Vascular actions of insulin with implications for endothelial dysfunction

Maria Assunta Potenza, Francesco Addabbo, and Monica Montagnani
Department of Pharmacology and Human Physiology, Medical School, University of Bari, Bari, Italy
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Potenza MA, Addabbo F, Montagnani M. Vascular actions of insulin with implications for endothelial dysfunction. Am J Physiol Endocrinol Metab 297: E568–E577, 2009. First published June 2, 2009; doi:10.1152/ajpendo.00297.2009.—Hemodynamic actions of insulin depend largely on the hormone’s ability to stimulate synthesis and release of endothelial mediators, whose balanced activity ensures dynamic control of vascular function. Nitric oxide (NO), endothelin-1 (ET-1), and reactive oxygen species (ROS) are important examples of endothelial mediators with opposing properties on vascular tone, hemostatic processes, and vascular permeability. Reduced NO bioavailability, resulting from either insufficient production or increased degradation of NO, characterizes endothelial dysfunction. In turn, endothelial dysfunction predicts vascular complications of metabolic and hemodynamic disorders. In the cardiovascular system, insulin stimulates the production and release of NO, ET-1, and ROS via activation of distinct intracellular signaling pathways. Under insulin-resistant conditions, increased insulin concentrations and/or impaired insulin-signaling pathways in the vasculature may contribute to imbalance in secretion of endothelial mediators that promote pathogenesis of vascular abnormalities. This short review describes signaling pathways involved in insulin-stimulated release of NO, ROS, and ET-1 and suggests possible molecular mechanisms by which abnormal insulin signaling may contribute to endothelial dysfunction.

Insulin has physiological actions in brain, pancreatic β-cells, and the cardiovascular system that contribute to its overall regulation of glucose and lipid homeostasis in muscles, liver, and adipocytes. The importance of insulin in hemodynamic homeostasis, acknowledged since the initial observation that insulin increases blood flow in skeletal muscle beds (8), has been progressively recognized during 15 years of investigations leading to the discovery of signaling pathways and molecular mechanisms that account for cardiovascular properties of insulin (see Ref. 95 for review).

The ability of endothelial cells to synthesize and release essential vascular mediators underlies the concept that a healthy endothelium is a signpost for vascular integrity and that imbalanced production/activity of endothelial mediators is one early marker of vascular abnormality (see Ref. 35 for review). In response to hormones, neurotransmitters, and physical and chemical stimuli, endothelial cells release mediators with opposing vascular effects to coordinate changes in regional blood flow, transport and distribution of nutrients, and disposal of metabolic waste products. Under physiological conditions, molecules including nitric oxide (NO), prostacyclin (PGI2), hydrogen peroxide (H2O2), endothelin-1 (ET-1), angiotensin II (Ang II), thromboxane A2, and several others act in concert to maintain hemodynamic balance, adequate oxygen supply, and nutrient delivery to tissues. This implies that changes in concentrations and/or activity for substances able to regulate endothelial cell activity would impair synthesis and/or release of endothelial mediators and therefore compromise vascular function and morphology. Since insulin physiologically stimulates production of endothelial factors with opposing hemodynamic activities, abnormal insulin concentrations and/or impaired insulin actions have a profound impact on vascular homeostasis.

Endothelial Mediators and Vascular Function

The endothelium forms a semipermeable barrier that prevents leaking of excessive plasma fluid through the monolayer and regulates selective delivery of nutrients and hormones to underlying tissues. Endothelial cells contribute to vascular homeostasis by releasing mediators with a wide range of vasodilator/vasoconstrictor, procoagulant/fibrinolytic, permeability/adhesion, and growth/differentiation properties (see Refs. 34, 35, 83, and 105 for more extensive reviews). For the purpose of this review, particular attention will be given to those endothelial factors directly involved in vascular effects of insulin and whose synthesis and/or function may be affected by impaired insulin actions (Fig. 1).

NO. The gaseous NO, generated from the conversion of the amino acid L-arginine by a family of NO synthases (NOS) (47, 90), is the prominent representative of the reactive nitrogen species (RNS). Once formed in endothelial cells, NO diffuses freely into adjacent vascular smooth muscle cells (VSMC), where it promotes vasorelaxation and inhibits migration, and into platelets, where it prevents their activation and aggregation (89). As its low chemical stability implies, NO has a very short half-life and tends to be quickly converted into stable...
byproducts. In the cellular environment, NO may react with several oxygen free radicals [reactive oxygen species (ROS)] to form strong oxidant intermediates such as peroxynitrites (ONOO\textsuperscript{-}). Under physiological conditions, adequate levels of intracellular NO are maintained by the efficiency of antioxidant enzymes that quench ROS production and therefore limit ONOO\textsuperscript{-} formation (2).

The endothelial NOS (eNOS) isoform, for a long time considered a strictly Ca\textsuperscript{2+}/calmodulin (CaM)-dependent enzyme, can be activated by Ca\textsuperscript{2+}-independent pathways requiring phosphorylation/dephosphorylation on specific residues (121). Ser\textsuperscript{1177} in the reductase domain is one regulatory site for eNOS catalytic activity. Phosphorylation of Ser\textsuperscript{1177} increases both the flux of electrons through the reductase domain of eNOS and the production of NO (43). Kinases known to phosphorylate eNOS on Ser\textsuperscript{1177} residue include protein kinase A (13), 5\textsuperscript{-}AMP-activated protein kinase (19), and serine/threonine kinase PKB/Akt (37, 46). Thr\textsuperscript{495} of eNOS represents a negative regulatory site whose phosphorylation causes a decreased enzymatic activity through interference with the binding of CaM to the CaM-binding domain (128). Although the role of Thr\textsuperscript{495} phosphorylation in eNOS activation remains controversial, several studies support the hypothesis that dephosphorylation on Thr\textsuperscript{495} and phosphorylation on Ser\textsuperscript{1177} act in concert to fully activate eNOS (43). Additional posttranslational mechanisms regulating eNOS activity include palmitoylation and myristoylation on several cysteine residues (85) as well as physical interaction with chaperone heat shock protein-90 (HSP90) (49) and caveolin-1 (84). Production of NO by eNOS is strictly dependant on availability of substrates and cofactors. In the absence of adequate levels of L-arginine or sufficient amounts of FAD, NADPH, and tetrahydrobiopterin, eNOS may become uncoupled and generate oxygen free radicals instead of nitrogen species (27, 54, 126). The complexity of mechanisms required for eNOS activation further emphasizes the importance of NO in endothelial and vascular function.

**ET-1.** As the natural counterpart of NO, endothelial cells release the polypeptide ET-1. Vascular shear stress or vasoconstrictors norepinephrine (NE) and Ang II upregulate bio-synthesis of ET-1, which in turn enhances vasoreactivity in response to NE. Ang II, and serotonin (80). ET-1 receptors are G protein-coupled receptors expressed on VSMC, cardiomyocytes, fibroblasts, and endothelial cells. Activation of ET\textsubscript{A} in VSMC culminates with intracellular calcium release and vasoconstriction in the short term but is also involved in control of cell growth, adhesion, and migration in both VSMC and cardiac cells in the long term (103, 115). Interestingly, ET-1 and NO exert a paracrine regulation on each other (14, 75, 139), and endogenous NO decreases ET-1 secretion (66). ET-1 enhances the activity of growth factors like PDGF or VEGF (135), promotes synthesis and secretion of thrombospondin and fibronectin, and increases platelet adhesion (115). ET\textsubscript{B} receptors on endothelium activate a negative feedback mechanism that promotes ET-1 clearance and favors release of NO and PGI\textsubscript{2}. Dysregulation of ET-1 synthesis, release, or activity represents another important mechanism underlying endothelial dysfunction.

**ROS.** Under aerobic conditions, endothelial cells produce ROS oxidant species, including superoxide anion (O\textsubscript{2-}) and H\textsubscript{2}O\textsubscript{2}. These are generated by transfer of electrons to molecular oxygen in the mitochondrial respiratory systems (2, 144) and by the activity of NADPH oxidases (12). As described before, intracellular ROS may also result from uncoupled eNOS activity that generates H\textsubscript{2}O\textsubscript{2} or O\textsubscript{2-} when amounts of substrates or essential cofactors are inadequate to produce NO (3, 102). In the cardiovascular system, ROS are involved in critical biological functions such as immune responses (52), regulation of vascular tone (53), and cell adhesion processes (21, 123). In response to PDGF (60, 122), epithelial growth factor (7), or proinflammatory cytokines (26), ROS activate signaling cascades such as the stress-activated protein kinases JNK and p38 mitogen-activated protein kinase (MAPK) (40, 111). These signaling pathways lead to reversible oxidative modifications that affect gene regulation and/or modulation of protein functions. Highly efficient antioxidant enzymes, including superoxide dismutase (which scavenges O\textsubscript{2-} to H\textsubscript{2}O\textsubscript{2}) and catalase (which reduces H\textsubscript{2}O\textsubscript{2} to H\textsubscript{2}O), are usually coactivated to prevent O\textsubscript{2-} overproduction and/or generation of abnormal species by interaction with RNS. For example, reaction of O\textsubscript{2-}
with NO generates ONOO− that irreversibly bind, oxidize, and transform nucleic acids, lipids, and proteins (38). Decreased antioxidant capacity and increased production of ROS result in oxidative stress, one of the most damaging mechanisms for impaired endothelial function in hemodynamic or metabolic diseases.

**Insulin Actions in the Vasculature**

In addition to crucial metabolic actions, insulin possesses physiological effects ranging from cell growth to regulation of cognitive functions such as learning and memory (145). In the cardiovascular system, insulin contributes to hemodynamic regulation by multiple mechanisms. In healthy lean subjects, physiological concentrations of insulin increase sympathetic nervous system activity and plasma NE levels. This may result in either vasoconstriction (113) or vasodilation (29), depending on the vascular district and the preexisting vascular tone. Insulin also favors renal sodium reabsorption with subsequent extracellular fluid expansion and increased systemic blood pressure. Vasodilation is one of the most important vascular effects produced by insulin. Vasodilation is related mainly to insulin-stimulated production of NO in endothelium (see below for signaling pathways). Overall vasodilator responses to insulin-mediated NO production result from integration of two distinct stages represented by capillary recruitment (the number of capillaries perfused) and increased total blood flow (131, 132). Initially, insulin stimulates dilation of terminal arterioles, which increases capillary recruitment without concomitant changes in total limb blood flow. Subsequently, physiological concentrations of insulin stimulate relaxation of larger resistance vessels and lead to increased limb blood flow (9).

**Insulin-mediated capillary recruitment.** A key component of insulin-mediated increase in microvascular perfusion is the ability of insulin to recruit nutritive capillary beds in skeletal muscle that are receiving little, intermittent, or no blood flow in the basal state (23, 24). Insulin-stimulated capillary recruitment has been studied in rat hindlimb (33, 109, 130) and in deep flexor muscles of the human forearm (25). Local intra-arterial insulin infusion (arterial insulin levels ~320 pM) caused a 25% increase in muscle capillary blood volume that is significantly higher than that observed after saline infusion in the same arm or in the contralateral arm not receiving insulin (25). Insulin-mediated capillary recruitment is consistent with a direct microvascular action of insulin (74), is blocked by inhibition of NO production, and temporally precedes stimulation of glucose disposal (130). By contrast with capillary recruitment, the time course for insulin-mediated increases in skeletal muscle blood flow requires several hours for a maximal effect to become evident (120). Although some controversy exists as to whether physiological concentrations of insulin cause significant increases in total limb flow (141), the preponderance of in vivo experimental evidence strongly suggests that increased blood flow in skeletal muscle results from insulin-stimulated capillary recruitment.

**Insulin-regulated endothelial barrier function.** In addition to its vasoactive actions in the microvasculature and resistance vessels, insulin may promote its own movement across the endothelial barrier (see Ref. 10 for review). Recent in vivo and in vitro findings suggest that insulin traverses the vascular endothelium via a transcellular, receptor-mediated pathway (134), and emerging data indicate that insulin may act on the endothelium to facilitate its own transendothelial transport. Thus, impaired activity of insulin on endothelium may also affect insulin delivery to muscles, the rate-limiting step for its metabolic actions (10).

**Signaling Involved in Insulin-Stimulated Release of Endothelial Mediators**

Following binding to its cognate tyrosine kinase receptor on the cell surface, insulin activates a complex, highly integrated signaling network. The two most critical signaling branches downstream from the insulin receptor are the phosphatidylinositol 3-kinase (PI3K) and the MAPK pathways (Fig. 2). In skeletal muscles and adipose tissues, the PI3K-dependent branch of insulin signaling controls mainly metabolic actions of insulin. The MAPK-dependent superfamily, ubiquitously involved in insulin signaling related to mitogenesis, growth, and differentiation (99), includes stress-dependent JNK and p38 MAPK molecules for acute response to environmental stressors (18, 118, 124).

**Signaling via PI3K pathways.** Insulin-signaling pathways regulating endothelial production of NO are dependent on PI3K and exhibit striking parallels with metabolic insulin-signaling pathways in skeletal muscle and adipose tissue (65, 143). This requires activation of insulin tyrosine kinase receptor (IR) (142, 143) and subsequent phosphorylation on tyrosine residues of insulin receptor substrates (IRSs). Both IRS-1 and IRS-2, the predominant members of the IRS family, are expressed on endothelial cells and involved in vascular effects of insulin (136). However, IRS-2 is implicated mainly in vascular protection in response to vessel injury (68), whereas IRS-1 is required for insulin-mediated production of NO in endothelium (93). Whereas phosphorylation of IRS-1 on tyrosine residues amplifies insulin signaling, phosphorylation of IRS-1 on specific serines represents a negative feedback mechanism (137).
Downstream from activated IRS-1, PI3K plays an essential role in mediating insulin-stimulated production of NO by phosphorylating and activating serine/threonine kinases, including phosphoinositide-dependent kinase 1 and Akt (142, 143). In turn, activated Akt directly phosphorylates eNOS at Ser1177, resulting in enhanced NO production (37). Akt is an essential molecule for insulin-mediated production of endothelial NO. 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 (91). Endothelial cells from mice with genetic deletion of the Akt1 isoform have significantly low levels of active eNOS (20). eNOS is activated by insulin in a calcium-independent manner that stimulates HSP90 binding and facilitates phosphorylation of eNOS at Ser1177 by Akt (Fig. 2) (55, 91).

**Signaling via MAPK pathways.** Activation of the IR by insulin favors the binding of the intracellular mediator Shc to the Src homology 2 domain of growth factor receptor-bound protein 2 (GRB2) (99). This, in turn, leads to activation of the preassociated guanine nucleotide exchange factor Son of Sevenless (SOS). When the GRB2-SOS complex docks to phosphorylated IR, SOS becomes activated. Activated SOS promotes the removal of GDP from Ras, which then initiates a kinase phosphorylation cascade involving Raf, MAPK/extracellular signal-regulated kinase, and MAPK. This MAPK-dependent branch of insulin-signaling pathways generally regulates biological actions related to growth, mitogenesis, and differentiation but is also involved in insulin-stimulated secretion of ET-1 (Fig. 2) (106, 107). Vasodilator actions of insulin are potentiated by ET-1 receptor blockade in animals (106) and humans (16) as well as by inhibitors of the MAPK signaling pathways (106). Endothelial insulin-signaling pathways related to ET-1 secretion upstream from MAPK activation still require further investigation. In addition to mediating ET-1 release, activation of the MAPK branch of insulin signaling in endothelium promotes other atherothrombotic effects mediated by prenyltransferases (92). Similarly, stimulation of MAPK signaling pathways by insulin and other growth factors via Ras and Rho may lead to upregulation of plasminogen activator inhibitor type 1 (PAI-1) (94), increase expression of vascular cell adhesion molecules like vascular cell adhesion molecule-1 (VCAM-1) and E-selectin (92), and increase interaction of endothelial cells with rolling monocytes in response to VEGF (92). Interestingly, insulin-mediated expression of VCAM-1 and E-selectin in endothelium is further enhanced by inhibition of PI3K branch of insulin signaling. This suggests that, in addition to stimulating production of NO, PI3K pathways may counteract expression of proatherogenic molecules, including PAI-1, VCAM-1, and E-selectin.

**Signaling via ROS.** Insulin is known to elicit generation of H$_2$O$_2$ in metabolic tissues (32, 79, 82), and increasing evidence suggests that a “burst” of intracellular ROS is promoted by insulin as soon as the receptor is activated (51, 116). Oxidation mediated by ROS (and by H$_2$O$_2$ in particular) reversibly inactivates protein and lipid phosphatases such as protein tyrosine phosphatase 1B, protein phosphatase 2A, and phosphatase and tensin homolog, all of which are negative regulators of insulin signaling (see Ref. 11 for review). Thus, when produced at physiological levels, ROS may act as positive modulators of the insulin-signaling cascade. Interestingly, natural polyphenols known for their insulin-mimicking effects, such as epigallocatechin gallate (96, 133), are able to acutely increase NO production in endothelial cells via signaling pathways that require H$_2$O$_2$ to activate PI3K and phosphorylate eNOS (63). In insulin-resistant hypertensive rats, chronic epigallocatechin gallate administration reduces systemic blood pressure, improves insulin-dependent vasodilation, and protects against myocardial ischemia/reperfusion injury (108). In adipocytes, production of ROS has been linked to the ability of insulin to activate a plasma membrane-associated enzyme system with the properties of a NADPH oxidase (67). In endothelial cells, detailed studies need to further characterize signaling pathways by which insulin activates production of ROS. Nevertheless, increasing evidence supports the concept that ROS are generated in response to insulin and are required for insulin to exert its full physiological actions. On the other hand, increased ROS generation has been implicated in the pathogenesis of insulin resistance. This apparent paradox may be explained by differences in concentrations of ROS. Low levels of ROS may promote signaling, whereas high levels promote insulin resistance and endothelial dysfunction.

**Abnormal Insulin Signaling and Endothelial Dysfunction**

The endothelium is the first tissue encountered by molecules released in the vascular system. However, this privileged position exposes endothelial cells to the consequences of altered composition/concentration of circulating factors that may abnormally stimulate endothelial cells and lead to impaired endothelial function. Under healthy conditions, endothelial cell injury resulting from exposure to risk factors is mitigated by endogenous reparative processes mediated by bone marrow-derived endothelial progenitor cells (EPC) (5). Reduced EPC availability and/or mobilization may disrupt the balance between endothelial damage and endothelial repair and accelerate the onset of endothelial dysfunction. A dysfunctional endothelium tends toward a vasoconstrictor, prothrombotic, and proinflammatory state secondary to loss of NO bioactivity (50). A decline in NO bioactivity may result from decreased eNOS protein expression and/or function (138), reduced or insufficient amounts of eNOS substrates and/or cofactors (28, 69), or increased release of endothelial mediators with opposing vascular effects (Fig. 3) (80, 81).

Molecular mechanisms by which insulin resistance, hyperinsulinemia, high glucose levels, hypertriglyceridemia, and inflammation induce endothelial dysfunction are overlapping and therefore hard to dissect (15, 65). Nevertheless, the role played by insulin in physiological regulation of vascular homeostasis may help explain how insulin resistance and sustained hyperinsulinemia contribute directly to endothelial dysfunction by promoting glyco- and lipotoxicity. Based on physiological effects of insulin in endothelium, insufficient PI3K-mediated activation of eNOS, with subsequent absolute decrease of NO bioavailability, may be among the mechanisms by which insulin resistance contributes to endothelial dysfunction. Conversely, excessive stimulation of MAPK-dependent pathways with subsequent increased release of mediators opposing NO function may explain how hyperinsulinemia induces a relative loss in NO bioactivity with subsequent endothelial dysfunction (Fig. 3). The fact that insulin resistance and hyperinsulinemia act concurrently makes their consequences on endothelial function more severe than the sum of each.
Insulin-resistance. Metabolic insulin resistance refers to a decreased ability of insulin to promote glucose uptake in skeletal muscle and adipose tissue and to suppress hepatic glucose output (110). Insulin resistance, based on both genetic components and acquired factors such as obesity and sedentary life style, most often precedes impaired glucose tolerance and hyperglycemia (36). Therefore, it is unlikely that endothelial dysfunction at the prediabetic stage is triggered by hyperglycemia per se. On the other hand, metabolic insulin resistance is often accompanied by dyslipidemia, which may in turn rapidly increase insulin resistance (70). On a molecular level, metabolic insulin resistance results from impaired PI3K-dependent signaling in metabolic targets of insulin (99). As discussed previously, activation of eNOS and production of NO in response to insulin requires activation of IR/IRS-1/PI3K/Akt-dependent signaling pathways. Up to Akt activation, this signaling pathway is overlapping with pathways mediating insulin-stimulated glucose uptake in target tissues for metabolic effects of insulin (112). The striking similarities between metabolic insulin signaling related to glucose uptake and insulin signaling related to vasodilation explain the parallel impairment of GLUT4 translocation in fat and muscles and of endothelial NO production in the vasculature, respectively, under insulin-resistant conditions (64).

Experimental and clinical evidence support the concept that defects immediately upstream or downstream from PI3K-signaling pathways result in impairment of insulin-mediated endothelial effects. For example, mice lacking IR specifically in endothelium (VENIRKO) have reduced expression of eNOS and ET-1 and develop insulin resistance and elevated blood pressure when exposed to a high-fat diet (129). Similarly, transgenic mice with a dysfunctional IR (Thr1134 substituted with Ala in the kinase domain) in endothelium (ESMIRO) show a significant reduction in NO bioavailability secondary to increased generation of ROS even in the absence of a metabolic phenotype (39). Mice that are homozygous null for the IRS-1 gene are not only insulin resistant but also have impaired endothelium-dependent vasodilation (1). Animal models of metabolic insulin resistance with impaired PI3K signaling have reduced NO-mediated vasodilation in response to insulin (106), enhanced VSMC calcium sensitivity (56) via RhoA activation (61, 97), and capillary rarefaction (45). In humans, the Thr1134 mutation of the IR is associated with metabolic and vascular abnormalities (88). A genetic polymorphism of IRS-1 that has been implicated in metabolic insulin resistance is also associated with genetically based endothelial dysfunction in subjects carrying the IRS-1 point mutation (42). Similarly, phosphorylation of Akt and eNOS are attenuated in vessels from patients with insulin resistance and glucose intolerance when compared with control subjects (100). Abnormal expression and activation of protein kinase C isoforms caused by insulin resistance (98) may further impair insulin’s ability to release endothelial NO both directly, through phosphorylation of serine residues on IRSs (58), and indirectly, via oxidative stress and the subsequent activation of JNK (101). Impaired cardiac function and reduced insulin-stimulated vasodilation are mediated, at least in part, by endothelial ROS overproduction in animal models of insulin resistance (62, 73). Altered expression of catalytic and regulatory subunits of NADPH complex is implicated in increased ROS production (11). Expression of NADPH oxidase subunits Nox-1, -2, and -4 is increased in the vasculature of insulin-resistant, diabetic db/db mice (57), and levels of NADPH oxidase catalytic subunit p47phox are elevated in endothelial cells of insulin-resistant overweight mice (48) and patients (119).

A direct pathophysiological consequence of reduced NO production under insulin-resistant conditions is impaired microvascular recruitment. Reduced capillary perfusion, resulting in a decreased surface area available for nutrient exchange, is unquestionably involved in lower glucose delivery and uptake in skeletal muscles (see Ref. 24 for review). Increasing evidence suggests that insulin’s transendothelial transport is the rate-limiting factor for insulin action in stimulating muscle glucose uptake (86, 140). Therefore, impaired capillary recruit-
ment caused by insulin resistance may not only reduce glucose delivery and uptake but also severely impair insulin’s own transendothelial transport to muscles (see Ref. 23 for review).

Loss of endothelial integrity under insulin-resistant conditions may be worsened by biochemical abnormalities with the potential to disrupt EPC-based vascular repair (see Ref. 30 for review). For example, reduced NO bioavailability, increased production of ROS, and downregulation of PI3K/Akt signaling pathways have been related to decreased mobilization of EPCs from bone marrow in individuals with diabetes (125) and the metabolic syndrome (41). The migratory defects associated with insulin resistance are at least partially attributable to cytoskeletal alterations of EPCs induced by reduced NO bioavailability (117). Insulin resistance may also affect the ability of EPCs to survive and diminish their capacity for adhesion, endothelial integration, proliferation, and differentiation. For example, the ability of EPCs to survive oxidative stress is impaired in diabetic animals with respect to wild-type controls (6).

Hyperinsulinemia. Hyperinsulinemia influences endothelial function both directly and indirectly. Animal studies show that rats chronically treated with insulin have increased levels of IRS1/2 serine phosphorylation and IR/ protein tyrosine phosphatase 1B association with subsequent impaired activation of PI3K/Akt pathways (127) and endothelial dysfunction. Chronic exposure to high insulin levels may induce insulin resistance, which in turn impairs signaling via PI3K pathways and reduces production of endothelial NO in response to insulin. However, rather than the cause, hyperinsulinemia is usually a consequence of metabolic insulin resistance that develops as a compensatory mechanism to maintain euglycemia. A key feature of insulin resistance is selective impairment in PI3K-dependent signaling pathways, whereas MAPK-dependent pathways are unaffected (31, 59, 92). Thus, hyperinsulinemia is predicted to override unaltered MAPK-dependent pathways. This results in an imbalance between PI3K- and MAPK-dependent vascular actions of insulin, as suggested by in vitro and in vivo studies. In endothelial cells in vitro, elevated insulin levels stimulate increased expression of adhesion molecules VCAM-1 and E-selectin via MAPK-dependent signaling pathway (92), thus facilitating the interaction of activated monocytes and favoring proatherogenic processes. Increased insulin signaling via MAPK-dependent pathways not only upregulates ET-1 gene expression and ET-1 release in endothelial cells (106) but also increases expression of ET_α receptors and proliferation of VSMC in culture (44). In animals as well as in humans, hyperinsulinemia increases secretion of ET-1 from endothelium and enhances ET-1 activity. This promotes vasoconstrictor tone that contributes to abnormal vascular function and increased systemic blood pressure (17, 104, 106). In addition, increased stimulation of MAPK-dependent pathways has been implicated in disruption of endothelial integrity as well as in increased vascular permeability and microvascular leak underlying retinopathy (87) and microalbuminuria (114).

Interestingly, sustained high levels of insulin may impair endothelial function even in the absence of insulin resistance. At pathophysiologically relevant concentrations, insulin exerts proinflammatory and proatherogenic effects on endothelial cells by enhancing VCAM-1 expression and monocyte-endothelial interactions via activation of p38 MAPK-signaling pathways (76–78). Sustained activation of the p38 MAPK pathway by elevated levels of proinflammatory cytokines may also inhibit EPC proliferation and differentiation in vitro (30).

Endothelial cells express more IGF-I receptors than insulin receptors (22, 72). At concentrations seen in insulin-resistant states, insulin potently activates both the insulin and IGF-I receptors on endothelial cells (72). Therefore, in the presence of intact MAPK-signaling pathways, hyperinsulinemia may activate both receptor types, enhance the expression of adhesion molecules, and further contribute to endothelial dysfunction and atherosclerosis (71).

A progressive decline in endothelium-dependent vasodilation has been observed in both femoral and brachial arteries of healthy subjects exposed to modest, prolonged hyperinsulinemia (4). Concomitant infusion of vitamin C completely reverses the endothelial dysfunction induced by elevated levels of insulin. This suggests that, particularly in large arteries, hyperinsulinemia per se may affect endothelial-mediated vasodilation via mechanisms increasing the rate of oxidative stress (4). Consistent with these findings, dose- and time-dependent increases in production of intracellular O₂− are detectable as early as 1 h after exposure of endothelial cells to insulin (Potenza MA and Montagnani M, unpublished observations). Thus, as for impaired insulin signaling during insulin-resistant conditions, increased concentrations of insulin may have direct consequences on endothelial function by disrupting mechanisms involved in maintenance of NO bioavailability.

Conclusions

Endothelial dysfunction is a key event in the pathogenesis of vascular complications for metabolic disorders, including diabetes, obesity, and the metabolic syndrome. Insulin resistance and hyperglycemia alter endothelial function by causing imbalance in release of endothelial mediators with opposite effects on vascular function. Loss of endothelial-derived NO bioavailability, increased production of ROS, and enhanced release of ET-1 are often observed under conditions characterized by perturbed insulin signaling. Understanding molecular mechanisms of insulin action in endothelium is critical to understanding the physiology coupling metabolic and cardiovascular homeostasis. Understanding the contribution of insulin resistance and hyperinsulinemia to the pathophysiology of endothelial dysfunction is essential for developing novel therapeutic approaches and targets for treatment and prevention of cardiovascular complications under diabetes, obesity, and metabolic syndrome.

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Review


