Review

Insulin Resistance and Skeletal Muscle Vasculature: Significance, Assessment and Therapeutic Modulators

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Insulin resistance · Skeletal muscle vasculature · Type 2 diabetes mellitus

Abstract

Overnutrition and sedentarism are closely related to the alarming incidence of obesity and type 2 diabetes mellitus (DM2) in the Western world. Resistance to the actions of insulin is a common occurrence in conditions such as obesity, hypertension and DM2. In the skeletal muscle vasculature, insulin promotes vasodilation and its own transport across the vascular wall to reach its target tissue. Furthermore, insulin resistance (IR) in the skeletal muscle vasculature results in impaired skeletal muscle glucose uptake and altered whole-body glucose homeostasis. The development of different invasive and noninvasive techniques has allowed the characterization of the actions of insulin and other vasoactive hormones in the skeletal muscle vasculature in both health and disease. Current treatment strategies for DM2 do not necessarily address the impaired effect of insulin in the vasculature. Understanding the effects of insulin and other metabolically active hormones in the vasculature should facilitate the development of new therapeutic strategies targeted at the modulation of IR and improvement of whole-body glucose tolerance.

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Introduction

The impact of type 2 diabetes mellitus (DM2) continues to grow, affecting 26 million individuals in the United States and 347 million worldwide [1]. Resistance to the metabolic actions of insulin is an important contributor to the pathogenesis of DM2 [2], and, despite the availability of different pharmacological strategies for the treatment of DM2, the options

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available to diminish insulin resistance (IR) in skeletal muscle are limited [2]. Furthermore, IR in the skeletal muscle vasculature is increasingly recognized as having a causative role in the impairment of insulin-induced glucose uptake in skeletal muscle [2]. Understanding the role of insulin in the vasculature in health and disease should help to elucidate new therapeutic strategies aimed at improving peripheral insulin sensitivity. In this review, we will discuss the available evidence of normal and pathological actions of insulin in the skeletal muscle vasculature, the methods used to assess its action, as well as its impact on glucose homeostasis.

Molecular Effects of Insulin in the Vasculature

Under normal conditions, the net effect of insulin signaling in the vasculature is vasodilation via increased nitric oxide (NO) production and increased bioavailable NO [3]. However, in conditions of IR such as DM2, insulin promotes vasoconstriction and vascular remodeling [4, 5]. Insulin action on the vasculature is determined by signaling pathways activated upon stimulation of the insulin receptor [4]. The insulin receptor has intrinsic tyrosine kinase activity that triggers its own phosphorylation and subsequent tyrosine phosphorylation of the insulin receptor substrate (IRS) proteins [3–5]. Moreover, phosphorylation of the IRS proteins creates Src homology 2 domain-binding motifs that serve as docking points for proteins containing Src homology 2 such as phosphatidylinositol 3-kinase (PI3K) [4]. PI3K activation by IRS-1 results in phosphatidylinositol 3,4,5-trisphosphate production, activation of 3-phosphoinositide-dependent protein kinase-1, and of different serine-threonine kinases such as protein kinase B (Akt) [3, 4]. Akt activation results in serine phosphorylation of the endothelial NO synthase (eNOS) [6, 7]. NO produced by eNOS decreases vascular tone, modulates vascular smooth muscle cell proliferation, and diminishes adhesion of inflammatory cells and platelet aggregation to the endothelium [3, 4]. NO production is controlled by other intracellular signaling pathways besides insulin [7–10]. Heat-shock protein 90 modulates eNOS activity, and an inadequate supply of tetrahydrobiopterin (eNOS cofactor) limits the enzyme activity resulting in eNOS uncoupling [11]. Under insulin-resistant conditions, stimulation of the vasculature can result in vasoconstriction [3, 4]. The mitogen-activated protein kinase (MAPK) cascade, which has vasoconstrictor and growth promoting effects, serves as one of the effectors that have deleterious effects on insulin in the vasculature [3, 4]. MAPK activation leads to increased endothelial production of endothelin-1 (ET-1) and activation of signaling through the vascular tissue renin-angiotensin-aldosterone system (RAAS) [3, 4]. ET-1 acts via the ET-1 receptor A to cause vasoconstriction, increased oxidative stress as well as cell growth and mitogenesis of vascular smooth muscle cells [12]. Supraphysiological levels of insulin have been shown to increase the production of ET-1 in cultured endothelial cells and in a rodent model [13].

Resistance to the metabolic and vasodilatory actions of insulin is a common denominator in clinical conditions such as obesity, hypertension, and DM2 [14–16]. IR has been linked to overnutrition and obesity via lipotoxicity, sustained low-grade inflammation, oxidative stress, and endoplasmic reticulum stress [15, 16]. Systemic and vascular IR have not only been related to overnutrition, but also specifically to an increased intake of high-fructose corn syrup [17, 18]. Furthermore, increased fructose content in the diet has been shown to impair insulin response in the vasculature [17, 18]. One of the critical insulin-signaling nodes that is impaired in IR is the tyrosine phosphorylation of IRS-1 protein [16]. Serine kinases such as C-Jun kinase, protein kinase C, and ribosomal p70 S6 kinase serine phosphorylated IRS-1 [13, 14]. IRS-1 serine phosphorylation impairs IRS-1 tyrosine phosphorylation, its activation of PI3K, and promotes proteasome-dependent degradation [14–16].

Skeletal Muscle Vasculature

The skeletal muscle is one of the main targets of insulin and its action is central for the maintenance of glucose homeostasis [19]. The insulin-mediated uptake of glucose in skeletal muscle depends on the effect of insulin on the skeletal muscle vasculature as well as on insulin action in the skeletal muscle fiber [19, 20]. The skeletal muscle vasculature behaves in an extremely flexible manner [21, 22]. Under nonexercising conditions, skeletal muscle receives 0.03–0.04 ml/min of blood flow per gram of tissue [21], but upon initiation of exercise, blood flow increases up to 100-fold [22]. Capillary recruitment is determined initially by vasomotor changes of the terminal arterioles [20]. Later, the increased metabolic demand posed by exercise results in a reduced tissue oxygen tension followed by an ascending vasodilatory response [22, 23]. This vasodilation depends on an intact endothelium and on the restriction of blood flow to the noncontracting areas of the muscle via alpha-adrenergic response [23]. Once the terminal arterioles are maximally dilated, capillary perfusion is determined by tissue metabolic demands and the anatomic arrangement of the vessels [20]. Adequate skeletal muscle capillary recruitment is crucial for the normal metabolic effects of insulin [24], and clinical conditions characterized by IR, such as obesity and DM2, manifest with impaired capillary recruitment [25].

Methodologies Used for Assessing the Effect of Insulin and Other Vasoactive Substances on the Skeletal Muscle Vasculature

Before examining the available evidence for the action of insulin on the skeletal muscle vasculature, we will review the main experimental methods that have been used to characterize the effect of insulin and other vasoactive hormones on the skeletal muscle vasculature of both animals and humans (table 1).

1-Methylxanthine Metabolism in the Determination of Capillary Recruitment

This technique relies on the idea that xanthine oxidase, the enzyme that metabolizes 1-methylxanthine (1-MX) to 1-methyltaurate, exists primarily in the capillary endothelium [26] and that the degree of metabolism of 1-MX is proportional to capillary recruitment [26]. Capillary recruitment is expressed as the arteriovenous difference across the skeletal muscle bed in 1-MX concentration multiplied by the total limb blood flow [27]. This technique has several limitations: as it requires measuring whole-limb blood flow, it cannot be used in humans and does not take into account changes in microvascular recruitment seen before changes in whole-limb blood flow [27].

Microdialysis

This method uses the principle of marker diffusion across a semipermeable membrane placed across the skeletal muscle area of interest [28, 29]. A probe is inserted in skeletal muscle and then perfused with a dialysate containing the marker [28]. The recovery across the marker of the substance employed is used to assess blood flow and distribution [29]. Limitations include an invasive nature and lack of spatial resolution [29].

Plethysmography

Veinocclusion plethysmography has been long established as a method for the assessment of whole-limb blood flow [30]. In this method, a cuff is inflated around the upper arm or thigh to obliterate the venous outflow without impacting the arterial inflow [30]. The volume of the limb increases over time, and that increase is parallel to the increase of the arterial blood flow as the venous system is collapsed (limb positioned at heart level) [30]. Water-filled plethysmographs, circumferential mercury-in-silastic strain gauges, and the Dohn plethysmograph are

Table 1. Methods for the assessment of skeletal muscle/limb vasculature

Methods	Advantages	Limitations	Invasive	Human/ animal use
1-MX metabolism	Does not require muscle or tissue biopsies; correlates with nutritive flow	Requires assessment of whole-limb blood flow	Yes	–/+
Microdialysis	Not widely available; can be used in a specific area of the limb/muscle	Lack of spatial resolution	Yes	+/+
Dye dilution and arteriovenous sampling	Extensively used	Does not give specific information about a region of interest in the muscle	Yes	+/+
CEU	Has been used widely to examine the effect of different vasoactive substances	Requires immobilization	No	+/+
Positron-emission tomography	Accesses the metabolic activity of the tissue examined	Limited availability; noise might obscure findings	No	+/+
Plethysmography	Provides volume flow; easy use in human studies	Does not give specific information about a region of interest in the muscle	No	+/+
Intravital microscopy	Allows direct visualization and pharmacological manipulation of microvasculature	Requires general anesthesia	No	–/+

some of the methods that have been used to measure changes in limb volume and whole-limb blood flow. This technique is minimally invasive and has been used widely in humans [29].

Dye Dilution and Arteriovenous Sampling

These methods rely on an injection of dye in the arterial system (e.g. brachial artery) followed by sampling in the vein system (e.g. antecubital vein). Spectrophotometric methods are used to evaluate limb blood flow based on the recovery of the tracer in the venous system [31]. Arteriovenous sampling using radiolabeled substrate and insulin has been extensively used to evaluate the metabolic events in skeletal muscle [29]. Other techniques available to measure muscle perfusion and metabolism include nuclear magnetic resonance in conjunction with arterial spin labeling. It correlates well with plethysmography and has been successfully used in both humans and rodents [32]. Nevertheless, this technique requires expensive equipment and specialized software for data analysis [29].

Intravital Microscopy

As an in vivo technique, intravital microscopy allows the direct visualization and manipulation of the skeletal muscle microvasculature [33]. It has been used to characterize the vasculature of cremaster and gluteus maximus muscle [33] and has been proven useful to delineate cellular pathways and intracellular signaling events in the skeletal muscle microvasculature [34–37]. Recently, investigators have used this technique in the gluteus maximus muscle of insulin-resistant mice showing arteriolar dysfunction in response to muscle contraction [37].

Positron-Emission Tomography

Positron-emission tomography and [^{15}O]H $_2$ O or [^{15}O]CO $_2$ are noninvasive methods used as a tracer to evaluate blood flow in a region of interest [37]. This technique has also been used in humans to determine blood volume in the heart and brain. In skeletal muscle, it is limited by the amount of noise produced by the arterial blood volume (in comparison with the actual region of interest) [25, 29]. Indeed, the skeletal uptake of ^{18}F -deoxyglucose can be evaluated in parallel with various techniques measuring blood flow to give a functional correlation of insulin metabolic signaling to changes seen in the blood flow measured [26, 38, 39].

Contrast-Enhanced Ultrasound

A noninvasive technique, contrast-enhanced ultrasound (CEU) is adapted from heart-muscle imaging [26] and uses lipid-coated microbubbles filled with perfluorocarbon that behave similarly to red blood cells and remain in the intravascular compartment [26, 38]. The microbubbles are destroyed using harmonic ultrasound imaging, and their rate of reappearance in the microcirculation parallels the microvascular blood volume and the rate of flow across the muscle area being studied [20]. This technique requires anesthesia when used in rodents and immobilization when performed in humans, which may interfere with the normal physiological function of the microvasculature [26, 38]. This method has been widely used by several investigators to study the response of the skeletal muscle vasculature to different stimuli [20, 26, 38], and most of the evidence that will be discussed in the present review regarding insulin action in skeletal muscle is based on work utilizing CEU.

Effects of Insulin on the Skeletal Muscle Vasculature

The impact of insulin action in the skeletal muscle vasculature has been examined in both resistance vessels and in the microvasculature.

Whole-Limb Blood Flow

The effects of insulin in the vasculature have been known for several decades [40], but the significance of insulin-mediated whole-limb vasodilation in relation to skeletal muscle glucose uptake remains controversial [41]. Initial studies in lean nondiabetic subjects, using the thermodilution method and the hyperinsulinemic euglycemic clamp [42–46], demonstrated an increase in whole-limb blood flow in a dose-dependent manner upon stimulation with insulin in correlation with increased skeletal muscle glucose uptake [47]. The effect of insulin on leg blood flow is mostly dependent on increased NO production/bioavailability and is blunted by eNOS inhibition [48]. Indeed, eNOS inhibition also results in a reduced insulin-mediated skeletal muscle glucose uptake [46]. Nonetheless, some investigators have challenged the significance of those findings and have reported that in lean individuals, an augmentation of limb blood flow through bradykinin infusion did not translate into increased insulin-mediated skeletal muscle glucose uptake [41]. In addition, insulin-induced increases in blood flow to certain areas of skeletal muscle was not concordant with the muscle areas where insulin was stimulating the glucose uptake [49]. The divergent evidence can partially be explained by the use of different techniques and highlights the need for further understanding the effect of insulin on the vasculature.

Microvasculature

Insulin is believed to elicit an exercise-like effect on the skeletal muscle vasculature [38]. During exercise, the recruitment of underperfused capillaries via vasodilation increases the endothelial surface available for nutrient exchange in concert with an increased metabolic

demand [38, 50]. Capillary recruitment promotes the delivery of insulin to its target tissues [38]. In healthy individuals, systemic and local infusion of insulin results in an increased forearm blood volume without significant changes in whole-limb blood flow [51]. The insulin-mediated increases in microvascular blood flow, which is believed to parallel the microvascular recruitment, antecede a rise in total limb blood flow [52] and are dependent of NO production [24]. Furthermore, the concentrations of insulin required to cause an increased microvascular blood flow are lower than those required to increase whole-limb blood flow [26]. The role of bioavailable NO in skeletal muscle glucose uptake has been further clarified [53]. Recently, an investigative group has infused an eNOS inhibitor in the hind leg of anesthetized rats fed a regular diet [53]. No changes were reported in blood pressure or heart rate, but eNOS inhibition significantly blunted the insulin-stimulated increase of ultrasound-assessed femoral blood flow [53]. In parallel, insulin-stimulated microvascular recruitment and glucose uptake were completely abolished [53]. Interestingly, noninsulin-stimulated microvascular recruitment, evaluated via 1-MX, and basal glucose uptake were not impacted by the local infusion of L-NG-nitroarginine methyl ester (L-NAME) [53]. This study highlights the importance of local capillary NO bioavailability in the regulation of insulin-stimulated skeletal microvascular recruitment and glucose uptake [53].

Other metabolically active hormones such as adiponectin and glucagon-like peptide-1 (GLP-1) regulate skeletal muscle vascular recruitment and glucose uptake [54, 55]. Globular adiponectin has been shown via CEU and hyperinsulinemic-euglycemic insulin clamp to augment skeletal muscle microvascular recruitment and glucose uptake, which are both effects mediated by the activation of eNOS and independent of changes in endothelial insulin uptake [54].

GLP-1 is an incretin produced in the gut in response to nutrients and acts as insulin secretagogue [56]. Two types of agents are used clinically to enhance or mimic incretin effects, the GLP-1 analogs and the dipeptidyl peptidase-4 inhibitors (dipeptidyl peptidase-4 metabolizes GLP-1) [56]. The vascular effects of incretins have been explored in different models and vascular beds [55, 56]. Recently, the effects of GLP-1 and GLP-1 receptor activation on the skeletal muscle vasculature and endothelial cells have been described [57]. Chai et al. [57] showed that GLP-1 treatment on cultured aortic endothelial cells results in increased Akt and eNOS phosphorylation, as well as cyclic adenosine monophosphate-dependent protein kinase activity. In adult Sprague-Dawley rats, in vivo GLP-1 infusion for 2 h resulted in marked increments of skeletal muscle blood flow and glucose extraction. These effects were abolished by the coadministration of L-NAME [57].

Besides the vasoactive substances mentioned above, capillary density is a determinant of glucose homeostasis [58]. Capillary density in skeletal muscle is altered in IR conditions [58, 59], and progressive deterioration of glucose homeostasis correlates with a decreased capillary density and decreased bioavailable NO [58, 59]. In rodents, knockout of the vascular endothelial growth factor results in a reduced capillary density in skeletal muscle and a significantly decreased insulin-stimulated glucose uptake [60].

Transendothelial Insulin Transport

The transport of insulin across the skeletal muscle vasculature in both health and disease has gained increasing attention in recent years [26]. Insulin is transported across the endothelium in a receptor-dependent manner [61]. In animal models, the transcapillary transport of insulin appears to be the rate-limiting step of insulin action in skeletal muscle [62, 63]. In obese and insulin-resistant humans, the appearance of insulin in the interstitial fluid secondary to transcapillary transport is delayed when compared with lean controls [64]. The molecular basis of the transendothelial transport of insulin relies on a functional caveolar transport and bioavailable NO [65]. Caveolae are required for the transport of multiple hormones and

proteins across the endothelium and serve as a site where insulin receptors are located [65]. Caveolin-1 is a plasma membrane protein required for caveola formation [65]. One investigative group has proposed that the delivery of insulin to skeletal muscle depends on binding insulin to its receptor in the caveolae, which results in the internalization of the vesicles and transendothelial transport of the hormone [20]. In bovine aortic endothelial cells, knockdown of caveolin-1 results in the decreased endothelial uptake of radiolabeled insulin and the decreased IR expression and insulin-dependent Akt phosphorylation [66]. Similarly, endothelial cells from caveolin-1 null mice do not uptake insulin and have an impaired insulin-signaling cascade [66]. Interestingly, insulin stimulates its own endothelial transport via PI3K and MAPK signaling, and, in an inflammatory milieu – like the one seen in insulin-resistant conditions – insulin signaling and transport are decreased [67]. More recently, Wang et al. [68] have examined the effect of NO on the endothelial transport of insulin and endothelial insulin signaling. In cultured endothelial cells, NO regulates the endothelial uptake of insulin and an NO donor rescues the inhibition of tyrosine kinases in the insulin-signaling cascade. Furthermore, in insulin-resistant rodents, NO reverses endothelial impaired insulin signaling and insulin transport [68].

RAAS – Dual Effects in the Skeletal Muscle Vasculature

As mentioned previously, multiple other hormones and peptides are known to regulate the skeletal muscle vasculature [4, 5]. The RAAS has a dual role, as it has both beneficial and deleterious effects in the skeletal muscle vasculature [4, 5]. The vascular effects of angiotensin II (Ang II) are well characterized [69]. Ang II signals through G-coupled membrane-bound receptors, type 1 and type 2 receptors (AT1R and AT2R, respectively). In the vasculature, AT1R activation increases oxidative stress and promotes vasoconstriction and remodeling [69]. In contrast, AT2R activation counteracts the deleterious effects of AT1R signaling in part via vasodilation induced by the activation of the bradykinin/NO system [70–72].

In the skeletal muscle vasculature, Ang II also promotes skeletal muscle vasodilation via AT2R and increased NO [73, 74]. Ang II infusion in rodents results in a 2-fold increase of microvascular blood volume in skeletal muscle. In addition, AT1R blockade causes a greater increment in blood flow and glucose extraction [73]. The increased blood flow and glucose extraction are NO-dependent as demonstrated by a blunting the effect of an eNOS inhibitor [73]. In parallel, AT2R blockade decreases microvascular blood flow and glucose extraction by 80% [73]. This same group noted that during continuous insulin infusion, AT1R blockade did not have an additive on muscle blood flow and glucose uptake effect; however, when AT2R was antagonized, it resulted in whole-body IR and attenuation of microvasculature recruitment [74]. These changes correlated with a decrease in plasma NO and skeletal muscle eNOS activation [74]. These studies present convincing evidence of the important role of A2TR activation in the maintenance of glucose homeostasis in skeletal muscle.

In IR conditions, such as obesity and DM2, there is an inappropriate activation of the RAAS with enhanced AT1R signaling [75]. Furthermore, in a rodent model of diabetes and IR, increased reactive oxygen species resulted in reduced AT2R-mediated dilatation in resistance arteries [70]. One-year treatment with an AT1R blocker in hypertensive diabetic patients resulted in increased AT2R expression and enhanced vasodilatory response in resistance arteries [71].

IR in the Vasculature

Obesity and DM2 are associated with impaired endothelial-dependent vasodilation [4, 5]. After a mixed meal, lean subjects exhibit increased brachial blood flow and forearm microvascular recruitment, and obese subjects have a blunted response in the postprandial state despite hyperinsulinemic conditions [45, 46, 76]. Endothelial dysfunction manifests as decreased NO bioavailability, abnormal vasoreactivity, increased oxidative stress, and increased expression of inflammatory, immune and prothrombotic mediators [75]. The impaired, endothelial-dependent skeletal muscle vasodilation in insulin-resistant models correlates with arteriolar remodeling and increased stiffness of the vessel wall [77]. Resistance to the vascular effects of insulin has been shown to selectively involve the impaired activation of the PI3K/Akt/NO pathway with intact signaling via the MAPK promitotic and procontractive pathway [76–79].

Therapeutic Strategies

Nonpharmacological Interventions: Physical Activity and Weight Loss

In subjects with DM2, weight loss and regular physical activity are known to positively modify metabolic abnormalities that are correlated with vascular disease [80]. In the Look AHEAD trial, 1 year of intensive life intervention resulted in a significant improvement in IR, fasting glucose, free fatty acids, and hepatic fat [80].

Increased physical activity improves insulin sensitivity in the vasculature [81]. In the spontaneously hypertensive insulin-resistant rat model, vascular insulin sensitivity, assessed as insulin-induced vasodilation of the mesenteric vasculature, and blood pressure improve significantly after 10 weeks of exercise [81]. Exercise also decreased the expression and activity of G-protein-coupled kinase-2 known to inhibit insulin-signaling vascular endothelium [81, 82]. Similarly, in the Otsuka Long Evans Tokushima Fatty (OLETF) rat, a model of obesity and IR, voluntary running results in improved insulin responsiveness in the skeletal muscle microvasculature [83].

Weight loss secondary to caloric restriction improves endothelial function in conduit and resistance arteries of overweight and obese adults with increased NO bioavailability [84]. In a recent trial examining 208 participants subjected to weight loss therapy, individuals with higher insulin levels at the beginning and a >10% weight loss showed a significant improvement in brachial artery flow-mediated vasodilation and microvascular reactive hyperemia [85]. The effects of lifestyle modifications are also seen in obese subjects with DM2 [86]. In this population, 6 months of caloric restriction (500-calorie negative balance) and 150 min of weekly exercise resulted in increased brachial artery flow-mediated dilation and improved systemic insulin sensitivity [86]. In addition, weight loss also decreases circulating levels on ET-1 in obese males [87].

As mentioned before, capillary density is also relevant for insulin sensitivity in skeletal muscle [88]. A 6-month intervention in overweight insulin-resistant subjects with exercise and caloric restrictions resulted in improved skeletal muscle capillary density and decreased IR [88].

Even though improved insulin sensitivity driven by lifestyle modifications has not been clearly translated into decreased cardiovascular mortality [89], other relevant clinical outcomes are clearly improved with these lifestyle interventions in DM2 subjects [89].

5'-Adenosine Monophosphate: Activated Kinase Activation/Metformin

Metformin, the most common clinically used insulin sensitizer, improves endothelial function in different vessels [90–93]. Data has shown that in rodents, the improvement in

endothelial-dependent vasodilation is independent of NO production [90], while in vitro evidence in cultured aortic endothelial cells points toward increased activation of eNOS in a PI3K-dependent manner [91]. The vascular effects of metformin require 5'-adenosine mono-phosphate-activated kinase, as this knockout mouse does not obtain these beneficial actions [92]. In women with polycystic ovary syndrome, a condition characterized by IR, metformin therapy has been shown to improve flow-mediated dilation and decreases levels of ET-1 [93].

RAAS Blockade

The RAAS system exerts beneficial effects on the skeletal muscle vasculature via AT2R; however, in insulin-resistant conditions, RAAS promotes vascular disease [75]. Therefore, RAAS blockade has been explored as an intervention to decrease vascular disease in patients with IR [75]. In subjects with metabolic syndrome, 4 weeks of treatment with irbesartan, an AT1R blocker, was associated with improved endothelial function assessed by flow-mediated vasodilation of the brachial artery and decreased systemic markers of oxidative stress and inflammation [94]. The vascular effects were potentiated by the use of an α -lipoic acid (an antioxidant) [94]. A similar study with quinapril, an angiotensin-converting enzyme, and an α -lipoic acid showed an improvement in endothelial function and albuminuria with 8 weeks of treatment in hypertensive patients [94].

Summary

Under physiologic conditions, insulin promotes vasodilation, capillary recruitment and its own transendothelial transport in the skeletal muscle vasculature. Insulin effect in the vasculature relies on an increased production of NO via activation of the PI3K/Akt/eNOS pathway. Insulin augments its own delivery to skeletal muscle and promotes nutrient delivery to skeletal muscle as well as glucose uptake. IR, which is present in obesity and DM2, manifests with impaired vasodilation, decreased capillary recruitment and diminished insulin-stimulated glucose uptake. Other hormones including Ang II, adiponectin and GLP-1 enhance insulin action or independently promote skeletal muscle capillary recruitment and glucose uptake. Among available therapeutic strategies for IR, exercise, weight loss, RAAS blockade and incretin-based therapy have potential vascular beneficial effects. Decreased intake of a diet high in fructose will likely result in improved insulin action in the skeletal muscle vasculature, but clinical trials are needed to clarify the specific role of this intervention on cardiovascular disease risk.

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References

- 1 Jeon JY, Ko SH, Kwon HS, Kim NH, Kim JH, Kim CS, Song KH, Won JC, Lim S, Choi SH, Jang M, Kim Y, Oh K, Kim DJ, Cha BY: Prevalence of diabetes and prediabetes according to fasting plasma glucose and HbA1c. *Diabetes Metab J* 2013;37:349–357.
- 2 DeFronzo RA, Ferrannini E: Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991;14:173–194.
- 3 Muniyappa R, Montagnani M, Koh KK, Quon MJ: Cardiovascular actions of insulin. *Endocr Rev* 2007;28:463–491.

- 4 Muniyappa R, Yavuz S: Metabolic actions of angiotensin II and insulin: a microvascular endothelial balancing act. *Mol Cell Endocrinol* 2013;378:59–69.
- 5 Manrique C, Lastra G, Sowers JR: New insights into insulin action and resistance in the vasculature. *Ann NY Acad Sci* 2014;1311:138–150.
- 6 Montagnani M, Ravichandran LV, Chen H, Esposito DL, Quon MJ: Insulin receptor substrate-1 and phosphoinositide-dependent kinase-1 are required for insulin-stimulated production of nitric oxide in endothelial cells. *Mol Endocrinol* 2002;16:1931–1942.
- 7 Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, Johansen M, Kucik DF, Quon MJ, Draznin B: Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. *J Biol Chem* 2002;277:1794–1799.
- 8 Michel JB, Feron O, Sacks D, Michel T: Reciprocal regulation of endothelial nitric-oxide synthase by Ca²⁺-calmodulin and caveolin. *J Biol Chem* 1997;272:15583–15586.
- 9 Lin MI, Fulton D, Babbitt R, Fleming I, Busse R, Pritchard KA Jr, Sessa WC: Phosphorylation of threonine 497 in endothelial nitric-oxide synthase coordinates the coupling of L-arginine metabolism to efficient nitric oxide production. *J Biol Chem* 2003;278:44719–44726.
- 10 Pritchard KA Jr, Ackerman AW, Gross ER, Stepp DW, Shi Y, Fontana JT, Baker JE, Sessa WC: Heat shock protein 90 mediates the balance of nitric oxide and superoxide anion from endothelial nitric-oxide synthase. *J Biol Chem* 2001;276:17621–17624.
- 11 Crabtree M, Channon K: Synthesis and recycling of tetrahydrobiopterin in endothelial function and vascular disease. *Nitric Oxide* 2011;25:81–88.
- 12 Khimji A, Rockey D: Endothelin – biology and disease. *Cell Signal* 2010;22:1615–1625.
- 13 Ferri C, Pittoni V, Piccoli A, Laurenti O, Cassone MR, Bellini C, Properzi G, Valesini G, De Mattia G, Santucci A: Insulin stimulates endothelin-1 secretion from human endothelial cells and modulates its circulating levels in vivo. *J Clin Endocrinol Metab* 1995;80:829–835.
- 14 Bender SB, McGraw AP, Jaffe IZ, Sowers JR: Mineralocorticoid receptor-mediated vascular insulin resistance: an early contributor to diabetes-related vascular disease? *Diabetes* 2013;62:313–319.
- 15 DeFronzo R: Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia* 2010;53:1270–1287.
- 16 Rask-Madsen C, Kahn CR: Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2012;32:2052–2059.
- 17 Sowers JR, Whaley-Connell A, Hayden MR: The role of overweight and obesity in the cardiorenal syndrome. *Cardiorenal Med* 2011;1:5–12.
- 18 Jia G, Aroor AR, Whaley-Connell AT, Sowers JR: Fructose and uric acid: is there a role in endothelial function? *Curr Hypertens Rep* 2014;16:434.
- 19 Thiebaud D, Jacot E, DeFronzo RA, Maeder E, Jequier E, Felber J-P: The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes* 1982;31:957–963.
- 20 Barrett EJ, Wang H, Upchurch CT, Liu Z: Insulin regulates its own delivery to skeletal muscle by feed-forward actions on the vasculature. *Am J Physiol Endocrinol Metab* 2011;301:E252–E263.
- 21 Saltin B, Radegran G, Koskolou M, Roach R: Skeletal muscle blood flow in humans and its regulation during exercise. *Acta Physiol Scand* 1998;162:421–436.
- 22 Klitzman B, Damon D, Gorczynski R, Duling B: Augmented tissue oxygen supply during striated muscle contraction in the hamster. Relative contributions of capillary recruitment, functional dilation, and reduced tissue PO₂. *Circ Res* 1982;51:711–721.
- 23 Segal SS, Jacobs TL: Role for endothelial cell conduction in ascending vasodilatation and exercise hyperaemia in hamster skeletal muscle. *J Physiol* 2001;536:937–946.
- 24 Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S: Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am J Physiol Endocrinol Metab* 2003;285:E123–E129.
- 25 Womack L, Peters D, Barrett E, Kaul S, Price W, Lindner J: Abnormal skeletal muscle capillary recruitment during exercise in patients with type 2 diabetes mellitus and microvascular complications. *J Am Coll Cardiol* 2009;53:2175–2183.
- 26 Clark MG: Impaired microvascular perfusion: a consequence of vascular dysfunction and a potential cause of insulin resistance in muscle. *Am J Physiol Endocrinol Metab* 2008;295:E732–E750.
- 27 Bradley EA, Clark MG, Rattigan S: Acute effects of wortmannin on insulin's hemodynamic and metabolic actions in vivo. *Am J Physiol Endocrinol Metab* 2007;292:E779–E787.
- 28 MacLean DA, Ettinger SM, Sinoway LI, Lanoue KF: Determination of muscle-specific glucose flux using radioactive stereoisomers and microdialysis. *Am J Physiol Endocrinol Metab* 2001;280:E187–E192.
- 29 Barrett EJ, Rattigan S: Muscle perfusion: its measurement and role in metabolic regulation. *Diabetes* 2012;61:2661–2668.
- 30 Joyner MJ, Dietz NM, Shepherd JT: From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *J Appl Physiol* 2001;91:2431–2441.
- 31 Andres R, Zierler KL, Anderson HM, Stainsby WN, Cader G, Gharryib AS, Lilienthal JL Jr: Measurement of blood flow and volume in the forearm of man; with notes on the theory of indicator-dilution and on production of turbulence, hemolysis, and vasodilatation by intra-vascular injection. *J Clin Invest* 1954;33:482–504.
- 32 Carlier PG, Bertoldi D, Baligand C, Wary C, Fromes Y: Muscle blood flow and oxygenation measured by NMR imaging and spectroscopy. *NMR Biomed* 2006;19:954–967.

- 33 Bagher P, Segal SS: The mouse cremaster muscle preparation for intravital imaging of the microcirculation. *J Vis Exp* 2011;pii:2874.
- 34 Wölfe SE, Schmidt VJ, Hoepfl B, Gebert A, Alcoléa S, Gros D, de Wit C: Connexin45 cannot replace the function of connexin40 in conducting endothelium-dependent dilations along arterioles. *Circ Res* 2007;101:1292–1299.
- 35 Bagher P, Duan D, Segal SS: Evidence for impaired neurovascular transmission in a murine model of Duchenne muscular dystrophy. *J Appl Physiol* (1985) 2011;110:601–609.
- 36 Tallini YN, Brekke JF, Shui B, Doran R, Hwang SM, Nakai J, Salama G, Segal SS, Kotlikoff MI: Propagated endothelial Ca²⁺ waves and arteriolar dilation in vivo: measurements in Cx40BAC GCaMP2 transgenic mice. *Circ Res* 2007;101:1300–1309.
- 37 Novielli NM, Jackson DN: Contraction-evoked vasodilation and functional hyperaemia are compromised in branching skeletal muscle arterioles of young pre-diabetic mice. *Acta Physiol (Oxf)* 2014;211:371–384.
- 38 Liu Z, Ko SH, Chai W, Cao W: Regulation of muscle microcirculation in health and diabetes. *Diabetes Metab J* 2012;36:83–89.
- 39 Ruotsalainen U, Raitakari M, Nuutila P, Oikonen V, Sipilä H, Teras M, Knuuti MJ, Bloomfield PM, Iida H: Quantitative blood flow measurement of skeletal muscle using oxygen-15- water and PET. *J Nucl Med* 1997;38:314–319.
- 40 Thiebaud D, Jacot E, Defronzo RA, Maeder E, Jequier E, Felber J-P: The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes* 1982;31:957–963.
- 41 Nuutila P, Raitakari M, Laine H, Kirvelä O, Takala T, Utriainen T, Mäkimattila S, Pitkänen OP, Ruotsalainen U, Iida H, Knuuti J, Yki-Järvinen H: Role of blood flow in regulating insulin-stimulated glucose uptake in humans. Studies using bradykinin, [150]water, and [18F]fluoro-deoxy-glucose and positron emission tomography. *J Clin Invest* 1996;97:1741–1747.
- 42 Clerk LH, Rattigan S, Clark MG: Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake in vivo. *Diabetes* 2002;51:1138–1145.
- 43 Baron AD: Hemodynamic actions of insulin. *Am J Physiol Endocrinol Metab* 1994;267:E187–E202.
- 44 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;73:637–643.
- 45 Baron AD, Laakso M, Brechtel G, Hoit B, Watt C, Edelman SV: Reduced postprandial skeletal muscle blood flow contributes to glucose intolerance in human obesity. *J Clin Endocrinol Metab* 1990;70:1525–1533.
- 46 Baron A, Steinberg H, Chaker H, Leaming R, Johnson A, Brechtel G: Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J Clin Invest* 1995;96:786–792.
- 47 Laakso M, Edelman S, Brechtel G, Baron A: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J Clin Invest* 1990;85:1844–1852.
- 48 Steinberg H, Brechtel G, Johnson A, Fineberg N, Baron A: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J Clin Invest* 1994;94:1172–1179.
- 49 Raitakari M, Nuutila P, Ruotsalainen U, Laine H, Teräs M, Iida H, Mäkimattila S, Utriainen T, Oikonen V, Sipilä H, Haaparanta M, Solin O, Wegelius U, Knuuti J, Yki-Järvinen H: Evidence for dissociation of insulin stimulation of blood flow and glucose uptake in human skeletal muscle: studies using [150]H₂O, [18F]fluoro-2-deoxy-D-glucose, and positron emission tomography. *Diabetes* 1996;45:1471–1477.
- 50 Ruotsalainen U, Raitakari M, Nuutila P, Oikonen V, Sipilä H, Teräs M, Knuuti MJ, Bloomfield PM, Iida H: Quantitative blood flow measurement of skeletal muscle using oxygen-15-water and PET. *J Nucl Med* 1997;38:314–319.
- 51 Coggins M, Lindner J, Rattigan S, Jahn L, Fasy E, Kaul S, Barrett E: Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. *Diabetes* 2001;50:2682–2690.
- 52 Vincent MA, Dawson D, Clark ADH, Lindner JR, Rattigan S, Clark MG, Barrett EJ: Skeletal muscle microvascular recruitment by physiological hyperinsulinemia precedes increases in total blood flow. *Diabetes* 2002;51:42–48.
- 53 Bradley EA, Richards SM, Keske MA, Rattigan S: Local NOS inhibition impairs vascular and metabolic actions of insulin in rat hindleg muscle in vivo. *Am J Physiol Endocrinol Metab* 2013;305:E745–E750.
- 54 Zhao L, Chai W, Fu Z, Dong Z, Aylor KW, Barrett EJ, Cao W, Liu Z: Globular adiponectin enhances muscle insulin action via microvascular recruitment and increased insulin delivery. *Circ Res* 2013;112:1263–1271.
- 55 Nikolaidis LA, Elahi D, Shen YT, Shannon RP: Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 2005;289:15.
- 56 Drucker DJ: The biology of incretin hormones. *Cell Metab* 2006;3:153–165.
- 57 Chai W, Dong Z, Wang N, Wang W, Tao L, Cao W, Liu Z: Glucagon-like peptide 1 recruits microvasculature and increases glucose use in muscle via a nitric oxide-dependent mechanism. *Diabetes* 2012;61:888–896.
- 58 Lillioja S, Young AA, Culter CL, Ivy JL, Abbott WG, Zawadzki JK, Yki-Järvinen H, Christin L, Secomb TW, Bogardus C: Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. *J Clin Invest* 1987;80:415–424.
- 59 Solomon TP, Haus JM, Li Y, Kirwan JP: Progressive hyperglycemia across the glucose tolerance continuum in older obese adults is related to skeletal muscle capillarization and nitric oxide bioavailability. *J Clin Endocrinol Metab* 2011;96:1377–1384.

- 60 Bonner JS, Lantier L, Hasenour CM, James FD, Bracy DP, Wasserman DH: Muscle-specific vascular endothelial growth factor deletion induces muscle capillary rarefaction creating muscle insulin resistance. *Diabetes* 2013; 62:572–580.
- 61 King GL, Johnson SM: Receptor-mediated transport of insulin across endothelial cells. *Science* 1985;227: 1583–1586.
- 62 Yang YJ, Hope ID, Ader M, Bergman RN: Insulin transport across capillaries is rate limiting for insulin action in dogs. *J Clin Invest* 1989;84:1620–1628.
- 63 Miles PD, Levisetti M, Reichart D, Khoursheed M, Moossa AR, Olefsky JM. Kinetics of insulin action in vivo. Identification of rate-limiting steps. *Diabetes* 1995;44:947–953.
- 64 Sjöstrand M, Gudbjörnsdóttir S, Holmäng A, Lönn L, Strindberg L, Lönnroth P: Delayed transcapillary transport of insulin to muscle interstitial fluid in obese subjects. *Diabetes* 2002;51:2742–2748.
- 65 Cohen AW, Combs TP, Scherer PE, Lisanti MP: Role of caveolin and caveolae in insulin signaling and diabetes. *Am J Physiol Endocrinol Metab* 2003;285:E1151–E1160.
- 66 Wang H, Wang AX, Barrett EJ: Caveolin-1 is required for vascular endothelial insulin uptake. *Am J Physiol Endocrinol Metab* 2011;300:E134–E144.
- 67 Wang H, Wang AX, Liu Z, Barrett EJ: Insulin signaling stimulates insulin transport by bovine aortic endothelial cells. *Diabetes* 2008;57:540–547.
- 68 Wang H, Wang AX, Aylor K, Barrett EJ: Barrett nitric oxide directly promotes vascular endothelial insulin transport. *Diabetes* 2013;62:4030–4042.
- 69 Mehta PK, Griendling KK: Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 2007;292:C82–C97.
- 70 Retaillieu K1, Belin de Chantemèle EJ, Chanoine S, Guihot AL, Vessièrès E, Toutain B, Faure S, Bagi Z, Loufrani L, Henrion D: Reactive oxygen species and cyclooxygenase 2-derived thromboxane A2 reduce angiotensin II type 2 receptor vasorelaxation in diabetic rat resistance arteries. *Hypertension* 2010;55:339–344.
- 71 Savoia C, Touyz RM, Volpe M, Schiffrin EL: Angiotensin type 2 receptor in resistance arteries of type 2 diabetic hypertensive patients. *Hypertension* 2007;49:341–346.
- 72 Funke-Kaiser H, Reinemund J, Steckelings UM, Unger T: Adapter proteins and promoter regulation of the angiotensin AT2 receptor – implications for cardiac pathophysiology. *J Renin Angiotensin Aldosterone Syst* 2010;11:7–17.
- 73 Chai W, Wang W, Liu J, Barrett EJ, Carey RM, Cao W, Liu Z: Angiotensin II type 1 and type 2 receptors regulate basal skeletal muscle microvascular volume and glucose use. *Hypertension* 2010;55:523–530.
- 74 Chai W, Wang W, Dong Z, Cao W, Liu Z: Angiotensin II receptors modulate muscle microvascular and metabolic responses to insulin in vivo. *Diabetes* 2011;60:2939–2946.
- 75 Muniyappa R, Sowers JR: Role of insulin resistance in endothelial dysfunction. *Rev Endocr Metab Disord* 2013; 14:5–12.
- 76 Keske MA, Clerk LH, Price WJ, Jahn LA, Barrett EJ: Obesity blunts microvascular recruitment in human forearm muscle after a mixed meal. *Diabetes Care* 2009;32:1672–1677.
- 77 Frisbee JC: Remodeling of the skeletal muscle microcirculation increases resistance to perfusion in obese Zucker rats. *Am J Physiol Heart Circ Physiol* 2003;285:H104–H111.
- 78 Jiang ZY, Lin YW, Clemont A, Feener EP, Hein KD, Igarashi M, Yamauchi T, White MF, King GL: Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. *J Clin Invest* 1999; 104:447–457.
- 79 Potenza MA, Marasciulo FL, Chieppa DM, Brigiani GS, Formoso G, Quon MJ, Montagnani M: 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:H813–H822.
- 80 Albu JB, Heilbronn LK, Kelley DE, Smith SR, Azuma K, Berk ES, Pi-Sunyer FX, Ravussin E; Look AHEAD Adipose Research Group: Metabolic changes following a 1-year diet and exercise intervention in patients with type 2 diabetes. *Diabetes* 2010;59:627–633.
- 81 Xing W, Li Y, Zhang H, Mi C, Hou Z, Quon MJ, Gao F: Improvement of vascular insulin sensitivity by downregulation of GRK2 mediates exercise-induced alleviation of hypertension in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* 2013;305:H1111–H1119.
- 82 Taguchi K, Matsumoto T, Kamata K, Kobayashi T: G protein-coupled receptor kinase 2, with beta-arrestin 2, impairs insulin-induced Akt/endothelial nitric oxide synthase signaling in ob/ob mouse aorta. *Diabetes* 2012; 61:1978–1985.
- 83 Mikus CR, Roseguini BT, Uptergrove GM, Morris EM, Rector RS, Libla JL, Oberlin DJ, Borengasser SJ, Taylor AM, Ibdah JA, Laughlin MH, Thyfault JP: Voluntary wheel running selectively augments insulin-stimulated vasodilation in arterioles from white skeletal muscle of insulin-resistant rats. *Microcirculation*. 2012;19:729–738.
- 84 Pierce GL, Beske SD, Lawson BR, Southall KL, Benay FJ, Donato AJ, Seals DR: Weight loss alone improves conduit and resistance artery endothelial function in young and older overweight/obese adults. *Hypertension* 2008;52:72–79.
- 85 Bigornia SJ, Farb MG, Tiwari S, Karki S, Hamburg NM, Vita JA, Hess DT, Lavalley MP, Apovian CM, Gokce N: Insulin status and vascular responses to weight loss in obesity. *J Am Coll Cardiol* 2013;62:2297–2305.
- 86 Hamdy O, Ledbury S, Mullooly C, Jarema C, Porter S, Ovalle K, Moussa A, Caselli A, Caballero AE, Economides PA, Veves A, Horton ES: Lifestyle modification improves endothelial function in obese subjects with the insulin resistance syndrome. *Diabetes Care* 2003;26:2119–2125.

- 87 Maeda S, Jesmin S, Iemitsu M, Otsuki T, Matsuo T, Ohkawara K, Nakata Y, Tanaka K, Goto K, Miyauchi T: Weight loss reduces plasma endothelin-1 concentration in obese men. *Exp Biol Med* 2006;231:1044–1047.
- 88 Prior SJ, Blumenthal JB, Katzel LI, Goldberg AP, Ryan AS: Increased skeletal muscle capillarization after aerobic exercise training and weight loss improves insulin sensitivity in adults with IGT. *Diabetes Care* 2014;37:1469–1475.
- 89 Look AHEAD Research Group; Wing RR, Bolin P, et al: Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *N Engl J Med* 2013;369:145–154.
- 90 Katakam PV, Ujhelyi MR, Hoenig M, Miller AW: Metformin improves vascular function in insulin-resistant rats. *Hypertension* 2000;35:108–112.
- 91 Davis BJ, Xie Z, Viollet B, Zou MH: Activation of the AMP-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. *Diabetes* 2006;55:496–505.
- 92 Diamanti-Kandarakis E, Alexandraki K, Protogerou A, Piperi C, Papamichael C, Aessopos A, Lekakis J, Mavrikakis M: Metformin administration improves endothelial function in women with polycystic ovary syndrome. *Eur J Endocrinol* 2005;152:749–756.
- 93 Hong J, Zhang Y, Lai S, Lv A, Su Q, Dong Y, Zhou Z, Tang W, Zhao J, Cui L, Zou D, Wang D, Li H, Liu C, Wu G, Shen J, Zhu D, Wang W, Shen W, Ning G; SPREAD-DIMCAD Investigators: Effects of metformin versus glipizide on cardiovascular outcomes in patients with type 2 diabetes and coronary artery disease. *Diabetes Care* 2013;36:1304–1311.
- 94 Sola S, Mir MQ, Cheema FA, Khan-Merchant N, Menon RG, Parthasarathy S, Khan BV: 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;111:343–348.