Published in final edited form as:

Endocrinol Metab Clin North Am. 2008 September ; 37(3): 685–x. doi:10.1016/j.ecl.2008.06.001.

An Integrated View of Insulin Resistance and Endothelial Dysfunction

Ranganath Muniyappa, MD, PhD, Micaela Iantorno, MD, and Michael J. Quon, MD, PhD*
Diabetes Unit, National Center for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, Maryland 20892

Synopsis

Endothelial dysfunction and insulin resistance are frequently co-morbid states. Vasodilator actions of insulin are mediated by phosphatidylinositol 3-kinase (PI3K)-dependent signaling pathways that stimulate production of nitric oxide from vascular endothelium. This helps to couple metabolic and hemodynamic homeostasis under healthy conditions. In pathological states, shared causal factors including glucotoxicity, lipotoxicity, and inflammation selectively impair PI3K-dependent insulin signaling pathways that contribute to reciprocal relationships between insulin resistance and endothelial dysfunction. We discuss implications of pathway-selective insulin resistance in vascular endothelium, interactions between endothelial dysfunction and insulin resistance, and therapeutic interventions that may simultaneously improve both metabolic and cardiovascular physiology in insulin-resistant conditions.

Keywords

Nitric Oxide; Insulin Resistance; Endothelial Dysfunction; Metabolic Syndrome

Introduction

Insulin resistance plays a major patho-physiological role in type 2 diabetes and is tightly associated with major public health problems including obesity, hypertension, coronary artery disease, dyslipidemias, and a cluster of metabolic and cardiovascular abnormalities that define the metabolic syndrome [1,2]. A global epidemic of obesity is driving the increased incidence and prevalence of insulin resistance and its cardiovascular complications [3]. Insulin regulates glucose homeostasis by promoting glucose disposal in skeletal muscle and adipose tissue and inhibiting gluconeogenesis in liver [4]. In addition to these classical insulin target tissues, insulin has important physiological functions in the brain, pancreatic beta cells, heart, and vascular endothelium that help coordinate and couple metabolic and cardiovascular homeostasis under healthy conditions [5]. For example, vasodilator actions of insulin to stimulate production of nitric oxide (NO) from vascular endothelium lead to increased blood

*Corresponding author for proof and reprints: Michael J. Quon, MD, PhD, Chief, Diabetes Unit, NCCAM, NIH, 9 Memorial Drive, Building 9, Room 1N-105 MSC 0920, Bethesda, MD 20892-0920, Tel: (301) 496-6269, Fax: (301) 402-1679, Email: quonm@nih.gov. Coauthors addresses: Ranganath Muniyappa, MD, PhD, Diabetes Unit, NCCAM, NIH, 10 Center Drive, 10-CRC, Room 4-1741, Bethesda, MD 20892-1632, Tel: (301) 451-7702, Fax: (301) 480 3159, Email: muniyapr@mail.nih.gov
Micaela Iantorno, MD, Diabetes Unit, NCCAM, NIH, 9 Memorial Drive, Building 9, Room 1N-105 MSC 0920, Bethesda, MD 20892-0920, Tel: (301) 435-5791, Fax: (301) 402-1679, Email: iantornm@mail.nih.gov

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

flow that further enhances glucose uptake in skeletal muscle [6,7]. The time- and dose-response for metabolic and cardiovascular actions of insulin are distinct and tissue-specific [5,8,9]. Insulin resistance is typically defined as decreased sensitivity or responsiveness to metabolic actions of insulin such as insulin-mediated glucose disposal. However, diminished sensitivity or resistance to the actions of insulin in vascular endothelium also contributes importantly to the clinical phenotype of insulin-resistant states [5,10,11].

Insulin binding to its cognate receptor at the cell surface activates two major branches of a complex insulin signal transduction network. Metabolic actions of insulin tend to be mediated by phosphatidylinositol 3-kinase (PI3K) –dependent signaling pathways whereas mitogen-activated protein kinase (MAPK) –dependent insulin signaling typically regulates mitogenesis, growth, and differentiation [12,13]. Insulin-signaling pathways regulating endothelial production of NO are PI3K-dependent and exhibit striking parallels with metabolic insulin signaling pathways in skeletal muscle and adipose tissue [14]. Insulin resistance is characterized by pathway-selective impairment in PI3K-dependent signaling in both metabolic and vascular insulin target tissues [15,16]. Consequently, glucotoxicity, lipotoxicity, and inflammation that contribute to development of insulin resistance also lead to endothelial dysfunction. Indeed, pathway-specific impairment in PI3K-dependent insulin signaling contributes to reciprocal relationships between insulin resistance and endothelial dysfunction that foster the clustering of metabolic and cardiovascular diseases in insulin-resistant states [14]. Herein, we discuss the implications of pathway-specific insulin resistance in vascular endothelium, effects of endothelial dysfunction on insulin resistance, and therapeutic interventions that may simultaneously improve both metabolic and endothelial function in insulin-resistant conditions.

NO and Endothelial Function

NO, an important determinant of endothelial function, is produced in vascular endothelium by activation of endothelial NO synthase (eNOS) [17]. Classical cholinergic vasodilators (e.g. acetylcholine) activate serpentine G protein-coupled receptors on endothelial cells that mediate a rise in intracellular calcium levels. Interaction of calcium/calmodulin with the calmodulin binding site on eNOS results in increased enzymatic activity. In addition, phosphorylation of eNOS at Ser¹¹⁷⁷ by serine kinases including Akt, AMPK, and PKA also stimulate production of NO in a calcium-independent manner. eNOS activity is also regulated by other posttranslational modifications including acylation (myristoylation and palmitoylation), and S-nitrosylation [18]. Availability of L-arginine (substrate for eNOS) and enzymatic cofactors (NADPH, flavin adenine dinucleotide [FAD], flavin mononucleotide [FMN], and tetrahydrobiopterin [BH₄]) also play a role in regulating NO production by eNOS [17]. Endothelial-derived NO diffuses into adjacent vascular smooth muscle cells (VSMC) where it activates guanylate cyclase. Increased levels of cGMP then lead to vasorelaxation. In addition to modulating vascular tone, NO attenuates production of proinflammatory cytokines, decreases expression of vascular cell adhesion molecules, limits leukocyte recruitment, inhibits VSMC proliferation, opposes apoptosis, attenuates platelet aggregation, and reduces monocyte adhesion to the vascular wall [19]. Inactivation of NO by enhanced production of reactive oxygen species (ROS) in the vasculature can significantly reduce NO bioavailability. This contributes to endothelial dysfunction and promotes the development of atherosclerosis. The term “endothelial dysfunction” refers to a maladapted endothelial phenotype characterized by reduced NO bioavailability, increased oxidative stress, elevated expression of pro-inflammatory and pro-thrombotic factors, and abnormal vasoreactivity [20]. Endothelial dysfunction is linked to insulin-resistant states including diabetes, obesity, and the metabolic syndrome. This increases the susceptibility of patients with these metabolic diseases to cardiovascular complications including accelerated atherosclerosis, coronary heart disease,

and hypertension. Importantly, endothelial dysfunction is independently associated with and predicts cardiac death, myocardial infarction, and stroke [21].

Clinical Assessment of Endothelial Function

Direct assessment of endothelial production of NO *in vivo* is challenging because of its short-half life (~5 s) and low physiological concentrations (pM range). Therefore, the vasodilator effect of endothelium-derived NO is often used to evaluate endothelial function in humans (Table 1)[22]. Changes in limb blood flow (assessed by plethysmography) or conduit artery diameter (assessed by ultrasound) in response to intra-arterial infusion of agents that stimulate endothelium-dependent production of NO such as acetylcholine are used primarily in research settings to evaluate endothelial function. Another less-invasive method involves shear stress-induced flow-mediated dilatation (FMD) of the brachial artery. High resolution doppler ultrasonography is used to measure changes in arterial diameter and blood flow in the brachial artery in response to shear stress induced by inflating and deflating a blood pressure cuff. Elevated circulating plasma concentrations of biomarkers for inflammation, hemostasis, and oxidative stress are also used as indicators that accompany and promote endothelial dysfunction [22].

Signaling Pathways Mediating Insulin-Stimulated Production of NO

One of the key vascular actions of insulin is to stimulate production of the potent vasodilator NO from endothelium. Recent studies have elucidated a complete biochemical insulin-signaling pathway in endothelium regulating production of NO [5,23]. Insulin binding to its receptor (a receptor tyrosine kinase) results in phosphorylation of IRS-1 which then binds and activates PI3K. Lipid products of PI3K (PI-3,4,5-triphosphate (PIP₃)) stimulate phosphorylation and activation of PDK-1 that in turn phosphorylates and activates Akt. Akt directly phosphorylates eNOS at Ser¹¹⁷⁷ resulting in increased eNOS activity and subsequent NO production. Although insulin-induced eNOS activation is calcium-independent [24], insulin stimulates calmodulin binding to eNOS. This requires HSP90 binding to eNOS which facilitates insulin-stimulated activation of eNOS mediated by phosphorylation of eNOS at Ser¹¹⁷⁷ by Akt. The Ras/MAP-kinase branch of insulin-signaling pathways does not contribute significantly to activation of eNOS in response to insulin [5] (Fig. 1).

Signaling Pathways Mediating Insulin-Stimulated Secretion of Endothelin-1 (ET-1), Plasminogen Activator Inhibitor Type-1 (PAI-1) and Adhesion Molecules

ET-1, a potent vasoconstrictor synthesized and secreted from vascular endothelium, plays an important role in endothelial dysfunction and may contribute to development of hypertension [25]. Insulin stimulates ET-1 production using MAPK-dependent (but not PI3K-dependent) signaling pathways [26] (Fig. 1). Increased endothelial expression of plasminogen activator inhibitor type-1 (PAI-1) and cellular adhesion molecules including intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), and E-selectin may contribute to accelerated atherosclerosis in insulin-resistant states [21]. Insulin stimulates increased expression of PAI-1, VCAM-1 and E-selectin on endothelium using MAPK-dependent pathway [27,28]. Inhibition of PI3K or Akt increases insulin-induced PAI-1 and expression of adhesion molecules [27]. These findings suggest that insulin-stimulated PI3K/Akt pathways oppose atherothrombotic factors in endothelium by multiple mechanisms including production of beneficial molecules such as NO and inhibition of pathogenic molecules including PAI-1, ICAM-1, VCAM-1, and E-selectin.

Endothelial Actions Link Metabolic Effects of Insulin

PI3K-dependent insulin signaling pathways in vascular endothelium, skeletal muscle, and adipose tissue regulate vasodilator and metabolic actions of insulin. However, MAPK-dependent insulin signaling pathways tend to promote pro-hypertensive and pro-atherogenic actions of insulin in various tissues. In humans, intravenous insulin infusion stimulates capillary recruitment, vasodilation, and increased blood flow in an NO-dependent fashion [7]. These actions of insulin occur in distinct stages. 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 that increases overall limb blood flow (maximum flow reached after 2 h) [29]. The overall vasodilator response to insulin is an integration of enhanced capillary recruitment and elevated total blood flow. Local intra-arterial infusion of insulin (arterial plasma levels of ~ 300 pM) results in a 25% increase in capillary blood volume in the deep flexor muscles of the human forearm [30]. Similarly, an hour after a mixed meal, microvascular volume in human forearm increases by ~ 45% [31]. Thus, physiological concentrations of insulin rapidly enhance skeletal muscle capillary recruitment. These vascular actions play an important role in augmenting the delivery of insulin and glucose to skeletal muscle. Glucose delivery to skeletal muscle is dependent on muscle blood flow as well as vascular capillary surface area and permeability. After a mixed meal, an oral glucose load, or infusion of insulin, recruitment of capillaries expands the capillary surface area and increases muscle blood flow which together substantially increase glucose and insulin delivery [30–32]. This enhances direct effects of insulin to stimulate glucose uptake and utilization in skeletal muscle. Indeed, the time course for insulin-stimulated capillary recruitment approximates the time course for insulin-mediated glucose uptake in skeletal muscle [7]. Moreover, inhibitors of NOS that block insulin-mediated capillary recruitment cause a concomitant 40% reduction in glucose disposal [7,33]. Thus, PI3K-dependent metabolic actions of insulin directly promote glucose uptake in skeletal muscle by stimulating translocation of insulin responsive glucose transporters (GLUT4). At the same time, 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 (Fig. 1).

Role of Insulin-Stimulated Secretion of Endothelin-1 (ET-1) to Oppose Metabolic Actions of Insulin

In humans, peripheral insulin infusion increases both vascular ET-1 production and circulating levels of ET-1 [34]. NO-dependent vasodilator actions of insulin are potentiated by ET-1 receptor blockade in healthy individuals. Conversely, ET-1 infusion decreases insulin-induced increases in blood flow in humans. In a recent animal study, ET-1 not only diminished insulin-mediated skeletal muscle capillary recruitment, but also decreased skeletal muscle glucose uptake by 50% [35]. In humans, insulin-stimulated ET-1 production may influence skeletal muscle glucose disposal. For example, ET-1 infusion in humans induces peripheral insulin resistance [36]. However, NO is known to inhibit ET-1 production and action [37]. Consequently, under healthy conditions, the effects of insulin-stimulated ET-1 on the metabolic actions of insulin are likely to be offset by insulin-stimulated production of NO. In support of this notion, endothelin-antagonism (by ET-1A receptor blocker) in healthy individuals fails to affect insulin-mediated whole body or limb glucose uptake [38]. However, in insulin-resistant states associated with impaired PI3K-dependent insulin signaling pathways, insulin-mediated ET-1 secretion is augmented and blockade of ET-1 receptors significantly improves insulin sensitivity and peripheral glucose uptake in the context of insulin resistance [38,39].

Potential Mechanisms Mediating Reciprocal Relationships Between Endothelial Dysfunction and Insulin Resistance

Endothelial dysfunction *per se* is associated with and predicts cardiovascular disease. Many established risk factors for coronary artery disease including dyslipidemia, hypertension, diabetes, obesity, and physical inactivity also increase the risk of developing endothelial dysfunction. Similarly, many risk factors for developing CVD also enhance the risk of developing insulin resistance [21]. Thus, endothelial dysfunction and insulin resistance frequently co-exist. In cross-sectional studies, endothelial dysfunction is consistently present in patients with insulin resistance [40–42]. This includes relatives of patients with type 2 diabetes, and patients with type 2 diabetes themselves [43,44]. Shared causal factors such as glucotoxicity, lipotoxicity, inflammation, and oxidative stress interact at multiple levels to create reciprocal relationships between insulin resistance and endothelial dysfunction that may help explain the frequent clustering of metabolic and cardiovascular disorders [14] (Fig. 2).

Role of Endothelial Dysfunction in Development of Insulin Resistance

Cross-sectional studies suggest that endothelial dysfunction independently predicts the incidence of diabetes [45–48]. In a prospective study (Framingham Offspring Study) of the children and spouses of children of the original Framingham Heart Study cohort, circulating plasma markers of endothelial dysfunction (plasminogen activator inhibitor-1 (PAI-1) and Willebrand factor (vWF)) increases the risk of developing diabetes independent of other risk factors for diabetes including obesity, insulin resistance, and inflammation [46]. Similarly, in a large, prospective, nested case-control study from an ethnically diverse cohort of U.S. postmenopausal women (Women's Health Initiative Observational Study), higher levels of circulating E-selectin and intercellular adhesion molecule-1 (ICAM-1) were consistently associated with increased risk of developing diabetes [47]. These studies support a potential causal role for endothelial dysfunction in insulin resistance. Rodent models of endothelial dysfunction provide additional important insights into this issue. The central role of endothelium in regulating metabolic actions of insulin is evident by the presence of insulin resistance and hypertension in eNOS knockout mice. These animals also demonstrate microvascular changes including reduced capillary density (rarefaction) [49,50]. Insulin-mediated glucose disposal is reduced by nearly 40% in these mice, an amount roughly equivalent to the contribution of capillary recruitment in insulin-mediated glucose disposal in healthy rodents. Moreover, these mice also have increased triglyceride and FFA levels, decreased energy expenditure, defective beta-oxidation, and impaired mitochondrial function [51]. These findings suggest that endothelium-derived NO has additional and direct metabolic effects on mitochondrial function. Although mice with partial eNOS deficiency (eNOS +/-) are insulin sensitive and normotensive, they develop insulin resistance and hypertension when challenged with high-fat diet [52]. Thus, partial defects in endothelial function characterized by reduced NO bioavailability are sufficient to cause cardio-metabolic abnormalities (insulin resistance and dyslipidemia) under pathogenic conditions (e.g., caloric excess, physical inactivity, inflammation), a situation not unlike that observed in humans.

Role of Insulin Resistance in the Development of Endothelial Dysfunction

In humans with metabolic insulin resistance there is simultaneous impairment in insulin's ability to induce vasodilation. Diminished effects of insulin to stimulate blood flow has been demonstrated in obese subjects, type 2 diabetes and polycystic ovarian syndrome [41,53–55]. Diminished insulin-stimulated blood flow and glucose uptake is also present in patients with various cardiovascular diseases such as essential hypertension, microvascular angina, and heart failure. Non-diabetic offspring of diabetic parents have both insulin resistance and endothelial dysfunction [43,44,56]. Thus, there may be similar genetic and acquired contributions to both

insulin resistance and endothelial dysfunction. At the cellular level, a key feature of insulin resistance is the pathway-selective impairment in PI3K-dependent signaling pathways while other insulin signaling branches including Ras/MAPK-dependent pathways are relatively unaffected. This has important pathophysiological implications because metabolic insulin resistance is typically accompanied by compensatory hyperinsulinemia to maintain euglycemia. In the vasculature and elsewhere, hyperinsulinemia will overdrive unaffected MAPK-dependent pathways leading to an imbalance between PI3K- and MAPK-dependent functions of insulin. Lipotoxicity, glucotoxicity, and inflammation that contribute to insulin resistant states differentially affect PI3K and MAPK pathways through multiple independent and interdependent mechanisms in the endothelium. The imbalance between PI3K/Akt/eNOS/NO and MAPK/ET-1 vascular actions of insulin provoked by dyslipidemia, hyperglycemia, and inflammatory cytokines may contribute to both impaired vascular and metabolic actions of insulin. That is, compensatory hyperinsulinemia that typically accompanies pathway-selective insulin resistance (in PI3K pathways) activates unopposed MAPK pathways leading to enhanced pro-hypertensive and atherogenic actions of insulin [14].

Proinflammatory Cytokines

Insulin resistance and endothelial dysfunction are characterized by elevated circulating markers of inflammation [14,57]. Visceral fat accumulation may play a key role in the development of the systemic pro-inflammatory state associated with insulin resistance [58]. The most extensively studied proinflammatory cytokine implicated in insulin resistance is TNF- α . TNF- α activates a variety of serine kinases including JNK, IKK β , and IL-1 β receptor-associated kinase that directly or indirectly increase serine phosphorylation of IRS-1/2, leading to decreased insulin-stimulated activation of PI3K/Akt/eNOS in endothelial cells [59–62]. In addition, TNF- α increases ET-1 secretion in a MAPK-dependent fashion [63]. IL-6, another cytokine elevated in insulin resistant states, also inhibits insulin-stimulated increases in eNOS activity and NO production in the endothelium [64]. Similarly, C-reactive protein (CRP), a marker of inflammation, has important biological actions to inhibit insulin-evoked NO production in endothelial cells through specific inactivation of the PI3K/Akt/eNOS pathway [65,66]. Similar to TNF- α , CRP simultaneously increases endothelial ET-1 production [66]. Systemic infusion of high doses of TNF- α results in the loss of insulin-induced increases in glucose uptake, limb blood flow, and capillary recruitment in rat hind limb [62]. In the presence of TNF- α , insulin constricts skeletal muscle arterioles. However, pre-treatment of these arterioles with the nonselective ET-1 receptor antagonist abolishes these vasoconstrictor actions of insulin [67]. In humans, high local concentrations of TNF- α achieved by intra-arterial infusion simultaneously inhibits both insulin-stimulated glucose uptake and endothelium-dependent vasodilation in the forearm [68]. These findings suggest that TNF- α specifically downregulates the insulin-dependent PI3K/Akt/eNOS vasodilator pathway without modulating insulin-stimulated ET-1-mediated vasoconstriction. Thus, proinflammatory cytokines may contribute to coupling of metabolic and vascular insulin resistance manifested by impaired insulin signaling and endothelial dysfunction.

Adipokines

Adipocyte-derived hormones such as leptin and adiponectin have both metabolic and vascular actions. Adiponectin is an anti-inflammatory peptide whose circulating levels are positively correlated with insulin sensitivity and that may serve to link obesity with insulin resistance [57]. 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. Similar to insulin, adiponectin has vasodilator actions to stimulate NO production in endothelial cells [69]. In addition, adiponectin enhances NO bioavailability by upregulating eNOS expression and reducing ROS production in endothelial cells [70]. Decreased plasma

adiponectin levels are observed in patients with obesity, type 2 diabetes, hypertension, metabolic syndrome, and coronary artery disease [71,72]. Moreover, low plasma adiponectin levels are significantly correlated with endothelial dysfunction [72]. These results suggest that low adiponectin levels may be a useful marker for early-stage atherosclerosis.

Leptin, a key regulator of appetite, body weight and energy balance in the CNS acts directly on the vasculature. Similar to insulin, leptin induces endothelium-dependent vasodilation through a PI3K/Akt/eNOS pathway [73]. Like insulin, leptin evoked vasodilation is opposed by sympathetically-induced vasoconstriction [74]. Angiotensin II and TNF- α stimulate the production of leptin. Consequently, insulin resistant states, characterized by elevated sympathetic activity, angiotensin II and TNF- α activity, leptin may potentiate pressor effects of hyperinsulinemia [75]. Therefore, interactions between angiotensin II and insulin with leptin may have deleterious cardiovascular effects in obesity. These actions may contribute to the pathogenesis of hypertension, atherosclerosis, and left ventricular hypertrophy. In fact, in the large, prospective, West of Scotland Coronary Prevention Study (WOSCOPS) elevated circulating leptin levels independently predicts increased risk of coronary events [76]. Thus, acute, beneficial vasodilator effects of leptin at low concentrations do not reflect the potentially detrimental effects of chronic elevations in leptin levels observed in the presence of insulin and leptin resistance. Human studies specifically examining interactions between cardiovascular actions of insulin and leptin in normal and pathological states are needed to fully understand potential beneficial and detrimental effects of leptin on metabolic and cardiovascular physiology.

Resistin, a proinflammatory peptide expressed in human macrophages, mononuclear leukocytes, and bone marrow cells has been implicated in insulin resistance. Recent studies suggest that resistin may adversely impact endothelial function and vascular relaxation by stimulating ET-1 production, inhibiting vasodilator actions of insulin, and decreasing eNOS expression [77,78]. Thus, resistin may participate in the reciprocal relationships between insulin resistance and endothelial dysfunction. In support of this notion, resistin expression in circulating monocytes independently predicts reduced flow-mediated vasodilation in individuals with insulin resistance [79].

Lipotoxicity

Patients with type 2 diabetes mellitus or the metabolic syndrome have a distinctive dyslipidemia characterized by hypertriglyceridemia, elevated blood levels of apolipoprotein B, small, dense LDL cholesterol, and low levels of HDL cholesterol. This contributes to endothelial dysfunction, atherosclerosis, and insulin resistance. Treatment of vascular endothelial cells with FFA impairs insulin-stimulated activation of PI3K, PDK1, Akt and eNOS [80–82]. Elevated cellular levels of lipid metabolites such as diacylglycerols, ceramide, and long-chain fatty acyl CoAs activate serine kinases such as PKC and IKK β that cause insulin resistance by increasing serine phosphorylation of IRS-1 [80–82]. In addition, FFAs increase production of reactive oxygen species (ROS) [80]. Thus, impaired PI3K signaling reduces eNOS activity, accentuates FFA-evoked oxidative stress, and diminishes NO bioavailability. In support of these findings, raising circulating FFA levels significantly impairs insulin-induced increases in skeletal muscle capillary recruitment with a concomitant decrease in glucose disposal [83]. Insulin's effects on capillary recruitment and glucose uptake are impaired when FFA levels are increased in healthy lean women [84]. Moreover, when FFA levels are lowered in obese women, vasodilator actions of insulin are improved suggesting that insulin's microvascular and metabolic effects are coupled in response to changes in FFA levels. Indeed, changes in capillary recruitment account for 30% of the association between changes in FFA levels and changes in insulin-mediated glucose uptake [84]. 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 with significant correlations between FFA-induced changes in glucose uptake and FFA-induced decreases in leg blood flow [85]. Moreover, FFA infusion in humans accentuates insulin-mediated ET-1 release [86]. This magnitude of this effect is significantly higher in insulin-resistant individuals (when compared with healthy controls). Decreasing forearm lipid oxidation reduces insulin-evoked ET-1 release while simultaneously increasing NO bioavailability and glucose uptake [87]. These studies suggest that in the context of pathway-selective impairment of PI3K signaling induced by elevated FFA levels, insulin stimulates increased ET-1 secretion through an unopposed MAPK signaling that leads to relative vasoconstriction and insulin resistance.

Glucotoxicity

Hyperglycemia associated with impaired glucose tolerance and diabetes causes insulin resistance and endothelial dysfunction by increasing oxidative stress, formation of advanced glycation end products (AGEs), and flux through the hexosamine biosynthetic pathway. In endothelial cells exposed to high glucose concentrations, insulin-stimulated activation of Akt and eNOS is significantly reduced [88]. Hyperglycemia induces increased ROS production, post-translational O-GlcNacylation, PKC activity, and AGE formation that are known to specifically inhibit the PI3K/Akt/eNOS pathway [5,88]. ROS decreases NO bioavailability, reduces cellular tetrahydrobiopterin levels, and promotes generation of superoxide by eNOS. Glucosamine, a product of the hexosamine biosynthetic pathway impairs insulin stimulated glucose uptake in skeletal muscle and production of NO in endothelium *in vitro*. *In vivo*, acute intravenous glucosamine administration causes metabolic insulin resistance and impairs insulin-mediated increases in femoral arterial blood flow and capillary recruitment [89]. Local hyperglycemia achieved by infusing concentrated glucose directly into the brachial artery of healthy humans diminishes agonist-induced vasodilation, an effect prevented by intraarterial administration of ascorbate (an antioxidant) [90]. Acute hyperglycemia consistently impairs endothelial function in individuals with insulin resistance or type 2 diabetes [91]. Collectively these data suggest that hyperglycemia impairs insulin action in skeletal and cardiac muscle as well as in vascular endothelium. Consistent with these findings, activity of Akt and eNOS in vasculature and muscle is significantly attenuated in patients with diabetes when compared with non-diabetics [92]. By contrast with deleterious effects on the PI3K/Akt/eNOS pathway, hyperglycemia enhances endothelial ET-1 secretion and thereby alters the balance between NO and ET-1 to favor vasoconstriction and endothelial dysfunction. An oral glucose load significantly increases plasma ET-1, in insulin-resistant, but not in healthy individuals [93]. In addition, selective ET_A receptor blockade in the forearm significantly increases forearm blood flow in patients with type 2 diabetes but not in healthy individuals [94]. Thus, a parallel increase in ET-1 activity and diminished NO bioactivity associated with hyperglycemia and insulin resistance may contribute to abnormal vascular function. This illustrates the altered balance between the vasodilator and vasoconstrictor actions of insulin in insulin resistant states that contributes to reciprocal relationships between insulin resistance and endothelial dysfunction.

Fatness and Fitness in Insulin Resistance and Endothelial Dysfunction

Increased adiposity and inadequate physical activity are strong and independent predictors of CAD. Many studies consistently report associations between obesity and endothelial dysfunction. In the largest study, the Framingham Heart Study, body mass index (BMI) independently predicts reduced brachial artery FMD [95]. Furthermore, increased abdominal adiposity (determined by waist-to-hip ratio (WHR)) is also a strong independent predictor of endothelial dysfunction even in overweight otherwise healthy adults [96]. Recently, Clerk et al. 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 glucose 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 [53]. These results demonstrate impaired insulin-mediated microvascular function in obesity. Obesity and accompanying changes in local and humoral adipocytokine profiles are frequently associated with insulin resistance. These alterations coupled with metabolic abnormalities that cause insulin resistance such as lipotoxicity and glucotoxicity all contribute importantly to endothelial dysfunction.

Physical inactivity and reduced exercise capacity predicts cardiovascular disease independent of conventional risk factors and is associated with endothelial dysfunction and insulin resistance [97,98]. Interestingly, when rats are segregated and bred based on aerobic exercise capacity, low-capacity runners are characterized by endothelial dysfunction and insulin resistance [99]. In humans, reduced $\dot{V}_{O2\text{ max}}$ along with impaired mitochondrial function and biogenesis are associated with insulin-resistance [100]. This is also observed in first-degree relatives of patients with type 2 diabetes [101]. Taken together, these data suggest a genetic component to the relationship between reduced aerobic capacity, endothelial dysfunction, and insulin resistance. In human skeletal muscle, NO-derived from eNOS plays an important role in mitochondrial biogenesis [102]. Moreover, skeletal muscle eNOS activity and capillary density is significantly reduced in insulin-resistant individuals [92,103]. Thus, physical inactivity is accompanied by reduced eNOS and NO activity that may contribute to impaired mitochondrial function. Further studies are required to determine if mitochondrial dysfunction is causally related to endothelial dysfunction and/or insulin resistance. Long-term inactivity in humans also results in a significant increase in ET-1-dependent vascular tone in skeletal muscle vascular beds [104]. Thus, physical inactivity is not only associated with impairment in the eNOS/NO pathway but augmented ET-1 secretion. This is consistent with animal studies where deficiency of eNOS promotes ET-1-induced endothelial dysfunction and hypertension [105].

Therapeutic Interventions Targeting Endothelial Dysfunction and Insulin Resistance

Acquired and genetic factors influence metabolic, vascular and inflammatory homeostasis that involve multiple cellular and physiological mechanisms to contribute, often simultaneously, to development of insulin resistance and endothelial dysfunction. There are no validated screening tools for assessing endothelial dysfunction in the clinical setting. Therefore, clinical assessment of conventional risk factors and a comprehensive management approach is needed to effectively treat and/or prevent endothelial dysfunction and insulin resistance. Interventions aimed at improving either insulin resistance or endothelial dysfunction, that raise plasma adiponectin levels, block renin angiotensin and endothelin systems, lower oxidative stress, and attenuate inflammation are predicted to have simultaneous beneficial effects on both metabolic and cardiovascular function.

Dietary and Lifestyle modifications

Diet, weight loss, and physical exercise decrease insulin resistance and improve endothelial dysfunction [102,106–130] (Table 2). Calorie restriction alone (25% less than baseline energy requirements) or a combination of calorie restriction and physical exercise for six months increases eNOS expression in human skeletal muscle [102]. Calorie restriction and/or exercise also improves NO-dependent vasodilation, reduces circulating ET-1 levels, and increases adiponectin levels in insulin resistant individuals [119–121]. Mediterranean-style diet significantly reduces serum concentrations of inflammatory markers, decreases insulin resistance, and improves endothelial function in patients with metabolic syndrome (when compared with matched subjects on a control diet [122]). Likewise, 2-year lifestyle intervention consisting of weight loss, physical exercise, and Mediterranean-style diet decreases BMI and inflammatory markers while increasing adiponectin levels in a cohort of obese women [123].

Increased physical activity/exercise enhances insulin sensitivity and NO-dependent vasodilatation in both conduit and resistance vessels of individuals characterized by endothelial dysfunction and insulin resistance [124–128]. Exercise increases insulin-stimulated blood flow in athletes, healthy controls, and type 2 diabetic individuals [129,130]. There appears to be a threshold for exercise-induced improvement in endothelial function. Moderate (50% VO₂ max), but not low (25% VO₂ max) or high intensity (75% VO₂ max) exercise for 12 weeks is associated with enhanced acetylcholine-mediated forearm vasodilation [131]. Physical exercise increases forearm skeletal muscle capillary recruitment in healthy individuals and may augment glucose uptake by enhancing nutritive blood flow [31]. The protective effects of exercise on the vasculature do not seem to be dependent on improvement of co-existing cardiovascular risk factors. Interestingly, regular exercise increases eNOS protein expression and activity via PI3K/Akt-dependent phosphorylation in human vasculature [132]. 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 balance between vasoconstrictor and vasodilator actions, and increased capillary density.

Pharmacological interventions

Routinely used pharmacotherapies such as insulin sensitizers, hypolipidemic agents, or angiotensin II antagonists improve endothelial dysfunction in insulin-resistant individuals [54,106,133–149](Table 3). These studies suggest that a combinatorial therapeutic strategy may be more effective in ameliorating endothelial dysfunction frequently observed in insulin-resistant states.

Primary prevention of endothelial dysfunction

It is well established that dysfunctional endothelium contributes to development and progression of atherosclerosis. Consequently, early detection and treatment of endothelial dysfunction may be an attractive strategy for preventing CHD. Unfortunately, established validated methods for assessment of endothelial dysfunction for CHD risk prediction in the clinical setting are not currently available. Current techniques to assess endothelial function (Table 1), are invasive, expensive, or suffer from lack of high sensitivity, specificity, reproducibility, or clinically defined cut-off values. Therefore, at this time, targeting established and modifiable risk factors for endothelial dysfunction and insulin resistance is the best primary strategy to prevent these conditions.

Conclusions

Pathway-specific impairment of PI3K-dependent insulin signaling pathways facilitates reciprocal relationships between endothelial dysfunction and insulin resistance that contribute to clustering of metabolic and cardiovascular diseases. Therapeutic interventions that target this pathway-selective impairment simultaneously ameliorate endothelial dysfunction and insulin resistance. Thus, an integrated approach that combines lifestyle modifications with pharmacotherapy to restore balance between vasodilator and vasoconstrictor actions of insulin may promote endothelial health, insulin sensitivity, and reduce the risk of metabolic and cardiovascular diseases

ACKNOWLEDGEMENTS

This research was supported by the Intramural Research Program of the NIH, NCCAM.

References

1. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for niddm, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991;14(3):173–194. [PubMed: 2044434]
2. Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S, et al. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc Natl Acad Sci U S A* 2007;104(31):12587–12594. [PubMed: 17640906]
3. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: Pathophysiology, evaluation, and effect of weight loss: An update of the 1997 american heart association scientific statement on obesity and heart disease from the obesity committee of the council on nutrition, physical activity, and metabolism. *Circulation* 2006;113(6):898–918. [PubMed: 16380542]
4. Accili D. Lilly lecture 2003: The struggle for mastery in insulin action: From triumvirate to republic. *Diabetes* 2004;53(7):1633–1642. [PubMed: 15220184]
5. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. *Endocr Rev* 2007;28(5):463–491. [PubMed: 17525361]
6. Zeng G, Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J Clin Invest* 1996;98(4):894–898. [PubMed: 8770859]
7. Vincent MA, Clerk LH, Lindner JR, Klibanov AL, Clark MG, Rattigan S, et al. Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. *Diabetes* 2004;53(6):1418–1423. [PubMed: 15161743]
8. Zhang L, Vincent MA, Richards SM, Clerk LH, Rattigan S, Clark MG, et al. Insulin sensitivity of muscle capillary recruitment in vivo. *Diabetes* 2004;53(2):447–453. [PubMed: 14747297]
9. Vicent D, Ilany J, Kondo T, Naruse K, Fisher SJ, Kisanuki YY, et al. The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *J Clin Invest* 2003;111(9):1373–1380. [PubMed: 12727929]
10. Baron AD, Laakso M, Brechtel G, Edelman SV. Mechanism of insulin resistance in insulin-dependent diabetes mellitus: A major role for reduced skeletal muscle blood flow. *J Clin Endocrinol Metab* 1991;73(3):637–643. [PubMed: 1874938]
11. Natali A, Taddei S, Quinones Galvan A, Camastra S, Baldi S, Frascerra S, et al. Insulin sensitivity, vascular reactivity, and clamp-induced vasodilatation in essential hypertension. *Circulation* 1997;96(3):849–855. [PubMed: 9264492]
12. Nystrom FH, Quon MJ. Insulin signalling: Metabolic pathways and mechanisms for specificity. *Cell Signal* 1999;11(8):563–574. [PubMed: 10433517]
13. Ver MR CH, Quon MJ. Insulin signaling pathways regulating translocation of glut4. *Curr. Med. Chem. - Immun. Endo. Metab. Agents* 2005;5:159–165.
14. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: Molecular and pathophysiological mechanisms. *Circulation* 2006;113(15):1888–1904. [PubMed: 16618833]
15. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, et al. Insulin resistance differentially affects the pi 3-kinase- and map kinase-mediated signaling in human muscle. *J Clin Invest* 2000;105(3):311–320. [PubMed: 10675357]
16. Jiang ZY, Lin YW, Clemont A, Feener EP, Hein KD, Igarashi M, et al. Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. *J Clin Invest* 1999;104(4):447–457. [PubMed: 10449437]
17. Dudzinski DM, Michel T. Life history of enos: Partners and pathways. *Cardiovasc Res* 2007;75(2):247–260. [PubMed: 17466957]
18. Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. *Am J Physiol Regul Integr Comp Physiol* 2003;284(1):R1–R12. [PubMed: 12482742]
19. Herman AG, Moncada S. Therapeutic potential of nitric oxide donors in the prevention and treatment of atherosclerosis. *Eur Heart J* 2005;26(19):1945–1955. [PubMed: 15911567]
20. Drexler H. Endothelial dysfunction: Clinical implications. *Prog Cardiovasc Dis* 1997;39(4):287–324. [PubMed: 9050817]

21. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: A marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol* 2003;23(2):168–175. [PubMed: 12588755]
22. Barac A, Campia U, Panza JA. Methods for evaluating endothelial function in humans. *Hypertension* 2007;49(4):748–760. [PubMed: 17309954]
23. Vincent MA, Montagnani M, Quon MJ. Molecular and physiologic actions of insulin related to production of nitric oxide in vascular endothelium. *Curr Diab Rep* 2003;3(4):279–288. [PubMed: 12866989]
24. Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-stimulated activation of enos is independent of Ca^{2+} but requires phosphorylation by akt at ser(1179). *J Biol Chem* 2001;276(32):30392–30398. [PubMed: 11402048]
25. Marasciulo FL, Montagnani M, Potenza MA. Endothelin-1: The yin and yang on vascular function. *Curr Med Chem* 2006;13(14):1655–1665. [PubMed: 16787211]
26. Potenza MA, Marasciulo FL, Chieppa DM, Brigiani GS, Formoso G, Quon MJ, et al. Insulin resistance in spontaneously hypertensive rats is associated with endothelial dysfunction characterized by imbalance between NO and $ET-1$ production. *Am J Physiol Heart Circ Physiol* 2005;289(2):H813–H822. [PubMed: 15792994]
27. Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, et al. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. *J Biol Chem* 2002;277(3):1794–1799. [PubMed: 11707433]
28. Mukai Y, Wang CY, Rikitake Y, Liao JK. Phosphatidylinositol 3-kinase/protein kinase akt negatively regulates plasminogen activator inhibitor type 1 expression in vascular endothelial cells. *Am J Physiol Heart Circ Physiol* 2007;292(4):H1937–H1942. [PubMed: 17172275]
29. Baron AD. Hemodynamic actions of insulin. *Am J Physiol* 1994;267(2 Pt 1):E187–E202. [PubMed: 8074198]
30. Coggins M, Lindner J, Rattigan S, Jahn L, Fasy E, Kaul S, et al. Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. *Diabetes* 2001;50(12):2682–2690. [PubMed: 11723050]
31. Vincent MA, Clerk LH, Lindner JR, Price WJ, Jahn LA, Leong-Poi H, et al. Mixed meal and light exercise each recruit muscle capillaries in healthy humans. *Am J Physiol Endocrinol Metab* 2006;290(6):E1191–E1197. [PubMed: 16682488]
32. Gudbjornsdottir S, Sjostrand M, Strindberg L, Wahren J, Lonnroth P. Direct measurements of the permeability surface area for insulin and glucose in human skeletal muscle. *J Clin Endocrinol Metab* 2003;88(10):4559–4564. [PubMed: 14557422]
33. Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S. Inhibiting NO blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am J Physiol Endocrinol Metab* 2003;285(1):E123–E129. [PubMed: 12791603]
34. Cardillo C, Nambi SS, Kilcoyne CM, Choucair WK, Katz A, Quon MJ, et al. Insulin stimulates both endothelin and nitric oxide activity in the human forearm. *Circulation* 1999;100(8):820–825. [PubMed: 10458717]
35. Ross RM, Kolka CM, Rattigan S, Clark MG. Acute blockade by endothelin-1 of haemodynamic insulin action in rats. *Diabetologia* 2007;50(2):443–451. [PubMed: 17187249]
36. Ahlborg G, Lindstrom J. Insulin sensitivity and big $ET-1$ conversion to $ET-1$ after ETA - or ETB -receptor blockade in humans. *J Appl Physiol* 2002;93(6):2112–2121. [PubMed: 12391119]
37. Kelly LK, Wedgwood S, Steinhorn RH, Black SM. Nitric oxide decreases endothelin-1 secretion through the activation of soluble guanylate cyclase. *Am J Physiol Lung Cell Mol Physiol* 2004;286(5):L984–L991. [PubMed: 14695117]
38. Lteif A, Vaishnava P, Baron AD, Mather KJ. Endothelin limits insulin action in obese/insulin-resistant humans. *Diabetes* 2007;56(3):728–734. [PubMed: 17327443]
39. Ahlborg G, Shemyakin A, Bohm F, Gonon A, Pernow J. Dual endothelin receptor blockade acutely improves insulin sensitivity in obese patients with insulin resistance and coronary artery disease. *Diabetes Care* 2007;30(3):591–596. [PubMed: 17327326]
40. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1996;27(3):567–574. [PubMed: 8606266]

41. Paradisi G, Steinberg HO, Hempfling A, Cronin J, Hook G, Shepard MK, et al. Polycystic ovary syndrome is associated with endothelial dysfunction. *Circulation* 2001;103(10):1410–1415. [PubMed: 11245645]
42. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J Clin Invest* 1996;97(11):2601–2610. [PubMed: 8647954]
43. Goldfine AB, Beckman JA, Betensky RA, Devlin H, Hurley S, Varo N, et al. Family history of diabetes is a major determinant of endothelial function. *J Am Coll Cardiol* 2006;47(12):2456–2461. [PubMed: 16781373]
44. Tesauro M, Rizza S, Iantorno M, Campia U, Cardillo C, Lauro D, et al. Vascular, metabolic, and inflammatory abnormalities in normoglycemic offspring of patients with type 2 diabetes mellitus. *Metabolism* 2007;56(3):413–419. [PubMed: 17292732]
45. Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. *Jama* 2004;291(16):1978–1986. [PubMed: 15113816]
46. Meigs JB, O'Donnell CJ, Tofler GH, Benjamin EJ, Fox CS, Lipinska I, et al. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: The framingham offspring study. *Diabetes* 2006;55(2):530–537. [PubMed: 16443791]
47. Song Y, Manson JE, Tinker L, Rifai N, Cook NR, Hu FB, et al. Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. *Diabetes* 2007;56(7):1898–1904. [PubMed: 17389327]
48. Donahue RP, Rejman K, Rafelson LB, Dmochowski J, Stranges S, Trevisan M. Sex differences in endothelial function markers before conversion to pre-diabetes: Does the clock start ticking earlier among women? The western new york study. *Diabetes Care* 2007;30(2):354–359. [PubMed: 17259507]
49. Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, et al. Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation* 2001;104(3):342–345. [PubMed: 11457755]
50. Kubis N, Richer C, Domergue V, Giudicelli JF, Levy BI. Role of microvascular rarefaction in the increased arterial pressure in mice lacking for the endothelial nitric oxide synthase gene (enos3pt-/-). *J Hypertens* 2002;20(8):1581–1587. [PubMed: 12172320]
51. Le Gouill E, Jimenez M, Binnert C, Jayet PY, Thalmann S, Nicod P, et al. Endothelial nitric oxide synthase (enos) knockout mice have defective mitochondrial beta-oxidation. *Diabetes* 2007;56(11):2690–2696. [PubMed: 17682093]
52. Cook S, Hugli O, Egli M, Menard B, Thalmann S, Sartori C, et al. Partial gene deletion of endothelial nitric oxide synthase predisposes to exaggerated high-fat diet-induced insulin resistance and arterial hypertension. *Diabetes* 2004;53(8):2067–2072. [PubMed: 15277387]
53. Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. *Diabetes* 2006;55(5):1436–1442. [PubMed: 16644702]
54. Hermann TS, Li W, Dominguez H, Ihlemann N, Rask-Madsen C, Major-Pedersen A, et al. Quinapril treatment increases insulin-stimulated endothelial function and adiponectin gene expression in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2006;91(3):1001–1008. [PubMed: 16352688]
55. Rask-Madsen C, Ihlemann N, Krarup T, Christiansen E, Kober L, Nervi Kistorp C, et al. Insulin therapy improves insulin-stimulated endothelial function in patients with type 2 diabetes and ischemic heart disease. *Diabetes* 2001;50(11):2611–2618. [PubMed: 11679442]
56. Balletshofer BM, Rittig K, Enderle MD, Volk A, Maerker E, Jacob S, et al. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 2000;101(15):1780–1784. [PubMed: 10769277]
57. Koh KK, Han SH, Quon MJ. Inflammatory markers and the metabolic syndrome: Insights from therapeutic interventions. *J Am Coll Cardiol* 2005;46(11):1978–1985. [PubMed: 16325028]
58. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;444(7121):860–867. [PubMed: 17167474]

59. Gustafson B, Hammarstedt A, Andersson CX, Smith U. Inflamed adipose tissue: A culprit underlying the metabolic syndrome and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007;27(11):2276–2283. [PubMed: 17823366]
60. Anderson HD, Rahmutula D, Gardner DG. Tumor necrosis factor- α inhibits endothelial nitric-oxide synthase gene promoter activity in bovine aortic endothelial cells. *J Biol Chem* 2004;279(2):963–969. [PubMed: 14581470]
61. Eringa EC, Stehouwer CD, van Nieuw Amerongen GP, Ouwehand L, Westerhof N, Sipkema P. Vasoconstrictor effects of insulin in skeletal muscle arterioles are mediated by erk1/2 activation in endothelium. *Am J Physiol Heart Circ Physiol* 2004;287(5):H2043–H2048. [PubMed: 15059773]
62. Zhang L, Wheatley CM, Richards SM, Barrett EJ, Clark MG, Rattigan S. Tnf- α acutely inhibits vascular effects of physiological but not high insulin or contraction. *Am J Physiol Endocrinol Metab* 2003;285(3):E654–E660. [PubMed: 12759220]
63. Sury MD, Frese-Schaper M, Muhlemann MK, Schulthess FT, Blasig IE, Tauber MG, et al. Evidence that n-acetylcysteine inhibits tnf- α -induced cerebrovascular endothelin-1 upregulation via inhibition of mitogen- and stress-activated protein kinase. *Free Radic Biol Med* 2006;41(9):1372–1383. [PubMed: 17023264]
64. Andreozzi F, Laratta E, Procopio C, Hribal ML, Sciacqua A, Perticone M, et al. Interleukin-6 impairs the insulin signaling pathway, promoting production of nitric oxide in human umbilical vein endothelial cells. *Mol Cell Biol* 2007;27(6):2372–2383. [PubMed: 17242212]
65. Schwartz R, Osborne-Lawrence S, Hahner L, Gibson LL, Gormley AK, Vongpatanasin W, et al. C-reactive protein downregulates endothelial nitric oxide synthase and attenuates reendothelialization in vivo in mice. *Circ Res* 2007;100(10):1452–1459. [PubMed: 17446434]
66. Xu JW, Morita I, Ikeda K, Miki T, Yamori Y. C-reactive protein suppresses insulin signaling in endothelial cells. ---role of syk tyrosine kinase. *Mol Endocrinol*. 2006
67. Eringa EC, Stehouwer CD, Walburg K, Clark AD, van Nieuw Amerongen GP, Westerhof N, et al. Physiological concentrations of insulin induce endothelin-dependent vasoconstriction of skeletal muscle resistance arteries in the presence of tumor necrosis factor- α dependence on c-jun n-terminal kinase. *Arterioscler Thromb Vasc Biol* 2006;26(2):274–280. [PubMed: 16322532]
68. Rask-Madsen C, Dominguez H, Ihlemann N, Hermann T, Kober L, Torp-Pedersen C. Tumor necrosis factor- α inhibits insulin's stimulating effect on glucose uptake and endothelium-dependent vasodilation in humans. *Circulation* 2003;108(15):1815–1821. [PubMed: 14530204]
69. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 2003;278(45):45021–45026. [PubMed: 12944390]
70. Motoshima H, Wu X, Mahadev K, Goldstein BJ. Adiponectin suppresses proliferation and superoxide generation and enhances enos activity in endothelial cells treated with oxidized ldl. *Biochem Biophys Res Commun* 2004;315(2):264–271. [PubMed: 14766203]
71. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: Molecular links between obesity and atherosclerosis. *Am J Physiol Heart Circ Physiol* 2005;288(5):H2031–H2041. [PubMed: 15653761]
72. Shimabukuro M, Higa N, Asahi T, Oshiro Y, Takasu N, Tagawa T, et al. Hypoadiponectinemia is closely linked to endothelial dysfunction in man. *J Clin Endocrinol Metab* 2003;88(7):3236–3240. [PubMed: 12843170]
73. Vecchione C, Maffei A, Colella S, Aretini A, Poulet R, Frati G, et al. Leptin effect on endothelial nitric oxide is mediated through akt-endothelial nitric oxide synthase phosphorylation pathway. *Diabetes* 2002;51(1):168–173. [PubMed: 11756337]
74. Rahmouni K, Correia ML, Haynes WG, Mark AL. Obesity-associated hypertension: New insights into mechanisms. *Hypertension* 2005;45(1):9–14. [PubMed: 15583075]
75. Skurk T, van Harmelen V, Blum WF, Hauner H. Angiotensin ii promotes leptin production in cultured human fat cells by an erk1/2-dependent pathway. *Obes Res* 2005;13(6):969–973. [PubMed: 15976138]
76. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, et al. Plasma leptin and the risk of cardiovascular disease in the west of scotland coronary prevention study (woscops). *Circulation* 2001;104(25):3052–3056. [PubMed: 11748099]

77. Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, et al. Resistin promotes endothelial cell activation: Further evidence of adipokine-endothelial interaction. *Circulation* 2003;108(6):736–740. [PubMed: 12874180]
78. Shen YH, Zhang L, Gan Y, Wang X, Wang J, LeMaire SA, et al. Up-regulation of pten (phosphatase and tensin homolog deleted on chromosome ten) mediates p38 mapk stress signal-induced inhibition of insulin signaling. A cross-talk between stress signaling and insulin signaling in resistin-treated human endothelial cells. *J Biol Chem* 2006;281(12):7727–7736. [PubMed: 16418168]
79. Lupattelli G, Marchesi S, Ronti T, Lombardini R, Bruscoli S, Bianchini R, et al. Endothelial dysfunction in vivo is related to monocyte resistin mrna expression. *J Clin Pharm Ther* 2007;32(4):373–379. [PubMed: 17635339]
80. Du X, Edelstein D, Obici S, Higham N, Zou MH, Brownlee M. Insulin resistance reduces arterial prostacyclin synthase and enos activities by increasing endothelial fatty acid oxidation. *J Clin Invest* 2006;116(4):1071–1080. [PubMed: 16528409]
81. Kim F, Tysseling KA, Rice J, Pham M, Haji L, Gallis BM, et al. Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by ikkbeta. *Arterioscler Thromb Vasc Biol* 2005;25(5):989–994. [PubMed: 15731493]
82. Wang XL, Zhang L, Youker K, Zhang MX, Wang J, LeMaire SA, et al. Free fatty acids inhibit insulin signaling-stimulated endothelial nitric oxide synthase activation through upregulating pten or inhibiting akt kinase. *Diabetes* 2006;55(8):2301–2310. [PubMed: 16873694]
83. Clerk LH, Rattigan S, Clark MG. Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake in vivo. *Diabetes* 2002;51(4):1138–1145. [PubMed: 11916937]
84. de Jongh RT, Serne EH, Ijzerman RG, de Vries G, Stehouwer CD. Free fatty acid levels modulate microvascular function: Relevance for obesity-associated insulin resistance, hypertension, and microangiopathy. *Diabetes* 2004;53(11):2873–2882. [PubMed: 15504968]
85. Steinberg HO, Paradisi G, Hook G, Crowder K, Cronin J, Baron AD. Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. *Diabetes* 2000;49(7):1231–1238. [PubMed: 10909983]
86. Piatti PM, Monti LD, Conti M, Baruffaldi L, Galli L, Phan CV, et al. Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. *Diabetes* 1996;45(3):316–321. [PubMed: 8593936]
87. Monti LD, Setola E, Fragasso G, Camisasca RP, Lucotti P, Galluccio E, et al. Metabolic and endothelial effects of trimetazidine on forearm skeletal muscle in patients with type 2 diabetes and ischemic cardiomyopathy. *Am J Physiol Endocrinol Metab* 2006;290(1):E54–E59. [PubMed: 16174656]
88. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the akt site. *J Clin Invest* 2001;108(9):1341–1348. [PubMed: 11696579]
89. Wallis MG, Smith ME, Kolka CM, Zhang L, Richards SM, Rattigan S, et al. Acute glucosamine-induced insulin resistance in muscle in vivo is associated with impaired capillary recruitment. *Diabetologia* 2005;48(10):2131–2139. [PubMed: 16059714]
90. Beckman JA, Goldfine AB, Gordon MB, Creager MA. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. *Circulation* 2001;103(12):1618–1623. [PubMed: 11273987]
91. Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: A clinical perspective. *Endocr Rev* 2001;22(1):36–52. [PubMed: 11159815]
92. Kashyap SR, Roman LJ, Lamont J, Masters BS, Bajaj M, Suraamornkul S, et al. Insulin resistance is associated with impaired nitric oxide synthase activity in skeletal muscle of type 2 diabetic subjects. *J Clin Endocrinol Metab* 2005;90(2):1100–1105. [PubMed: 15562034]
93. Desideri G, Ferri C, Bellini C, De Mattia G, Santucci A. Effects of ace inhibition on spontaneous and insulin-stimulated endothelin-1 secretion: In vitro and in vivo studies. *Diabetes* 1997;46(1):81–86. [PubMed: 8971086]
94. Cardillo C, Campia U, Bryant MB, Panza JA. Increased activity of endogenous endothelin in patients with type ii diabetes mellitus. *Circulation* 2002;106(14):1783–1787. [PubMed: 12356630]

95. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF Jr, et al. Clinical correlates and heritability of flow-mediated dilation in the community: The framingham heart study. *Circulation* 2004;109(5):613–619. [PubMed: 14769683]
96. Williams IL, Chowienczyk PJ, Wheatcroft SB, Patel A, Sherwood R, Momin A, et al. Effect of fat distribution on endothelial-dependent and endothelial-independent vasodilatation in healthy humans. *Diabetes Obes Metab* 2006;8(3):296–301. [PubMed: 16634989]
97. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature* 2006;444(7121):875–880. [PubMed: 17167476]
98. Hamburg NM, McMackin CJ, Huang AL, Shenouda SM, Widlansky ME, Schulz E, et al. Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. *Arterioscler Thromb Vasc Biol* 2007;27(12):2650–2656. [PubMed: 17932315]
99. Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science* 2005;307(5708):418–420. [PubMed: 15662013]
100. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, et al. Pgc-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 2003;34(3):267–273. [PubMed: 12808457]
101. Befroy DE, Petersen KF, Dufour S, Mason GF, de Graaf RA, Rothman DL, et al. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. *Diabetes* 2007;56(5):1376–1381. [PubMed: 17287462]
102. Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, Deutsch WA, et al. Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. *PLoS Med* 2007;4(3):e76. [PubMed: 17341128]
103. Mather K, Verma S. Function determines structure in the vasculature: Lessons from insulin resistance. *Am J Physiol Regul Integr Comp Physiol* 2005;289(2):R305–R306. [PubMed: 16014446]
104. Thijssen DH, Ellenkamp R, Kooijman M, Pickkers P, Rongen GA, Hopman MT, et al. A causal role for endothelin-1 in the vascular adaptation to skeletal muscle deconditioning in spinal cord injury. *Arterioscler Thromb Vasc Biol* 2007;27(2):325–331. [PubMed: 17122448]
105. Quaschnig T, Voss F, Relle K, Kalk P, Vignon-Zellweger N, Pfab T, et al. Lack of endothelial nitric oxide synthase promotes endothelin-induced hypertension: Lessons from endothelin-1 transgenic/endothelial nitric oxide synthase knockout mice. *J Am Soc Nephrol* 2007;18(3):730–740. [PubMed: 17287431]
106. Sola S, Mir MQ, Cheema FA, Khan-Merchant N, Menon RG, Parthasarathy S, et al. Irbesartan and lipoic acid improve endothelial function and reduce markers of inflammation in the metabolic syndrome: Results of the irbesartan and lipoic acid in endothelial dysfunction (island) study. *Circulation* 2005;111(3):343–348. [PubMed: 15655130]
107. Heitzer T, Finckh B, Albers S, Krohn K, Kohlschutter A, Meinertz T. Beneficial effects of alpha-lipoic acid and ascorbic acid on endothelium-dependent, nitric oxide-mediated vasodilation in diabetic patients: Relation to parameters of oxidative stress. *Free Radic Biol Med* 2001;31(1):53–61. [PubMed: 11425490]
108. Chen H, Karne RJ, Hall G, Campia U, Panza JA, Cannon RO 3rd, et al. High-dose oral vitamin c partially replenishes vitamin c levels in patients with type 2 diabetes and low vitamin c levels but does not improve endothelial dysfunction or insulin resistance. *Am J Physiol Heart Circ Physiol* 2006;290(1):H137–H145. [PubMed: 16126809]
109. Perticone F, Ceravolo R, Candigliota M, Ventura G, Iacopino S, Sinopoli F, et al. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: Protective effect of vitamin c. *Diabetes* 2001;50(1):159–165. [PubMed: 11147782]
110. Lekakis JP, Anastasiou EA, Papamichael CM, Stamatelopoulos KS, Dagre AG, Alevizaki MC, et al. Short-term oral ascorbic acid improves endothelium-dependent vasodilatation in women with a history of gestational diabetes mellitus. *Diabetes Care* 2000;23(9):1432–1434. [PubMed: 10977048]

111. Darko D, Dornhorst A, Kelly FJ, Ritter JM, Chowienczyk PJ. Lack of effect of oral vitamin c on blood pressure, oxidative stress and endothelial function in type ii diabetes. *Clin Sci (Lond)* 2002;103(4):339–344. [PubMed: 12241530]
112. Regensteiner JG, Popylisen S, Bauer TA, Lindenfeld J, Gill E, Smith S, et al. Oral l-arginine and vitamins e and c improve endothelial function in women with type 2 diabetes. *Vasc Med* 2003;8(3):169–175. [PubMed: 14989557]
113. Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF Jr, Creager MA. Oral antioxidant therapy improves endothelial function in type 1 but not type 2 diabetes mellitus. *Am J Physiol Heart Circ Physiol* 2003;285(6):H2392–H2398. [PubMed: 12881209]
114. Pena AS, Wiltshire E, Gent R, Piotto L, Hirte C, Couper J. Folic acid does not improve endothelial function in obese children and adolescents. *Diabetes Care* 2007;30(8):2122–2127. [PubMed: 17519435]
115. Title LM, Ur E, Giddens K, McQueen MJ, Nassar BA. Folic acid improves endothelial dysfunction in type 2 diabetes--an effect independent of homocysteine-lowering. *Vasc Med* 2006;11(2):101–109. [PubMed: 16886840]
116. Heitzer T, Krohn K, Albers S, Meinertz T. Tetrahydrobiopterin improves endothelium-dependent vasodilation by increasing nitric oxide activity in patients with type ii diabetes mellitus. *Diabetologia* 2000;43(11):1435–1438. [PubMed: 11126415]
117. Nystrom T, Nygren A, Sjöholm A. Tetrahydrobiopterin increases insulin sensitivity in patients with type 2 diabetes and coronary heart disease. *Am J Physiol Endocrinol Metab* 2004;287(5):E919–E925. [PubMed: 15265759]
118. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, et al. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension* 2005;46(2):398–405. [PubMed: 16027246]
119. Hamdy O, Ledbury S, Mullooly C, Jarema C, Porter S, Ovalle K, et al. Lifestyle modification improves endothelial function in obese subjects with the insulin resistance syndrome. *Diabetes Care* 2003;26(7):2119–2125. [PubMed: 12832323]
120. Maeda S, Jesmin S, Iemitsu M, Otsuki T, Matsuo T, Ohkawara K, et al. Weight loss reduces plasma endothelin-1 concentration in obese men. *Exp Biol Med (Maywood)* 2006;231(6):1044–1047. [PubMed: 16741046]
121. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000;20(6):1595–1599. [PubMed: 10845877]
122. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: A randomized trial. *Jama* 2004;292(12):1440–1446. [PubMed: 15383514]
123. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: A randomized trial. *Jama* 2003;289(14):1799–1804. [PubMed: 12684358]
124. Lavrencic A, Salobir BG, Keber I. Physical training improves flow-mediated dilation in patients with the polymetabolic syndrome. *Arterioscler Thromb Vasc Biol* 2000;20(2):551–555. [PubMed: 10671187]
125. Maiorana A, O'Driscoll G, Cheetham C, Dembo L, Stanton K, Goodman C, et al. The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. *J Am Coll Cardiol* 2001;38(3):860–866. [PubMed: 11527646]
126. De Filippis E, Cusi K, Ocampo G, Berria R, Buck S, Consoli A, et al. Exercise-induced improvement in vasodilatory function accompanies increased insulin sensitivity in obesity and type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2006;91(12):4903–4910. [PubMed: 17018657]
127. Sasaki S, Higashi Y, Nakagawa K, Kimura M, Noma K, Sasaki S, et al. A low-calorie diet improves endothelium-dependent vasodilation in obese patients with essential hypertension. *Am J Hypertens* 2002;15(4 Pt 1):302–309. [PubMed: 11991214]
128. Meyer AA, Kundt G, Lenschow U, Schuff-Werner P, Kienast W. Improvement of early vascular changes and cardiovascular risk factors in obese children after a six-month exercise program. *J Am Coll Cardiol* 2006;48(9):1865–1870. [PubMed: 17084264]

129. Dela F, Larsen JJ, Mikines KJ, Ploug T, Petersen LN, Galbo H. Insulin-stimulated muscle glucose clearance in patients with niddm. Effects of one-legged physical training. *Diabetes* 1995;44(9):1010–1020. [PubMed: 7657022]
130. Hardin DS, Azzarelli B, Edwards J, Wigglesworth J, Maianu L, Brechtel G, et al. Mechanisms of enhanced insulin sensitivity in endurance-trained athletes: Effects on blood flow and differential expression of glut 4 in skeletal muscles. *J Clin Endocrinol Metab* 1995;80(8):2437–2446. [PubMed: 7629239]
131. Goto C, Higashi Y, Kimura M, Noma K, Hara K, Nakagawa K, et al. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: Role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 2003;108(5):530–535. [PubMed: 12874192]
132. Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, et al. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 2003;107(25):3152–3158. [PubMed: 12810615]
133. Diamanti-Kandarakis E, Alexandraki K, Protogerou A, Piperi C, Papamichael C, Aessopos A, et al. Metformin administration improves endothelial function in women with polycystic ovary syndrome. *Eur J Endocrinol* 2005;152(5):749–756. [PubMed: 15879361]
134. Mather KJ, Verma S, Anderson TJ. Improved endothelial function with metformin in type 2 diabetes mellitus. *J Am Coll Cardiol* 2001;37(5):1344–1350. [PubMed: 11300445]
135. de Aguiar LG, Bahia LR, Villela N, Laflor C, Sicuro F, Wiernsperger N, et al. Metformin improves endothelial vascular reactivity in first-degree relatives of type 2 diabetic patients with metabolic syndrome and normal glucose tolerance. *Diabetes Care* 2006;29(5):1083–1089. [PubMed: 16644641]
136. Martens FM, Visseren FL, de Koning EJ, Rabelink TJ. Short-term pioglitazone treatment improves vascular function irrespective of metabolic changes in patients with type 2 diabetes. *J Cardiovasc Pharmacol* 2005;46(6):773–778. [PubMed: 16306801]
137. Mittermayer F, Schaller G, Pleiner J, Krzyzanowska K, Kapiotis S, Roden M, et al. Rosiglitazone prevents free fatty acid-induced vascular endothelial dysfunction. *J Clin Endocrinol Metab* 2007;92(7):2574–2580. [PubMed: 17473072]
138. Wang TD, Chen WJ, Lin JW, Chen MF, Lee YT. Effects of rosiglitazone on endothelial function, c-reactive protein, and components of the metabolic syndrome in nondiabetic patients with the metabolic syndrome. *Am J Cardiol* 2004;93(3):362–365. [PubMed: 14759393]
139. Esposito K, Ciotola M, Carleo D, Schisano B, Saccomanno F, Sasso FC, et al. Effect of rosiglitazone on endothelial function and inflammatory markers in patients with the metabolic syndrome. *Diabetes Care* 2006;29(5):1071–1076. [PubMed: 16644639]
140. Ceriello A, Assaloni R, Da Ros R, Maier A, Piconi L, Quagliaro L, et al. Effect of atorvastatin and irbesartan, alone and in combination, on postprandial endothelial dysfunction, oxidative stress, and inflammation in type 2 diabetic patients. *Circulation* 2005;111(19):2518–2524. [PubMed: 15867169]
141. Economides PA, Caselli A, Tiani E, Khaodhiar L, Horton ES, Veves A. The effects of atorvastatin on endothelial function in diabetic patients and subjects at risk for type 2 diabetes. *J Clin Endocrinol Metab* 2004;89(2):740–747. [PubMed: 14764790]
142. Shimabukuro M, Higa N, Asahi T, Oshiro Y, Takasu N. Fluvastatin improves endothelial dysfunction in overweight postmenopausal women through small dense low-density lipoprotein reduction. *Metabolism* 2004;53(6):733–739. [PubMed: 15164320]
143. Evans M, Anderson RA, Graham J, Ellis GR, Morris K, Davies S, et al. Ciprofibrate therapy improves endothelial function and reduces postprandial lipemia and oxidative stress in type 2 diabetes mellitus. *Circulation* 2000;101(15):1773–1779. [PubMed: 10769276]
144. Avogaro A, Miola M, Favaro A, Gottardo L, Pacini G, Manzato E, et al. Gemfibrozil improves insulin sensitivity and flow-mediated vasodilatation in type 2 diabetic patients. *Eur J Clin Invest* 2001;31(7):603–609. [PubMed: 11454015]
145. Koh KK, Han SH, Quon MJ, Yeal Ahn J, Shin EK. Beneficial effects of fenofibrate to improve endothelial dysfunction and raise adiponectin levels in patients with primary hypertriglyceridemia. *Diabetes Care* 2005;28(6):1419–1424. [PubMed: 15920062]

146. Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, et al. Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. *Circulation* 2004;110(24):3687–3692. [PubMed: 15569835]
147. O'Driscoll G, Green D, Maiorana A, Stanton K, Colreavy F, Taylor R. Improvement in endothelial function by angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1999;33(6):1506–1511. [PubMed: 10334415]
148. Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, et al. Additive beneficial effects of fenofibrate combined with atorvastatin in the treatment of combined hyperlipidemia. *J Am Coll Cardiol* 2005;45(10):1649–1653. [PubMed: 15893182]
149. Koh KK, Quon MJ, Han SH, Ahn JY, Jin DK, Kim HS, et al. Vascular and metabolic effects of combined therapy with ramipril and simvastatin in patients with type 2 diabetes. *Hypertension* 2005;45(6):1088–1093. [PubMed: 15883229]

Figure 1.

General features of insulin signal transduction pathways. PI 3-kinase branch of insulin signaling regulates GLUT4 translocation and glucose uptake in skeletal muscle and NO production and vasodilation in vascular endothelium. MAP-kinase branch of insulin signaling generally regulates growth and mitogenesis and controls secretion of endothelin-1 (ET-1) in vascular endothelium. eNOS, endothelial nitric oxide synthase; GLUT, glucose transporter; INS, insulin; IRS, insulin receptor substrate; MEK, MAPK kinase; PDK, phosphoinositide-dependent protein kinase.

Figure 2.

Left, Parallel PI 3-kinase–dependent insulin-signaling pathways in metabolic and vascular tissues synergistically couple metabolic and vascular physiology under healthy conditions. Right, Parallel impairment in insulin-signaling pathways under pathological conditions contributes to synergistic coupling of insulin resistance and endothelial dysfunction. AGE, advanced glycation end-products; PKC ζ , atypical protein kinase C; eNOS, endothelial nitric oxide synthase; GlcNAc, N-acetylglucosamine; GLUT, glucose transporter; IKK, inhibitor of I κ B kinase; INS, insulin; IRS, insulin receptor substrate; JNK, c-jun aminoterminal kinase; MEK, MAPK kinase; PDK, phosphoinositide-dependent protein kinase; PKC, protein kinase C; ROS, reactive oxygen species.

Measure of Endothelial Function	Method	Stimuli
Endothelium-Dependent Vascular Tone		
Coronary arteries	Coronary Angiography to follow changes in vessel diameter	Acetylcholine, bradykinin or substance-P
Peripheral arteries	High-resolution Ultrasonography with Doppler to follow changes in vessel diameter and blood flow	Flow-mediated dilation induced by adenosine or papaverine
	Forearm perfusion technique where blood flow is measured noninvasively using strain gauge plethysmography	Shear stress-induced NO production-Flow-mediated dilation
	Applanation tonometry to measure changes in pulse wave form and augmentation index	Acetylcholine, bradykinin or substance-P
	Peripheral artery tonometry with plethysmography	β 2-agonist
		Reactive hyperemia
Surrogate Markers of Endothelial Activation		
Vascular cell adhesion molecule-1 (VCAM-1)	Plasma, ELISA	
Intercellular adhesion molecule-1 (ICAM-1)	Plasma, ELISA	
P- and E-selectin	Plasma, ELISA	
Circulating endothelial progenitor cells (EPCs)	Immunomagnetic separation or flow cytometry	
von Willebrand factor (vWf)	Plasma, ELISA	

Table 2
Summary of studies involving lifestyle and dietary modifications that target endothelial dysfunction in insulin resistance

Study	Target Population	Therapeutic Intervention	Treatment Duration	Outcome Measures	Treatment Effect
Life Style Modification					
Esposito [122]	Metabolic syndrome	Mediterranean-style diet	2 yr	L-arginine test + Adenosine-induced decrease in blood pressure	↑
Esposito [123]	Metabolic syndrome	Mediterranean-style diet +exercise	2 yr	Adiponectin, CRP, IL-6, IL-18	Adiponectin ↑, CRP ↓, IL-6↓, IL-18↓
Hamdy [115]	Obesity	Low-calorie diet (500 calories/day negative balance)+ Exercise, 30 min/wk	6 mo	FMD	↑
Lawrence [124]	Metabolic syndrome	Bicycle exercise, 3 times/wk	12 wk	FMD	↑
Maiorana [125]	Type 2 Diabetes	Circuit training, 3 times/wk	8 wk	FMD	↑
De Filippis [126]	Obesity/Type 2 Diabetes	Cycle exercise, 45 min, 4 times/wk	8 wk	ACH-induced vasodilation	↑
Sasaki [127]	Obesity/Hypertension	Low-calorie diet (800 kcal/d)	2 wk	ACH-induced vasodilation	↑
Meyer AA [128]	Obesity	Structured exercise 1 h, 3 times/wk	6 mo	FMD	↑
Antioxidants/Nutritional Supplements					
Sola [106]	Metabolic Syndrome	Lipoic acid, 300 mg/d, Orally	4 wk	FMD, IL-6	↑
Heitzer [107]	Type 2 Diabetes	Lipoic acid, 0.7 mg/min, Intra-arterial	Acute (5 min)	ACH-induced vasodilation	↑
Heitzer [107]	Type 2 Diabetes	Vitamin C, 24 mg/min, Intra-arterial	Acute (10 min)	ACH-induced vasodilation	↑
Chen [108]	Type 2 Diabetes	Vitamin C, 800 mg/day, Orally	4 wk	ACH-induced vasodilation	↔
Perticone [109]	Obesity	Vitamin C, 24 mg/min, Intra-arterial	Acute (5 min)	ACH-induced vasodilation	↑
Lekakis [110]	History of GDM	Vitamin C, 2g, Orally	Acute (120 min)	FMD	↑
Darko [111]	Type 2 Diabetes	Vitamin C, 1.5 g/d, Orally	3 wk	ACH-induced vasodilation	↔
Regensteiner [112]	Type 2 Diabetes	Vit E (1.8 g/d) + Vit C (1.0 g/d), Orally	1 wk	FMD	↑
Beckman [113]	Type 2 Diabetes	Vit E (800 IU/d) + Vit C (1.0 g/d), Orally	6 mo	FMD	↔
Pena [114]	Obesity	Folic acid, 5 mg/d, Orally	8 wk	FMD	↔
Title [115]	Type 2 Diabetes	Folic acid, 10 mg/d, Orally	2 wk	FMD	↑
Regensteiner [112]	Type 2 Diabetes	L-arginine, 9 g/d, Orally	1 wk	FMD	↑
Heitzer [116]	Type 2 Diabetes	BH4, 500 µg/min, Intra-arterial	Acute (30 min)	ACH-induced vasodilation	↑
Nystrom [117]	Type 2 Diabetes	BH4, 500 µg/min, Intra-arterial	Acute (2 h)	Insulin-induced ΔFMD	↔
Grassi D [118]	Insulin-resistant Hypertensives	Dark cocoa (flavanols, 88 mg/d), Orally	15 d	FMD	↑

FMD, flow-mediated dilation; CRP, c-reactive protein; IL-6, interleukin-6; IL-18, interleukin-18; BH4, tetrahydrobiopterin; GDM, gestational diabetes mellitus; CAD, coronary heart disease; Ach, acetylcholine

Summary of studies involving pharmacological interventions that target endothelial dysfunction in insulin resistance

Table 3

Study	Target Population	Therapeutic Intervention	Treatment Duration	Outcome Measures	Treatment Effect
Insulin Sensitizers					
Diamanti-Kandarakis [133]	PCOS	Metformin, 1700 mg/d, Orally	6 mo	FMD and plasma ET-1	FMD ↑ and plasma ET-1 ↓
Mather KJ [134]	Type 2 Diabetes	Metformin, 1000 mg/d, Orally	12 wk	ACh-induced vasodilation	↑
de Aguiar LG [135]	Metabolic Syndrome	Metformin, 1000 mg/d, Orally	3 mo	ACh-induced vasodilation	↑
Martens FM [136]	Type 2 Diabetes	Pioglitazone, 30 mg/d, Orally	4 wk	FMD	↑
Mittermayer [137]	Free fatty Acid-induced Endothelial Dysfunction	Rosiglitazone, 8 mg/d, Orally	3 wk	ACh-induced vasodilation	↑
Wang [138]	Metabolic Syndrome	Rosiglitazone, 4 mg/d, Orally	8 wk	FMD	↑
Esposito [139]	Metabolic Syndrome	Rosiglitazone, 4 mg/d, Orally	12 mo	FMD, Adiponectin, CRP, IL-6, IL-18	FMD ↑, Adiponectin ↑, CRP ↓, IL-6 ↓, IL-18 ↓
Hypolipidemic Agents					
Ceriello [140]	High-Fat and Glucose-induced endothelial dysfunction	Atorvastatin, 40 mg/d, Orally	4 d	FMD, ICAM-1, nitrotyrosine, IL-6	FMD ↑, ICAM-1 ↓, nitrotyrosine ↓, IL-6 ↓
Economides PA [141]	Type 2 Diabetes	Atorvastatin, 40 mg/d, Orally	12 wk	FMD	↑
Shimabukuro [142]	Obesity	Fluvastatin, 20 to 40 mg/d, Orally	12 wk	Peak FBF during reactive hyperemia	↑
Evans [143]	Type 2 Diabetes	Ciprofibrate, 100 mg/d, Orally	3 mo	FMD	↑
Avogaro [144]	Type 2 Diabetes	Gemfibrozil, 1200 mg/d, Orally	12 wk	FMD	↑
Koh [145]	Metabolic Syndrome	Fenofibrate, 200 mg/d, Orally	2 mo	FMD	↑
Renin-Angiotensin System Blockers					
Hermann [54]	Type 2 Diabetes	Quinapril, 20 mg/d, Orally	2 mo	Insulin-induced Δ forearm blood flow	↑
Koh [146]	Metabolic Syndrome	Losartan, 100 mg/d, Orally	2 mo	FMD	↑
O'Driscoll [147]	Type 2 Diabetes	Enalapril, 20 mg/d, Orally	4 wk	ACh-induced vasodilation	↑
Combination Therapies					
Koh [148]	Metabolic Syndrome	Atorvastatin, 10 mg/d + Fenofibrate, 200 mg/d	2 mo	FMD	↑
Koh [149]	Type 2 Diabetes	Simvastatin 20 mg/d + Ramipril 10 mg/d	2 mo	FMD	↑
Ceriello [140]	High-Fat and Glucose-induced endothelial dysfunction	Atorvastatin, 40 mg/d + Irbesartan, 300 mg/d	4 d	FMD, ICAM-1, nitrotyrosine, IL-6	FMD ↑, ICAM-1 ↓, nitrotyrosine ↓, IL-6 ↓
Sola [106]	Metabolic Syndrome	Irbesartan, 150 mg/d + Lipoic acid, 300 mg/d	4 wk	FMD	↑

FMD, flow-mediated dilation; CRP, c-reactive protein; IL-6, interleukin-6; IL-18, interleukin-18; BH4, tetrahydrobiopterin; GDM, gestational diabetes mellitus; CAD, coronary heart disease; PCOS, polycystic ovarian syndrome; ACh, acetylcholine; ET-1, endothelin-1; ICAM-1, intercellular adhesion molecule-1; FBF, forearm blood flow