similar to that observed in skeletal muscle. Exposure to FFA increases cellular levels of diacylglycerols, ceramide, and long-chain fatty acyl coenzyme A (CoA). These lipid metabolites activate serine kinases such as PKC and inhibitory kB kinase (IKK$\beta$) that regulate activation of nuclear factor-$\kappa$B (NF-$\kappa$B), a transcription factor associated with inflammation (216). Interestingly, activation of PKC$\beta$1

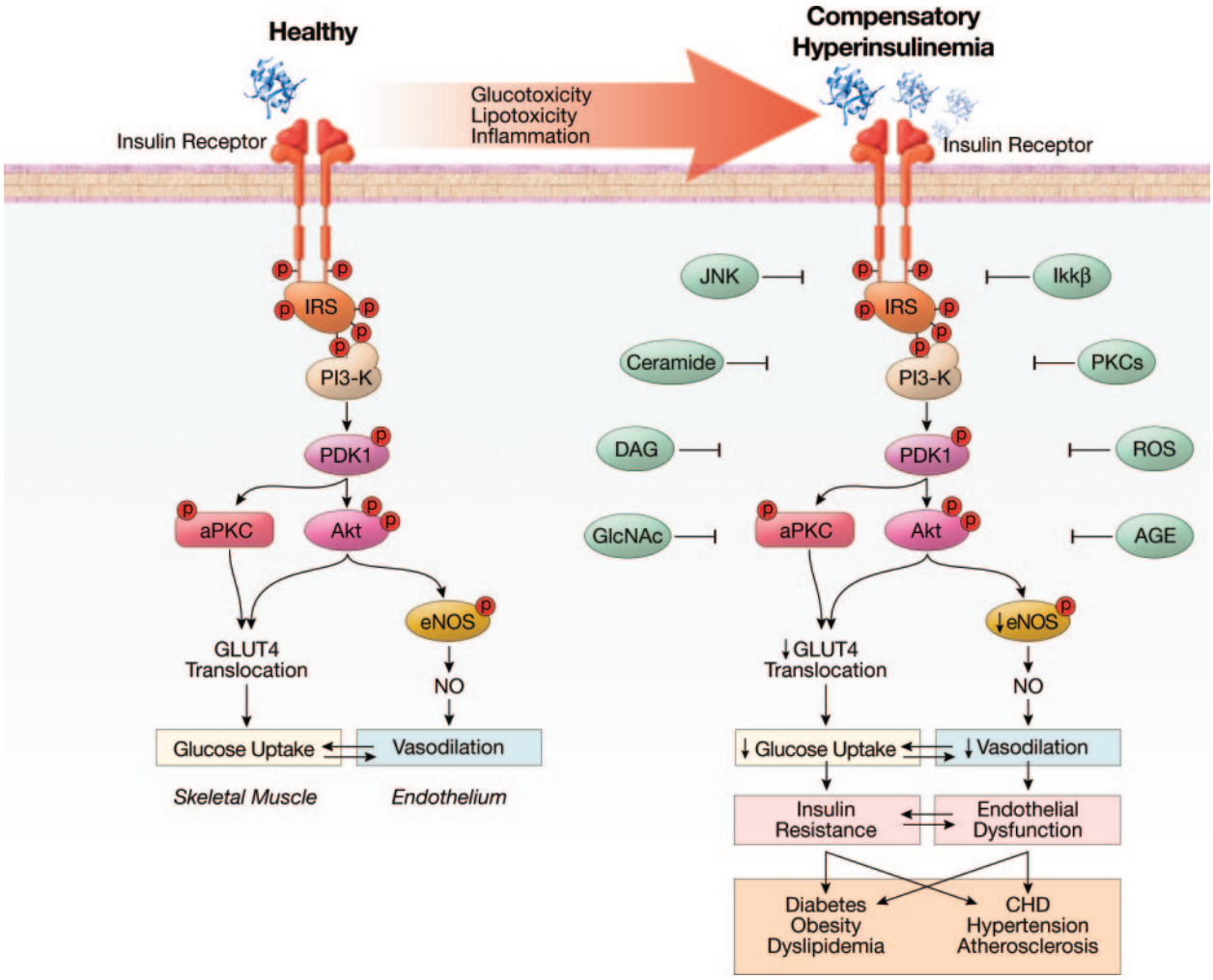


FIG. 4. Shared and interacting mechanisms of glucotoxicity, lipotoxicity, and inflammation underlie reciprocal relationships between insulin resistance and endothelial dysfunction that contribute to linkage between metabolic and cardiovascular diseases. *Left*, Parallel PI3K-dependent insulin-signaling pathways in metabolic and vascular tissues synergistically couple metabolic and vascular physiology under healthy conditions. *Right*, Parallel impairment in PI3K-dependent insulin-signaling pathways under pathological conditions contributes to synergistic coupling of insulin resistance and endothelial dysfunction. CHD, Coronary heart disease; DAG, diacylglycerol.

and -$\beta$2 isoforms results in increased serine phosphorylation of IRS-1 that leads to reduced insulin-stimulated Akt and eNOS activities (217). Likewise, palmitate activates IKK$\beta$ and Jun N-terminal kinase (JNK), which increases serine phosphorylation of IRS-1 and decreases insulin-stimulated production of NO (218). Inhibitory effects of FFA treatment on insulin signaling and NO production in endothelial cells can be blocked by overexpression of a dominant inhibitory mutant of IKK$\beta$. Moreover, deleterious effects of FFA treatment on endothelial cells are recapitulated by overexpression of wild-type IKK$\beta$. Treatment of endothelial cells with FFAs up-regulates expression of the lipid phosphatase PTEN, a negative regulator of PI3K-dependent signaling (211). These same cellular signaling pathways are impacted by FFA in the heart (219). In cardiomyocytes, FFA treatment is associated with reduced PI3K/Akt activity, resulting in diminished insulin-stimulated glucose uptake (220), eNOS activation, and contractile function (92). In addition to effects on insulin signaling, long-chain acyl-CoA esters directly stimulate opening of ATP-sensitive potassium ($K_{ATP}$) channels leading to $K^+$ efflux, shortened action potentials, reduced $Ca^{2+}$ influx, and decreased contractile force (221). In coculture studies of adipocytes and cardiomyocytes, adipocyte-derived factors directly depress intracellular systolic $Ca^{2+}$ peaks and cardiac contraction (222). Moreover, intracellular fatty acid accumulation is associated with local generation of TNF-$\alpha$, which is known to inhibit cardiac contraction (219).

*2. Oxidative stress.* FFAs increase reactive oxygen species (ROS) production in the vasculature. Two primary sources of ROS in the vasculature are nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (213) and the mitochondrial electron transport chain (212). Insulin treatment decreases FFA-induced ROS production in a PI3K-dependent manner. This suggests that specific impairment of PI3K signaling may accentuate FFA-evoked oxidative stress (212). Reduction in eNOS activity due to FFA is reversed by preventing fatty acid oxidation, uncoupling oxidative phosphorylation, or scavenging locally increased superoxide production. In addition, FFA-associated ROS production enhances PKC activity, activates the hexosamine biosynthetic pathway, and increases formation of advanced glycation end-products (AGEs). All of these mechanisms may independently contribute to inhibition of insulin-stimulated NO production in vascular endothelium (212). The increased mitochondrial superoxide generation was shown to inhibit $PGI_2$ activity by nitration. Consistent with these *in vitro* studies, *in vivo* exposure to high concentrations of FFA reduced eNOS and $PGI_2$ activity in the aorta. Administration of antilipolytic agents or ROS scavengers (superoxide dismutase mimetics) to rodents with PI3K pathway-specific insulin resistance normalized the impaired eNOS and $PGI_2$ activity (212). In addition, PKC-dependent activation of NADPH oxidase has been demonstrated in endothelial and VSMCs exposed to FFA (213). Superoxide may react with NO, generating peroxynitrite and reducing NO bioavailability. Peroxynitrite in turn can oxidize tetrahydrobiopterin, "uncouple" eNOS, nitrate and inhibit $PGI_2$, and alter NO/cGMP signaling in VSMCs by altering the expression of soluble guanylyl cyclase (sGC) and by inhibiting the activity of the sGC and cGKI (223). Furthermore, arterial content of eNOS dimers is significantly reduced in a rodent model of high-fat-diet-induced obesity and diabetes (224). This reduction in eNOS dimerization was associated with an increase in nitrotyrosine content, suggesting that oxidative stress may have contributed to eNOS disruption and endothelial dysfunction. Interestingly, insulin-stimulated eNOS phosphorylation was unaltered and does not appear to contribute to endothelial dysfunction in this model of diabetes. However, in a similar rodent model of high-fat-induced diabetes, insulin-mediated vasorelaxation is significantly reduced (225). Indeed, a high-fat meal is associated with elevated circulating levels of nitrotyrosine in insulin-resistant individuals (226). ROS production due to "uncoupled" eNOS in circulating endothelial progenitor cells is significantly increased in patients with diabetes (227). In summary, impaired phosphorylation, activity, expression, and "uncoupling" of eNOS may all act in concert to mediate FFA-provoked endothelial dysfunction.

*3. Altered sympathetic activity and the RAS.* Elevated FFAs secondary to lipid infusion in healthy individuals are known to increase sympathetic nerve activity, plasma catecholamine concentrations (228, 229), vascular $\alpha$-adrenergic reactivity (215), and local RAS activity (214). These changes may counteract vasodilator actions of insulin and potentiate vasoconstriction, resulting in enhanced vascular tone in insulin-resistant states.

*4. Effects of lipotoxicity on cardiovascular function.* FFAs concomitantly reduce insulin's vasodilator and metabolic effects. Infusion of intralipids in rats raises circulating FFA levels causing significant impairment in skeletal muscle capillary recruitment with a concomitant 40% decrease in glucose disposal during a glucose clamp procedure with steady-state plasma insulin levels of approximately 600 pM (230). Likewise, the effects of insulin on capillary recruitment and glucose uptake are impaired when FFA levels are increased in healthy lean women (231). Moreover, when FFA levels are lowered in obese women, vasodilator actions of insulin are improved, suggesting that insulin's microvascular and metabolic effects may be coupled during changes in exposure to FFAs. In fact, changes in capillary recruitment account for 30% of the association between changes in FFA levels and changes in insulin-mediated glucose uptake (231). Infusion of a lipid emulsion in conjunction with heparin to elevate circulating FFA concentrations simultaneously decreases glucose uptake and attenuates insulin-induced increases in leg blood flow and NO flux (161, 208), with significant correlations between FFA-induced changes in glucose uptake and FFA-induced decreases in leg blood flow. Thus, vascular and metabolic actions of insulin are tightly coupled such that impairment of the PI3K/Akt pathway by FFAs in the vascular wall contributes to insulin resistance in skeletal muscle. FFAs also induce cardiac insulin resistance. Six weeks of high-fat feeding in dogs induces systemic insulin resistance and decreased coronary hyperemia in response to exercise (232). This suggests direct lipotoxic effect(s) on cellular mechanisms that mediate metabolic coronary vasodilation and may explain diminished cardiac flow reserve in insulin-resistant individuals with dyslipidemia.

### *C. Glucotoxicity*

Long-term glycemic control is an important predictor of both micro- and macrovascular disease (233–236). Hyperglycemia impairs both metabolic and vascular actions of insulin by multiple biochemical and cellular mechanisms (237–239). These include elevated oxidative stress, increased flux through polyol and hexosamine biosynthetic pathways, formation of AGEs, and activation of diacylglycerol and PKC.

*1. Oxidative stress.* Hyperglycemia increases the production of ROS. In endothelial cells exposed to high glucose concentrations, insulin-stimulated activation of Akt and eNOS is significantly reduced (240, 241). Overexpression of uncoupling protein-1 or manganese superoxide dismutase prevents these inhibitory effects of glucose and restores vasodilator actions of insulin (240). In addition to impairing insulin-signaling pathways, ROS decreases NO bioavailability, reduces cellular tetrahydrobiopterin levels, and promotes generation of superoxide by eNOS. ROS also activates PKC-$\alpha$, PKC-$\beta$, and PKC-$\delta$, leading to decreased expression of eNOS and increased expression of ET-1, VEGF, and TGF-$\beta$ in endothelial cells (237).

*2. Hexosamine biosynthetic pathway.* Increased flux through the hexosamine biosynthetic pathway is another mechanism by which hyperglycemia may impair both metabolic and vascular actions of insulin (237, 242). Glutamine:fructose-6-phosphate amidotransferase (GFAT) is the rate-limiting enzyme for this pathway. Overexpression of GFAT in transgenic mice causes insulin resistance (243). The hexosamine biosynthetic pathway 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. For example, increased O-GlcN acylation of IRS-1 may lead to reduced insulin-stimulated translocation of GLUT4 and decreased glucose uptake (244, 245). In endothelial cells, hyperglycemia increases O-GlcN acylation of eNOS at the Akt phosphorylation site at $Ser^{1179}$, resulting in impaired eNOS activity. These defects are reversed by decreasing GFAT expression (240).

*3. AGEs.* AGEs are proteins or lipids that become nonenzymatically glycated and oxidized after contact with aldose sugars (246, 247). Enhanced AGE formation associated with hyperglycemia and oxidative stress inhibits both vascular and metabolic actions of insulin (248). Human glycated end-products inhibit insulin-stimulated tyrosine phosphorylation of IRS-1 and IRS-2 leading to impaired activation of PI3K and Akt (249). Moreover, AGE produces ROS and increases oxidative stress by activation of NADPH oxidase through specific receptors for AGE (250). In endothelial cells, AGEs decrease NO bioavailability and eNOS expression by accelerating eNOS mRNA degradation (251–254). AGEs also enhance expression of ET-1 in endothelial cells through the activation of NF-$\kappa$B (255). Thus, AGEs alter the balance of NO and ET-1 to favor vasoconstriction and endothelial dysfunction.

In cardiomyocytes, increased AGE, hexosamine and polyol flux, oxidative stress, and PKC activation have negative effects on function by prolonging action potentials, reducing relaxation kinetics, and altering myofilament $Ca^{2+}$ sensitivity through changes in expression and function of various ion channels, receptors (ryanodine receptor, $\beta$-adrenergic receptor), and ion pumps (SERCA, sodium-pump) (for review, see Refs. 219 and 220). These changes are predicted to result in ventricular stiffening and impaired ventricular filling (diastolic dysfunction), characteristics frequently observed in rodent and human models of diabetes.

*4. Effects of glucotoxicity on cardiovascular function.* In animal studies, acute hyperglycemia impairs endothelial function in both macro- and microvascular beds (256, 257). Local hyperglycemia achieved by infusing concentrated glucose directly into the brachial artery of healthy humans diminishes agonist-induced vasodilation, an effect prevented by antioxidants (258–260). Similarly, moderate hyperglycemia after an oral glucose load is associated with reduced flow-mediated vasodilation in healthy individuals (261). Acute hyperglycemia consistently impairs endothelial function in individuals with insulin resistance or type 2 diabetes (262, 263). Glucosamine, a product of the hexosamine biosynthetic pathway, impairs insulin stimulated glucose uptake in skeletal muscle and production of NO in endothelium *in vitro* (242, 264). *In vivo*, acute iv glucosamine administration causes metabolic insulin resistance (265, 266) and impairs insulin-mediated increases in femoral arterial blood flow (266, 267) and capillary recruitment (266). Collectively, these data suggest that hyperglycemia impairs insulin action in skeletal and cardiac muscle as well as in vascular endothelium.

### *D. Proinflammatory signaling and adipocytokines*

Insulin resistance and endothelial dysfunction are pathological states that are both characterized by increased circulating markers of inflammation (268). Visceral fat accumulation may play a key role in development of the systemic proinflammatory state associated with insulin resistance (268–270). Adipose tissue (and infiltrated resident macrophages) secretes a plethora of peptide hormones including leptin, adiponectin, TNF-$\alpha$, IL-6, resistin, angiotensinogen, and plasminogen activator inhibitor-1 that play crucial roles in metabolic and vascular homeostasis.

*1. Cytokines.* A number of potential biochemical mechanisms may explain the contribution of proinflammatory signaling to insulin resistance. The most extensively studied proinflammatory cytokine implicated in insulin resistance is TNF-$\alpha$. FFAs are important determinants of adipose tissue TNF-$\alpha$ activity and expression (271). Increased ROS in response to FFA activates NF-$\kappa$B, which further stimulates production of other proinflammatory cytokines including TNF-$\alpha$ and IL-6 (272–275). TNF-$\alpha$ activates a variety of serine kinases including JNK, IKK$\beta$, and IL-1$\beta$ receptor-associated kinase (276–279) that directly or indirectly increase serine phosphorylation of IRS-1/2, leading to decreased binding and activity of PI3K in response to insulin stimulation. For example, TNF-$\alpha$-activates JNK, resulting in increased phosphorylation of IRS-1 at $Ser^{307}$ (280). This reduces insulin-stimulated activation of PI3K/Akt/eNOS in endothelial cells

(281). In addition to modulating eNOS activity, JNK and IKK$\beta$ (through activation of activator protein-1 and NF-$\kappa$B) also inhibit insulin-stimulated expression of eNOS (282). Thus, insulin resistance reduces bioavailability of NO under basal conditions, and this may be an additional pathogenic factor in chronic diseases including atherosclerosis, hypertension, and diabetes with inflammatory components. Furthermore, suppressors of cytokine-signaling proteins are induced by treatment of cells with TNF-$\alpha$, IL-1$\beta$, or IL-6. Increased expression of suppressors of cytokine-signaling proteins interferes with interaction of the IR and IRS-1 and enhances proteasomal degradation of IRS-1 in adipose tissue (283). TNF-$\alpha$ also stimulates expression of other inflammatory proteins including C-reactive protein (CRP) and IL-6. CRP is an important marker of vascular inflammation whose plasma levels are correlated with risk of cardiovascular disease. In addition to being a marker of inflammation, CRP may have biological actions to inhibit insulin-evoked NO production in endothelial cells through mechanisms involving phosphorylation of IRS-1 at $Ser^{307}$ (mediated by syk, RhoA, and JNK) (284) and decreased expression of eNOS (285). In addition, CRP simultaneously increases ET-1 production and may directly promote cardiovascular disease by modulating expression of proinflammatory cytokines (286), up-regulating angiotensin receptor type 1 expression (287), and increasing expression of intercellular adhesion molecule, VCAM, E-selectin, and monocyte chemoattractant protein-1 in vascular endothelium (288, 289).

In animal models, administration of TNF-$\alpha$ induces insulin resistance (290), whereas neutralization of TNF-$\alpha$ improves insulin sensitivity (291). By contrast, neutralization of circulating TNF-$\alpha$ in patients with type 2 diabetes fails to alter insulin sensitivity significantly (292). Although this argues against a systemic role for TNF-$\alpha$ in mediating insulin resistance, the autocrine/paracrine nature of TNF-$\alpha$ action may have important pathophysiological significance. Indeed, systemic infusion of high doses of TNF-$\alpha$ results in loss of insulin-induced increases in glucose uptake, limb blood flow, and capillary recruitment in rat hind limb (293). This inhibitory action of TNF-$\alpha$ is specific to insulin-mediated, but not to exercise-mediated, hemodynamic and metabolic changes (293). TNF-$\alpha$ specifically down-regulates the insulin-dependent PI3K/Akt/eNOS vasodilator pathway while simultaneously augmenting ET-1-mediated vasoconstriction in skeletal muscle arterioles (280). In humans, high local concentrations of TNF-$\alpha$ achieved by intraarterial infusion simultaneously inhibit both insulin-stimulated glucose uptake (294) and endothelium-dependent vasodilation in the forearm (294, 295). Similarly, systemic infusion of TNF-$\alpha$ to achieve circulating concentrations slightly higher than those observed in chronic inflammation such as diabetes reduced glucose disposal in healthy individuals (296). Thus, proinflammatory cytokines may contribute to coupling of metabolic and vascular insulin resistance manifested by impaired insulin signaling and endothelial dysfunction.

*2. Adipokines and related peptide hormones.* Adipocyte-derived hormones such as leptin and adiponectin have both metabolic and vascular actions. Leptin, a key regulator of appetite, body weight, and energy balance in the central nervous system acts directly on the vasculature. Similar to insulin, leptin induces endothelium-dependent vasodilation (297, 298) through a PI3K/Akt/eNOS pathway (299). Insulin enhances leptin-induced eNOS activation, NO production, and vasorelaxation suggesting cross-talk between the insulin and leptin signaling pathways (300). Like insulin, leptin-evoked vasodilation is opposed by sympathetically induced vasoconstriction (301). Leptin replacement partially ameliorates cardiac contractile dysfunction that is present in hypoleptinemic (*ob/ob*) mice (302). Similarly, administration of leptin improves cardiac dysfunction in transgenic mice with cardiac-restricted steatosis caused by overexpression of acyl-CoA synthase (303). This salutary effect of leptin may be mediated by activation of AMP-activated protein kinase and may be secondary to mobilization of myocardial lipid. In contrast to these potentially beneficial actions of leptin, in a large prospective study, the West of Scotland Coronary Prevention Study (WOSCOPS), leptin was an independent risk factor for coronary artery disease (304). This suggests that hyperleptinemia and/or leptin resistance may have deleterious vascular and metabolic effects. In support of this concept, leptin enhances cytokine (TNF-$\alpha$ and IL-6) (305) and ROS production (306), up-regulates expression of angiotensinogen and ET-1 (307, 308), and negatively regulates insulin signaling (309) and glucose uptake by increasing serine phosphorylation of IRS-1 (310). Interestingly, leptin also suppresses cardiac contractile function in ventricular myocytes by an ET-1-dependent pathway (302). Angiotensin II increases leptin secretion from cultured human fat cells (311). Leptin may potentiate pressor effects of hyperinsulinemia in insulin-resistant states. Therefore, interactions between angiotensin II and insulin with leptin may have deleterious cardiovascular effects in obesity (311). Additionally, hyperleptinemia is associated with vascular inflammation, oxidative stress, and vascular smooth muscle hypertrophy that may contribute to the pathogenesis of hypertension, atherosclerosis, and left ventricular hypertrophy (312). Consequently hyperleptinemia and/or leptin resistance may alter the balance between the beneficial and harmful effects of leptin to impact adversely the cardiac, vascular, and metabolic actions of insulin leading to insulin resistance and endothelial and cardiac dysfunction (313, 314). However, human studies specifically examining the interaction of cardiovascular actions of insulin and leptin in normal and pathological states are lacking.

Adiponectin is an antiinflammatory peptide whose circulating levels are positively correlated with insulin sensitivity and that may serve to link obesity with insulin resistance (315–317). Adiponectin mimics vascular as well as metabolic actions of insulin, and the interaction between these two hormones may play a part in determining the cardiac, vascular, and metabolic phenotype in insulin-resistant states such as diabetes, obesity, and hypertension. Low circulating adiponectin levels are associated with insulin resistance, type 2 diabetes, premature vascular disease, and myocardial infarction (318, 319). Similar to insulin, adiponectin has vasodilator actions to stimulate NO production in endothelial cells (320, 321). In addition, adiponectin enhances NO bioavailability by up-regulating eNOS expression and reducing ROS production in endothelial cells (322, 323). Consistent

with these studies, adiponectin-knockout mice develop hypertension on a high-salt diet (324). Adenoviral expression of adiponectin in obese mice lowers blood pressure (324). In the heart, adiponectin exerts beneficial actions to protect against ischemia-reperfusion injury (325). However, the effects of adiponectin on insulin signaling and function in the heart are unknown.

Ghrelin is an orexigenic peptide hormone released from the stomach that has important metabolic and vascular actions. Circulating ghrelin levels are low in insulin-resistant conditions (326, 327). Some polymorphisms in the ghrelin gene are associated with increased prevalence of diabetes, impaired glucose tolerance, and hypertension (328–330). Ghrelin acutely stimulates production of NO in endothelium using a signaling pathway that involves ghrelin receptor (GHSR-1a), PI3K, Akt, and eNOS (331). In patients with the metabolic syndrome who have lower circulating ghrelin levels than healthy subjects, intraarterial ghrelin infusion acutely improves their endothelial dysfunction by increasing bioavailability of NO (332). Thus, vasodilator actions of ghrelin that mimic those of insulin may help to oppose the reciprocal relationships between insulin resistance and endothelial dysfunction.

Resistin is a proinflammatory peptide expressed in human macrophages, mononuclear leukocytes, and bone marrow cells that has been implicated in insulin resistance (333, 334). Recent studies suggest that resistin may adversely impact on endothelial function and vascular relaxation by stimulating ET-1 production, inhibiting vasodilator actions of insulin, and decreasing eNOS expression (335–338). Resistin also upregulates cytokine expression (TNF-$\alpha$ and IL-6) and increases oxidative stress (337). Thus, resistin may participate in the reciprocal relationships between insulin resistance and endothelial dysfunction.

### *E. Effects of compensatory hyperinsulinemia on blood pressure*

Insulin resistance is typically accompanied by compensatory hyperinsulinemia that serves to maintain euglycemia. Pathway-selective impairment in PI3K signaling underlying metabolic and vascular insulin resistance blunts NO-dependent vasodilator actions of insulin. Under these conditions, compensatory hyperinsulinemia may contribute to development of hypertension through antinatriuretic and sympathoexcitatory effects as well as activation of the RAS and enhanced secretion of ET-1 that are regulated by unimpaired MAPK-dependent insulin-signaling pathways (45, 339). Insulin sensitivity correlates with insulin-induced increases in glomerular filtration rate and renal plasma flow (188). Insulin-stimulated activation of serum- and glucocorticoid-inducible kinase-1 (SGK1) may mediate distal sodium reabsorption (340). In mice, disruption of SGK1 does not affect basal or salt-induced increases in blood pressure. However, on a high-fat diet that induces insulin resistance, high-salt conditions fail to elevate blood pressure in SGK1-deficent mice (341). Thus, it is possible that SGK1 may play a role in sodium retention during hyperinsulinemia in the presence (but not in the absence) of insulin resistance.

## V. Insights from Genetics and Therapeutic Interventions

### *A. Animal models*

Rodent models of insulin resistance provide important insights into the cardiovascular actions of insulin. In the vasculature of heterozygous IR knockout mice with metabolic insulin resistance, insulin-stimulated phosphorylation and activation of eNOS are impaired, resulting in reduced basal and insulin-stimulated NO release with increased blood pressure (342). Mice lacking IRs specifically in vascular endothelium (VENIRKO) have normal metabolic insulin responsiveness and blood pressure, but reduced expression of eNOS and ET-1 in endothelium (171). When challenged with a high-salt diet, VENIRKO mice develop insulin resistance and elevated blood pressure. This suggests that complex interactions between insulin action, eNOS, and ET-1 determine the metabolic and cardiovascular phenotype in these mice. Tissue-specific knockout of the IR in cardiomyocytes leads to diminished glucose and fatty acid oxidation in the heart, decreased cardiac size, contractile dysfunction, and reduced VEGF expression and capillary density (19, 87, 88, 103, 343). VEGF expression and vascular density are also significantly lower in myocardium of muscle IR knockout mice (344). Indeed, impaired myocardial insulin signaling in cardiomyocyte IR knockout mice predisposes to a rapid development of cardiac contractile dysfunction associated with pressure overload, a condition frequently associated with hypertension (345). Collectively, these studies suggest that insulin signaling in the myocardium plays an important role in the cardiac response to stresses such as dyslipidemia, hypertension, heightened sympathetic activity, and oxidative stress (87). Highlighting the important role of insulin signaling in the vasculature, IRS-1 (IRS-$1^{-/-}$) and IRS-2 (IRS-$2^{-/-}$) deficient mice not only exhibit resistance to the metabolic actions of insulin, but also demonstrate diminished endothelial NO activity and elevated blood pressure (346). The central role of NO in regulating the metabolic actions of insulin is evident in the presence of insulin resistance and hypertension in eNOS knockout mice (347, 348). These animals also demonstrate microvascular changes including reduced capillary density (rarefaction) (349). Although mice with partial eNOS deficiency (eNOS$^{+/-}$) are insulin sensitive and normotensive, they develop insulin resistance and hypertension when challenged with a high-fat diet (350). Thus, partial defects in insulin signaling or NO activity are sufficient to cause cardiometabolic abnormalities under pathogenic conditions (*e.g.*, nutritional stress, inflammation). The obese Zucker rat carrying a recessive mutation in the gene for the leptin receptor is a commonly used animal model of insulin resistance that exhibits many characteristics of the metabolic syndrome in humans. In particular, insulin-mediated attenuation of vascular contractility (351) and increases in limb blood flow and capillary recruitment (352) are substantially reduced in these animals. Obese Zucker rats have pathway-selective insulin resistance in PI3K-dependent signaling (with intact MAPK signaling) in the vasculature (202) and myocardium (344). This results in impaired NO-mediated vasodilation and augmented ET-1-mediated vaso-

constriction in response to insulin as well as enhanced VSMC calcium sensitivity (353) via RhoA activation (354, 355). Reduced NO bioavailability may contribute to capillary rarefaction observed in these animals (356). Of note, calorie restriction in mice increases eNOS expression and NO-dependent mitochondrial biogenesis (357). One functional consequence of this is improved insulin action in both cardiovascular and metabolic tissues.

The spontaneously hypertensive rat (SHR) is a genetic model of hypertension that is also insulin resistant (358). 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 (45, 359). When compared with age-matched normotensive Wistar-Kyoto control rats, SHRs at 12 wk of age are overweight, hypertensive, hyperinsulinemic, and insulin resistant, with normal fasting glucose. Thus, SHRs may be an informative model of the human metabolic syndrome that is useful for evaluating the contribution of pathway-specific insulin resistance to coupling between insulin resistance and endothelial dysfunction. In the mesenteric vascular bed of SHRs, the *ex vivo* vasodilator response to acetylcholine is comparable to that in Wistar-Kyoto 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. In the vasculature of SHRs, PI3K-dependent pathways are blunted, consistent with insulin resistance. Moreover, inhibiting MAPK-dependent pathways unmasks vasodilator actions of insulin in the mesenteric vascular bed of SHRs. Similar findings are evident after treatment of vessels with the ET-1 receptor antagonists BQ788 and BQ123. Taken together, these findings suggest that in SHRs, impaired PI3K pathways associated with insulin resistance lead to decreased endothelial production of NO, whereas increased insulin signaling through MAPK-dependent 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 insulin-resistant SHRs. Decreased bioavailability of NO together with increased secretion of ET-1 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 (45).

Additional evidence to support the concept of a reciprocal relationship between insulin resistance and endothelial dysfunction comes from therapeutic interventions in SHRs with insulin-sensitizers (rosiglitazone), angiotensin-converting enzyme (ACE) inhibitors (enalapril), or bioactive polyphenols in green tea [epigallocatechin gallate (EGCG)] (47, 360). Treatment of SHRs with these agents simultaneously lowers blood pressure, improves insulin sensitivity, decreases insulin levels, decreases ET-1 levels, and improves endothelial function with normalization of vasodilator responses to insulin. The improvement in metabolic and hemodynamic phenotypes resulting from therapeutic interventions with insulin sensitizers and/or antihypertensives in SHR is accompanied by a restored balance between PI3K- and MAPK-dependent branches of insulin-signaling pathways in metabolic and vascular tissues (Fig. 5).

### *B. Human studies*

*1. Clinical states characterized by insulin resistance and endothelial dysfunction.* In humans with metabolic insulin resistance, there is simultaneous impairment in the ability of insulin to induce vasodilation. Diminished effects of insulin to stimulate blood flow have been demonstrated in obese subjects (132, 134, 190, 361–363), type 1 diabetes (364–366), type 2 diabetes (89, 367–370), and polycystic ovarian syndrome (371). Diminished insulin-stimulated blood flow and glucose uptake are also present in patients with various cardiovascular diseases such as essential hypertension (157, 372–376), microvascular angina (377), and heart failure (378). Nondiabetic offspring of diabetic parents have both insulin resistance and endothelial dysfunction (379). Thus, there may be similar genetic and acquired contributions to both insulin resistance and endothelial dysfunction.

It is clear that defects in insulin-stimulated production of NO are directly related to insulin sensitivity. Baron *et al.* (120) examined effects of insulin to stimulate femoral venous NOx flux in subjects exhibiting a wide range of insulin sensitivity. Basal NOx flux rates are not different between subject groups despite 4-fold differences in insulin sensitivity. However, during insulin stimulation, athletes exhibit a significant in-

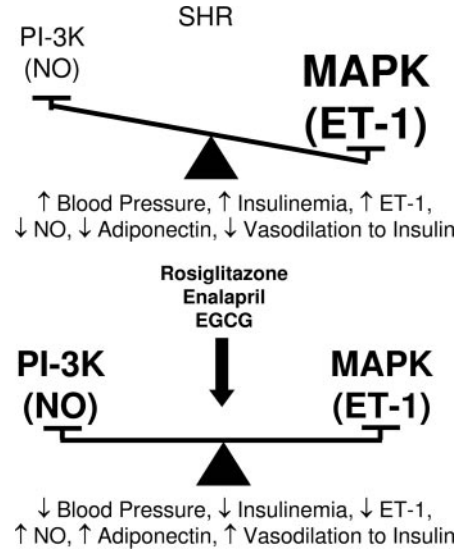


FIG. 5. SHRs are an animal model of the metabolic syndrome with hypertension, hyperinsulinemia, insulin resistance, overweight, elevated ET-1 levels, and decreased adiponectin levels. There is decreased vasodilator response to insulin due to decreased PI3K tone and elevated ET-1 levels due to increased MAPK tone. After treatment of SHRs with rosiglitazone, enalapril, or EGCG for 3 wk, blood pressure, insulin levels, and ET-1 levels are lower, whereas adiponectin levels and insulin sensitivity are increased. Increased vasodilator response to insulin is consistent with rebalancing between PI3K and MAPK branches of insulin signaling.

crease in NOx production, whereas diabetic subjects fail to augment NOx production above basal. Insulin-stimulated capillary recruitment (independent of total blood flow) accounts for more than 50% of insulin-mediated glucose uptake. It is well recognized that reduced capillary density is associated with insulin resistance (380). Recently, Clerk *et al.* (132) directly measured capillary recruitment using contrast-enhanced ultrasonography in the forearm flexor muscles of lean and obese adults before and during a 120-min euglycemic-hyperinsulinemic clamp. When compared with baseline measurements, insulin significantly increased microvascular blood volume (an index of microvascular recruitment) in the lean group but not in the obese group. These results demonstrate impaired insulin-mediated microvascular function in obesity. Direct measurements of the permeability surface area of insulin and glucose have been performed utilizing forearm arteriovenous cannulation in combination with microdialysis and blood flow measurements. Under steady-state glucose clamp conditions, the increase in permeability surface area to glucose was significantly attenuated in insulin-resistant type 2 diabetic subjects (368). This is consistent with reduced muscle capillary recruitment in response to insulin stimulation in subjects with type 2 diabetes. In patients with type 2 diabetes, ingestion of a mixed meal reduces MBF as opposed to a significant increase observed in normal insulin-sensitive controls (196). With respect to myocardial capillary recruitment, contrast-enhanced ultrasonography demonstrates that insulin can normalize reduced MBF in subjects with type 2 diabetes by enhancing capillary recruitment (194). This suggests that impaired actions of insulin in the heart are also linked with metabolic insulin resistance.

In related studies, phosphorylation of Akt, a key metabolic insulin-signaling molecule, is significantly attenuated in internal mammary arteries obtained from patients with diabetes when compared with vessels from nondiabetics (381). Similarly, the absolute level of phospho-eNOS ($Ser^{1177}$) is also decreased in vascular tissue from diabetic patients. Taken together, these findings are consistent with the concept that impaired vascular insulin signaling and reduced NO activity in individuals with type 2 diabetes contributes to endothelial dysfunction in insulin-resistant states. Genetic contributions to defective insulin signaling and NO activity in the vasculature are likely to contribute to diminished vascular actions of insulin that play a role in the pathogenesis of insulin resistance and endothelial dysfunction *per se* in type 2 diabetes (263) and obesity (382).

*2. Genetic polymorphisms.* Shared relationships between NO-dependent endothelial function and metabolic actions of insulin are also evident in clinical studies examining gene polymorphisms. The most commonly detected polymorphism in IRS-1 (glycine to arginine at codon 972) is associated with an increased risk of type 2 diabetes and insulin resistance as well as endothelial dysfunction (383). In human endothelial cells carrying the G972R-IRS-1 variant, insulin-mediated PI3K/Akt/eNOS activation is diminished (39). Conversely, eNOS gene polymorphisms are associated with insulin resistance, hyperinsulinemia, and type 2 diabetes (384, 385).

*3. Insulin resistance and increased vasoconstrictor tone.* As previously discussed, in states of metabolic insulin resistance, PI3K-dependent pathways are impaired, whereas MAPK-dependent pathways are intact. This pathway-specific insulin resistance results in enhanced effects of insulin to stimulate ET-1 production and promote increased vasoconstrictor tone. The increased ET-1 activity in parallel with diminished NO bioactivity contributes to abnormal vascular function. Human studies in overweight (386), obese (205), hypertensive (387, 388), and diabetic (205, 389) subjects support this notion. Combined $ET_A$/$ET_B$ receptor blockade in the forearm circulation significantly increases endothelium-dependent vasodilatation in overweight, insulin-resistant subjects or individuals with hypertension, but not in lean, healthy controls (386, 387). Similarly, selective $ET_A$ receptor blockade in the forearm significantly increases forearm blood flow in patients with type 2 diabetes (389). Of particular interest, $ET_A$ receptor blockade not only normalizes endothelium-dependent vasodilation, but acutely restores NO bioavailability (205). This suggests that increased ET-1 action in the vasculature may be proximal to reductions in NO bioavailability observed in insulin-resistant states, and it is possible that diminished NO may result in enhanced ET-1 production. Hyperinsulinemia stimulates ET-1 secretion (176), and accentuated ET-1 activity may cause insulin resistance (390). Thus, human studies support the idea that increased endogenous activity of ET-1 in the vasculature is a feature of vascular dysfunction and impaired vascular insulin response present in insulin resistance, obesity, hypertension, and diabetes mellitus.

Vascular smooth muscle dysfunction has also been demonstrated in insulin-resistant states including obesity and diabetes (381, 391–396). This suggests that impaired vasodilatory actions of insulin are not limited simply to reduction in NO bioavailability. Akt mediates insulin-stimulated vasodilation in VSMC as well as in endothelium. Consequently, reduced Akt activity in the vasculature of diabetic patients may play a role in the impaired VSMC relaxation. In addition, superoxide production and NADPH oxidase expression are significantly higher in internal mammary arteries from individuals with diabetes when compared with matched nondiabetic individuals (397). Increases in ROS not only diminish NO availability, but also diminish activity and expression of sGC and the cGKI in VSMC (223). This is consistent with diminished nitroglycerin/SNP-mediated vasodilation in individuals with insulin resistance (392–394, 396). In addition, altered VSMC cation concentrations (74) and sensitization to $Ca^{2+}$ through the Rho-kinase pathway (398) may contribute to elevated vascular tone in obesity and diabetes.

*4. Nonpharmacological lifestyle interventions.* Lifestyle modifications including diet, weight loss, and physical exercise decrease insulin resistance (378, 396, 399), increase adiponectin levels (400), and improve endothelial dysfunction (396, 401). Calorie restriction not only increases insulin sensitivity but also improves NO-dependent vasodilation in obese or hypertensive individuals (401, 402). Parallel to the increase in NO activity, calorie-restriction also reduces circulating ET-1

levels in obese individuals (403). Moreover, 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 (400). Consuming a Mediterranean-style diet significantly reduces serum concentrations of inflammatory markers, decreases insulin resistance, and improves endothelial function in patients with metabolic syndrome (compared with matched subjects on a controlled diet) (404). Similarly, in a cohort of obese women, a 2-yr lifestyle intervention consisting of weight loss, physical exercise, and Mediterranean-style diet decreases BMI and inflammatory markers while increasing adiponectin levels (compared with matched controls in a nonintervention group) (405).

Increased physical activity/exercise enhances insulin sensitivity and NO-dependent vasodilatation in both conduit and resistance vessels of sedentary individuals characterized by endothelial dysfunction and insulin resistance (406). Exercise increases insulin-stimulated blood flow in athletes, healthy controls, and type 2 diabetic individuals (407, 408). Physical exercise increases forearm skeletal muscle capillary recruitment in healthy individuals (111) and may augment glucose uptake by enhancing nutritive flow. The salutary effects of exercise on vascular actions of insulin may involve enhanced insulin signaling, accentuated eNOS activity/expression, reduced oxidative and inflammatory stress, enhanced NO availability, restoration of the imbalance in vasoconstrictor and vasodilator actions, and increased capillary density. A combination of diet and exercise significantly improves NO bioavailability in insulin-resistant hypertensive men and is accompanied by a reduction in levels of serum insulin and 8-iso-$PGF_{2\alpha}$, a marker of oxidative stress (409). After this intervention, there was a significant correlation between decreases in serum insulin and increases in urinary excretion of NO metabolites. This is consistent with the idea that exercise and dietary intervention may simultaneously enhance metabolic and vascular actions of insulin by reducing oxidative stress and enhancing NO bioavailability. In related clinical studies, regular exercise training increases eNOS protein expression and activity via PI3K/Akt-dependent phosphorylation and reduces NADPH oxidase and angiotensin-II type 1 receptor AT1-R expression in tissue specimens of the left internal mammary artery harvested during coronary bypass surgery (410, 411).

*5. Pharmacological therapies targeting insulin resistance and/or endothelial dysfunction.* Insulin resistance, inflammatory and oxidative stress, activation of the RAS and endothelin system, and low plasma adiponectin levels characteristic of metabolic disorders play an important role in endothelial dysfunction, whereas endothelial dysfunction contributes to metabolic insulin resistance. Thus, therapies aimed at improving either insulin resistance or endothelial dysfunction that raise plasma adiponectin levels, block renin angiotensin and endothelin systems, and lower oxidative stress are predicted to have simultaneous beneficial effects on both metabolic and cardiovascular function.

Thiazolidinediones [synthetic peroxisome proliferator-activated receptor (PPAR)-$\gamma$ ligands] are insulin sensitizers that also improve the action of insulin in the endothelium in insulin-resistant individuals (412–415). In individuals with recently diagnosed type 2 diabetes, rosiglitazone therapy further enhances the endothelium-dependent vasodilator response to insulin (415). This may be one mechanism by which thiazolidinediones attenuate both macro- (415) and microvascular dysfunction (412) in insulin-resistant individuals. Thiazolidinediones also have antiatherogenic properties mediated by antiinflammatory mechanisms to inhibit vascular smooth muscle cell proliferation and decrease accumulation of lipids by macrophages (416). Four-week treatment with pioglitazone protects against acute endothelial dysfunction induced by local infusion of TNF-$\alpha$ in individuals with type 2 diabetes (295). Moreover, administration of thiazolidinediones significantly increases adiponectin levels in patients with insulin resistance or type 2 diabetes without affecting body weight (417). In the PROactive study (prospective randomized trial in patients with preexisting cardiovascular disease and type 2 diabetes mellitus), pioglitazone significantly reduces a composite endpoint of all-cause mortality and nonfatal myocardial infarction (418).

Metformin, another agent that improves insulin sensitivity, also improves endothelium-dependent vasodilation in patients with insulin resistance (419–421). Metformin treatment results in increased production of NO by increasing AMP-activated protein kinase-dependent activation of eNOS (422). In addition to enhancing NO production, metformin decreases circulating ET-1 levels in insulin-resistant women (421). Moreover, therapy with thiazolidinediones or metformin lowers blood pressure in insulin-resistant patients who are also hypertensive (423, 424). Taken together, these studies suggest that drugs that improve insulin sensitivity may have both direct and indirect beneficial effects on the cardiovascular system.

Some drugs used for treatment of hypertension also have beneficial metabolic effects. ACE inhibitors reduce circulating angiotensin II levels, whereas angiotensin-II receptor blockers (ARBs) block the actions of angiotensin II. These effects lower blood pressure, improve endothelial function, and reduce circulating markers of inflammation. In patients with type 2 diabetes, quinapril treatment increases insulin-stimulated endothelial function and vascular expression of adiponectin (369). Moreover, in the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) study, ramipril significantly reduced postchallenge glucose levels and increased the likelihood of regression to normoglycemia in subjects with impaired glucose tolerance and impaired plasma glucose levels (425). This and other studies suggest that ACE inhibitors and ARBs may improve glucose metabolism (426). These beneficial metabolic effects may be mediated, in part, by blocking inhibitory cross-talk between angiotensin II receptor signaling and IR signaling at the level of IRS-1 and PI3K (427). ACE inhibitors and ARBs may also have direct effects (*e.g.*, inducing PPAR-$\gamma$ activity) that augment insulin-stimulated glucose uptake (428). Treatment of patients with ACE inhibitors or ARBs significantly increases adiponectin levels and improves insulin sensitivity without changing BMI (429, 430). Losartan (ARB) therapy significantly increases plasma adiponectin levels and insulin sensitivity relative to baseline measurements in hypercholesterolemic hypertensive patients (429).

Of note, these findings significantly correlate with improvements in endothelial function and inflammatory markers.

ACE inhibition reduces plasma levels of ET-1 and insulin-stimulated ET-1 secretion in individuals with hypertension (431). PPAR-$\alpha$ agonists, such as fenofibrate, significantly improve endothelial dysfunction, reduce levels of inflammatory markers, increase adiponectin levels, and enhance insulin sensitivity in hypertriglyceridemic patients (432). Moreover, fenofibrate therapy significantly lowers blood pressure in hypertriglyceridemic hypertensive patients (433). Similarly, 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) have also been shown to improve endothelial dysfunction, reduce inflammation, and reduce circulating ET-1 levels in individuals with type 2 diabetes and insulin resistance (434). However, treatment with simvastatin does not increase adiponectin levels or improve insulin sensitivity (429, 430). 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.

Sympathetic nervous system activation in states of obesity, hypertension, diabetes, and heart failure may lower insulin-stimulated glucose disposal through vasoconstriction (via $\alpha$1-adrenergic receptors) and reduced blood flow. Indeed, $\alpha$1 adrenergic receptor blockade increases insulin sensitivity (435), whereas unopposed $\alpha$1-activity during use of conventional $\beta$-blocking agents may negatively influence insulin sensitivity by enhancing vascular tone (436). Nonselective $\beta$-blockers with $\alpha$1-blocking properties such as carvedilol are precapillary vasodilators that increase blood flow and improve insulin sensitivity (437, 438). Carvedilol treatment in patients with heart failure increases glucose oxidation and improves myocardial energy efficiency (439).

## VI. Summary and Conclusions

Cardiovascular actions of insulin play an important physiological role in coupling metabolic and cardiovascular homeostasis under healthy conditions. The balance between NO-dependent vasodilator actions and ET-1-dependent vasoconstrictor actions of insulin is regulated by PI3K- and MAPK-dependent signaling in vascular endothelium, respectively. Under insulin-resistant conditions, pathway-specific impairment in PI3K-dependent signaling and enhanced MAPK-dependent signaling in vascular endothelium may contribute to reciprocal relationships between endothelial dysfunction and insulin resistance that underlie the close associations between metabolic and cardiovascular diseases. Genetic studies and therapeutic interventions in both animals and humans support these concepts. Pharmacological and lifestyle modifications may simultaneously improve both endothelial function and insulin resistance, in part, by restoring balance between vasodilator and vasoconstrictor actions of insulin that serve to couple hemodynamic and metabolic homeostasis.

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