



Obesity-related changes in the vascular actions of insulin [☆]

Francesca Schinzari ^a, Manfredi Tesaro ^b, Carmine Cardillo ^{a,c,*}

^a Departments of Aging at Policlinico A. Gemelli IRCCS, Rome, Italy

^b Systems Medicine at University of Tor Vergata, Rome, Italy

^c Translational Medicine and Surgery at Catholic University, Rome, Italy



ARTICLE INFO

Keywords:

Insulin
Endothelium
Obesity
Insulin resistance
Atherosclerosis

ABSTRACT

Over the past 2 to 3 decades, research has focused on the changes in the vascular effects of insulin occurring in insulin resistant states like obesity. Consistent evidence indicates that obesity results in reduced endothelial release of nitric oxide in response to insulin, associated with concomitant enhancement in the production of endothelin-1. More recent work has pointed toward reduced vascular permeability and changes in the physical-chemical characteristics of the perivascular extracellular matrix as additional mechanisms of impaired insulin sensitivity in obesity. All these perturbations are important, because they contribute to impaired delivery of insulin itself and metabolic substrates to the target tissues and may play a role in the development of both diabetes and vascular damage. This review will describe the physiological vascular actions of insulin and their changes in obesity, focusing on some established pathophysiological determinants of the derangement of vascular insulin signaling, such as the lipid overflow from expanded fat depots and signals originating from inflamed obese adipose tissue (both distant and perivascular). Also, it will outline novel evidence underscoring the contribution of dysregulated adipokine secretion and changes in intestinal permeability and gut microbiome. Finally, it will touch upon some unresolved issues, such as the potential role of vascular insulin resistance in obesity-driven adipose tissue remodeling, and will discuss perspectives for future studies, regarding in particular possible therapeutic strategies with translational implications for the patients care.

Introduction

The central role of insulin resistance in the pathophysiology of metabolic and cardiovascular disease has been known for decades, since Reaven first described insulin resistance as the common denominator of a number of metabolic and hemodynamic abnormalities collectively termed as “Syndrome X” (Reaven, 1988). The combination of the clinical features of insulin resistance, including central adiposity, high blood pressure, hypertriglyceridemia, reduced high-density lipoprotein (HDL) cholesterol and elevated fasting glucose, is now defined as the metabolic syndrome, a condition affecting an ever-growing number of individuals worldwide in parallel with the obese epidemic (Mozaffarian et al., 2016). In fact, in the presence of overnutrition and excess adiposity, insulin receptor signaling is downregulated, thus diminishing insulin’s physiological action on those intracellular pathways that regulate nutrient metabolism and fluid homeostasis. In addition to its consequences on glucose metabolism, insulin resistance has been shown to be associated with a several-fold increase in cardiovascular morbidity and mortality (Reaven, 2008). At no surprise, these observations have inspired

thorough investigation about possible direct effects of insulin on blood vessels.

Vascular actions of insulin: a journey from the bloodstream to the target tissues

A seminal study by Baron et al. demonstrated that infusion of insulin into the femoral artery of healthy individuals during euglycemic clamp results in a substantial increase in leg blood flow, associated with a concurrent rise in leg glucose uptake; these findings suggested that insulin-induced vasodilation plays a role in glucose disposal by the skeletal muscle (Baron et al., 1995). The same group of investigators had previously reported that the vasodilator response to insulin mimics that of methacholine, an endothelium-dependent vasodilator, as both are inhibited by the nitric oxide (NO) synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) (Steinberg et al., 1994). Similar results had been independently obtained by Scherrer et al., who observed that, in healthy subjects, insulin-induced increase in forearm flow can be inhibited by L-NMMA, but not by other vasoconstrictors, such as norepinephrine

[☆] This article was sent to Matthias Barton, MD, Guest Editor, for review by expert referees, editorial decision, and final disposition.

* Corresponding author at: Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Largo Gemelli 8, 00168 Rome, Italy.
E-mail address: carmine.cardillo@unicatt.it (C. Cardillo).

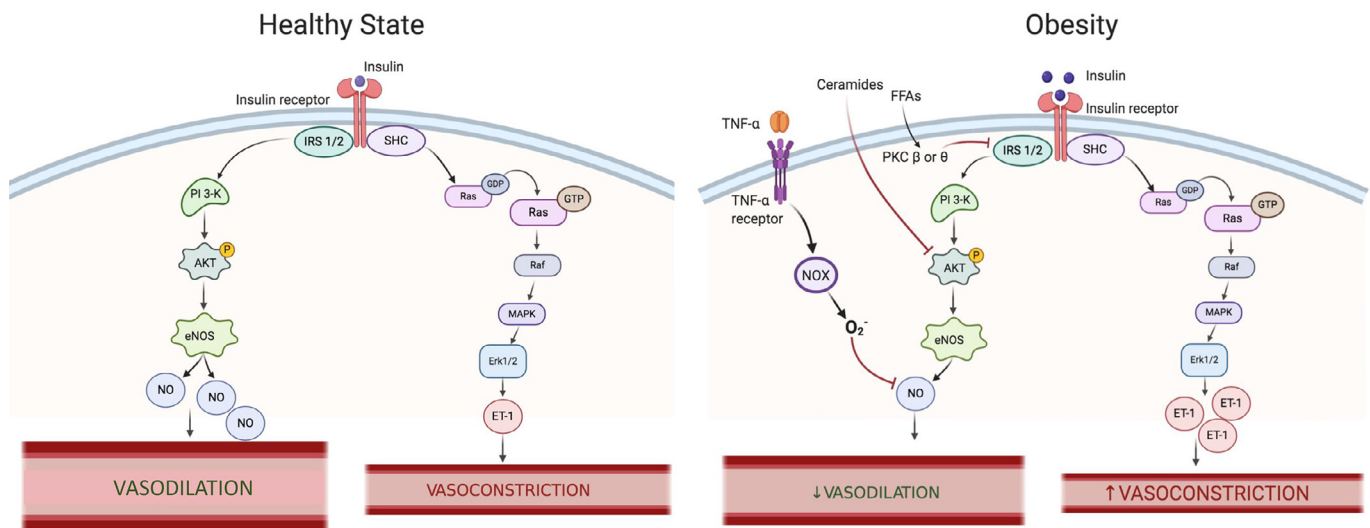


Fig. 1. Signaling transduction pathways of insulin in endothelial cells under healthy states (left panel) and changes occurring in obesity (right panel). FFAs, free fatty acids; PKC, phosphokinase C; TNF- α , tumor necrosis factor α ; NOX, NADPH oxidase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; ET-1, endothelin-1.

(Vollenweider et al., 1994). Taken together, these observations hinted a functional coupling between insulin-induced tissue perfusion and glucose utilization, fostering further laboratory work to ascertain the cellular and molecular mechanisms involved. In fact, Zeng and Quon demonstrated that the intracellular signaling pathways activated by insulin to stimulate NO production in endothelial cells are similar to those mediating glucose transport in canonical target tissues, involving the activation of IRS-1 and phosphatidylinositol 3-kinase (PI 3-K), with phosphorylation of protein kinase B (Akt) (Zeng and Quon, 1996). These findings were strengthened by the observations of another group, reporting that defective insulin signaling in mice with *Irs 1* knockout results in both endothelial dysfunction and insulin resistance (Abe et al., 1998) (Fig. 1).

By use of a microbubble-enhanced ultrasound technique, Vincent et al. first demonstrated that the NO-dependent insulin-induced vasodilation acts on precapillary resistance vessels to recruit capillary flow and hence enhance glucose disposal. Thus, in the hindlimb circulation of Sprague-Dawley rats, hyperinsulinemia results in increased total blood flow, skeletal muscle microvascular volume and glucose uptake; NO synthase inhibition, by contrast, completely blocks the effects of insulin on total blood flow and microvascular recruitment, concurrently blunting glucose uptake (Vincent et al., 2003). Based on these findings, it has been proposed that, under insulin action, microvascular flow is redirected from nonnutritive to nutritive routes, resulting in increased delivery of insulin, glucose and other nutrients to the skeletal muscle, a phenomenon defined as increased microvascular perfusion (Clark et al., 2003; Clark, 2008). This “redistribution” view of insulin vascular action has helped to settle a controversy regarding the mechanistic role, or lack thereof, of insulin-stimulated blood flow in skeletal muscle glucose delivery and uptake (Yki-Jarvinen and Utriainen, 1998). This “flow controversy” had been fueled by the discrepancy between studies demonstrating a significant increase in forearm or leg blood flow in response to exogenous insulin and studies in which insulin-induced flow changes had not been significant. The proponents of the notion that insulin induces vasodilation only at pharmacological doses, after reviewing 75 studies, came to the conclusion that the dose of insulin infused intravenously (systemically) is an important determinant of the blood flow response, but the duration of infusion is even more relevant; they calculated an “infusion index” (dose of insulin multiplied by the time of its infusion) and, when all studies were pooled, they observed a highly significant correlation between this index and the percent changes in blood flow. The same conclusion was drawn from the analysis of 23 studies assessing

the effect of intraarterial (local) hyperinsulinemia. Also, a different time course of the effects of insulin on glucose extraction (calculated as glucose arteriovenous difference) had been reported in the forearm tissues of healthy subjects, given that glucose extraction increases over 10-fold in 60 minutes, but little changes in blood flow are observed in this time; by contrast, continuing insulin infusion for longer time determines a progressive increase in blood flow, while glucose extraction has reached a plateau (Utriainen et al., 1995). To add more complexity to this picture were the results of a study performed in our laboratory, showing that high-physiological plasma levels of insulin result in a significant increase in forearm blood flow in healthy subjects when insulin is infused for 2 hours systemically, but not when it is given locally, hence suggesting that mechanisms independent of the direct effect of the hormone on the vessel wall may play a role in determining its hemodynamic action (Cardillo et al., 1998). Interestingly, Jahn et al. have characterized the different time-courses of insulin-mediated vasodilation at various locations of the arterial tree; they have shown that dilation of precapillary arterioles and capillary recruitment occurs 2-5 minutes after infusion of insulin, followed by relaxation of resistance arteries (30-60 minutes) and then flow-mediated dilation in conduit arteries (Jahn et al., 2016). This widespread vasodilator effect of insulin in healthy humans lends support from previous demonstration that insulin receptor is expressed on vascular endothelium at all levels of the arterial tree (Jialal et al., 1985). This bulk of evidence has been reviewed by Barrett et al., who concluded that, at physiological concentrations, insulin regulates the volume of the perfused vasculature within the skeletal and the cardiac muscle, and could also increase total muscle blood flow; both effects involve endothelial NO generation and smooth muscle cell relaxation, leading to increased delivery of insulin and nutrients to target tissues (Barrett et al., 2011).

Barrett et al. have also suggested that, in addition to regulating local blood flow through NO generation in resistance arterioles and precapillary arterioles, insulin induces NO release from the capillary endothelium to activate a vesicular transport system that allows its own delivery to the muscle interstitium (Fig. 2). Because the transit of insulin from plasma to muscle is a rate-limiting step for its metabolic action (Barrett et al., 2009), insulin crossing from plasma to interstitium represents an important first step in glucose disposal. This notion is supported by experimental data demonstrating that the metabolic action of insulin correlates more closely with its interstitial concentrations than with its plasma levels (Sjostrand et al., 2002). Studies in cultured endothelial cells and in rat muscle *in vivo* have shown that

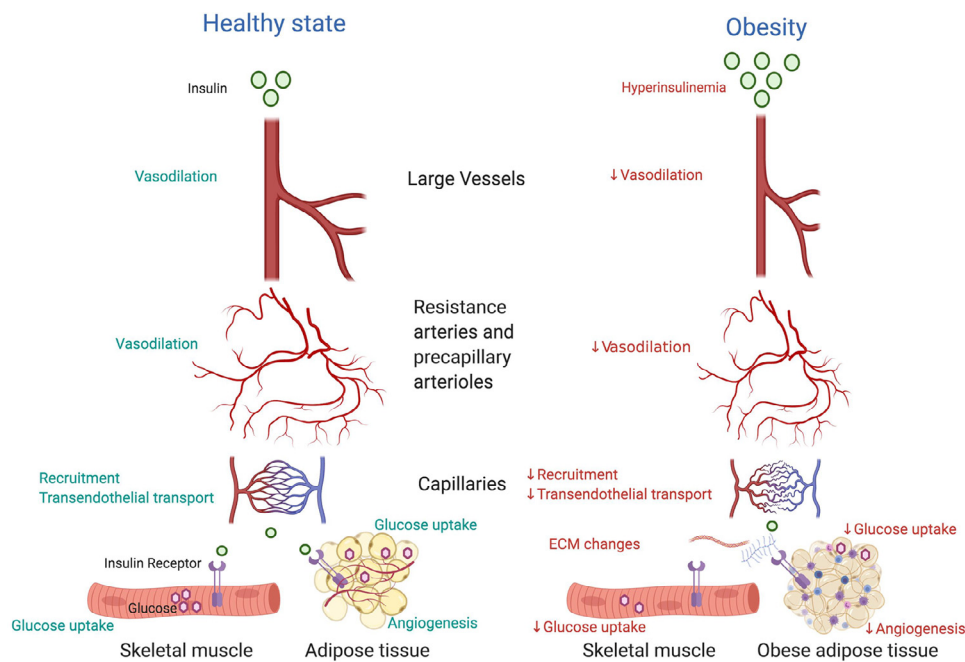


Fig. 2. Physiological vascular effects of insulin (left panel) and main obesity-related abnormalities that limit the availability of insulin at some of its target tissues (right panel).

this process involves insulin binding to insulin receptors and requires intact insulin signaling to endothelial NO synthase (eNOS) (Barrett and Eringa, 2012). This is probably because the non-fenestrated microvascular endothelial monolayer forms a tight barrier, restricting free access to the subendothelial interstitium of those plasma molecules, including insulin, whose diameter approaches that of the paracellular spaces. Models for transendothelial transport have shown insulin binding to its receptor on the endothelial plasma membrane, endocytosis via either a caveolin- or a clathrin-dependent entry into caveolae or caveolae-like vesicles, transcytosis facilitated by NO generated by insulin signaling to endothelial NO synthase, and final exocytosis into the interstitium (Wang et al., 2011; Azizi et al., 2015). The notion that insulin promotes its own delivery to the target tissues through transcytosis, rather than paracellular gaps, is corroborated by the observation that insulin-induced glucose uptake in vivo is maximal after more than 1 hour, whereas it occurs within minutes in isolated adipocytes or skeletal muscle cells (Artunc et al., 2016). This view, however, has been challenged by Williams et al., who used confocal microscopy to directly visualize the passage of fluorescent insulin across the endothelium of capillaries in the mouse muscle; they observed that insulin can cross the endothelial barrier by a receptor-independent mechanism that is unsaturable and conforms to the properties of fluid-phase transport, being thus determined by the balance between hydrostatic and oncotic pressures. This transport could also occur through paracellular junctions, not only through transcytosis, and could be facilitated by increased microvascular blood flow and capillary hydrostatic pressure (Williams et al., 2018).

Obesity-related changes in insulin vascular actions and tissue availability

Blood vessel wall

An early study by Laakso et al. demonstrated that, compared to lean individuals, obese patients have a dose-response curve to intravascular infusion of insulin flattened and shifted to the right; this is associated with reduced whole-body and leg glucose uptake, thus suggesting that insulin resistance in human obesity is characterized not only by reduced glucose disposal, but also by impaired vasodilation (Laakso et al., 1990). The same group of investigators obtained similar results in insulin resistant patient with type 2 diabetes (Laakso et al., 1990). A few years later, Steinberg et al. observed that intra-femoral infusion of exogenous insulin under euglycemic clamp conditions is associated with a substantial

potentiation of the vasodilator response to graded doses of methacholine in healthy subjects, but not in insulin resistant patients with obesity or type 2 diabetes; they also found a significant inverse relationship between maximal leg blood flow changes and body mass index, thereby suggesting that defective insulin potentiation of endothelium-dependent vasodilation goes in parallel with increased body weight (Steinberg et al., 1996). In an investigation performed in the forearm vasculature of obese patients, we have observed that the defective enhancement of vasodilator reactivity by insulin extends beyond endothelium-dependent vasorelaxation. Thus, local hyperinsulinemia potentiates the responses to vasodilators acting through disparate mechanisms (such as acetylcholine, sodium nitroprusside and verapamil) in lean subjects, but not in obese patients (Schinzari et al., 2010). These results have been later reproduced by Christou et al., who have reported reduced vasodilator reactivity to exogenous NO in healthy adults with increased adiposity, thereby confirming that vascular insulin resistance involves not only endothelial, but also smooth muscle responsiveness (Christou et al., 2012). More recently, Jahn et al., by use of a combination of various methodologies (brachial flow-mediated dilation, forearm post-ischemic flow velocity, and contrast-enhanced ultrasound) to measure blood flow at different locations of the arterial tree, have shown that insulin enhances endothelial function in conduit artery, resistance arteriole, and microvascular arteriolar in lean subjects, but not in patients with the metabolic syndrome (Jahn et al., 2016).

In a seminal study performed in our laboratory, we demonstrated that insulin not only stimulates NO production, but also promotes the endothelial synthesis and release of endothelin (ET)-1. Thus, we observed that separate infusion in the brachial artery of healthy volunteers of either insulin or a combination of BQ-123 (a selective endothelin type A receptor blocker) and BQ-788 (a selective endothelin type B receptor blocker) does not affect resting forearm blood flow; by contrast, concurrent infusion of insulin and the 2 blockers of ET-1 receptors was associated with a significant vasodilator response, consistent with increased ET-1-dependent vasoconstrictor tone; furthermore, during ET-1 receptor blockade, the vasoconstrictor response to NO synthesis inhibition with L-NMMA was significantly higher after insulin infusion than in the absence of hyperinsulinemia, indicating an increment in NO bioavailability (Cardillo et al., 1999). Based on these findings, we postulated that, in the skeletal muscle circulation under physiological conditions, insulin stimulates endothelial release of both NO and ET-1, albeit with a neutral

hemodynamic response to the hormone due to an equilibrium between these opposing vasoactive stimuli. This hypothesis was later supported by the experimental work of Vicent et al., who reported reduced endothelial NO synthase (eNOS) and ET-1 mRNA levels in endothelial cells from a vascular endothelial cell insulin receptor knockout (VENIRKO) mouse model (Vicent et al., 2003).

Moving from the observation that, in parallel to activation of the IRS/PI 3-K/Akt pathway, insulin signaling activates a phosphorylation cascade involving Raf, extracellular signal-regulated kinase (MEK), mitogen-activated protein (MAP)-kinase, and ERK 1/2 (Saltiel and Kahn, 2001), studies in endothelial cell cultures have demonstrated that insulin increases ET-1 levels in conditioned media through a MAP-kinase-dependent signaling pathway (Potenza et al., 2005). Similarly, other investigators have reported that activation of MEK/ERK1/2 signaling in microvascular endothelial cells mediates a vasoconstrictor effect of insulin on skeletal muscle first-order arterioles (Eringa et al., 2004). Interestingly, a study conducted by euglycemic clamp and muscle biopsies has demonstrated that a pivotal biochemical abnormality in insulin resistant individuals with obesity or type 2 diabetes is a dramatic reduction of IRS phosphorylation and PI 3-K activation compared to lean controls, whereas the activity of the MAP kinase pathway is preserved (Cusi et al., 2000). The potential pathophysiological implications of this abnormality are relevant, since insulin resistance is usually accompanied by a compensatory hyperinsulinemic state to maintain euglycemia. Hyperinsulinemia may overstimulate the normally functioning MAPK-dependent pathways, an effect that cannot be balanced by the abnormal PI 3-K cascade. At the vascular level, this pathway-specific insulin resistance would lead to an increased endothelial production of ET-1 and to a reduced synthesis of NO, with consequent endothelial dysfunction and increased vascular tone (Kim et al., 2006). This hypothesis is supported by the findings of Lteif et al., who demonstrated that insulin-resistant patients with obesity have enhanced ET-1-mediated vascular tone, which contributes to skeletal muscle insulin resistance by impairing insulin-mediated vasodilation; remarkably, intra-femoral infusion of BQ-123 augmented skeletal muscle responses to insulin through changes in both leg blood flow and glucose extraction in obese patients, but not in lean controls, thereby suggesting that increased ET-1 activity contributes to insulin resistance in obesity via both vascular and tissue effects (Lteif et al., 2007) (Fig. 1).

Importantly, these perturbations of insulin signaling at the level of endothelial cell not only impact vascular reactivity, but may also bear clinical implications by promoting both diabetes and atherosclerosis. Thus, impaired PI 3K/Akt signaling downstream of the insulin receptor results in reduced GLUT-1 translocation into membrane of vascular smooth muscle cells, with consequent decrease in glucose uptake (Schulman and Zhou, 2009); similarly, defective insulin-mediated vasodilation results in reduced delivery of insulin and glucose to the peripheral tissues, such as skeletal muscle and fat, thereby favoring obesity-related metabolic syndrome and type 2 diabetes. At the same time, reduced availability of NO and increased production of ET-1 may promote both early atherogenesis and advanced plaque progression, hence contributing to vascular disease (Bornfeldt and Tabas, 2011).

Transendothelial transport

Studies in animal models of tissue-selective insulin resistance have directly demonstrated that impaired insulin-mediated capillary recruitment may also impact the transendothelial transport of insulin. Thus, knockout mice lacking endothelial IRS-2, an IRS isoform expressed in endothelial cells, have impaired Akt phosphorylation and lack of Ser1177 phosphorylation of endothelial NO synthase; in these animals, both insulin-mediated vasodilation and insulin transport are reduced, concurrently with decreased glucose uptake by skeletal muscle (Kubota et al., 2011). Interestingly, in this model, insulin delivery is not reduced in the liver, likely because of a different capillary structure (the fenestrated endothelium of sinusoids allows free access of insulin to hepatocytes, whereas the occluded conjugations between endothelial cells

of the capillaries in the skeletal muscle do not). The notion that defective capillary perfusion in insulin resistant states may impact insulin egress and delivery to the skeletal muscle had been previously suggested by a study showing delayed transcapillary transport of insulin to muscle interstitial fluid in obese patients (Sjostrand et al., 2002). Similarly, Broussard et al., by measuring lymph fluid as a representation of the interstitial space to assess insulin access to skeletal muscle, observed decreased insulin concentration in the interstitial fluid of mongrel dogs during hyperinsulinemic euglycemic clamp following long-term high-fat diet (Broussard et al., 2016). In keeping with these experimental findings, a clinical study has demonstrated that insulin delivery to adipose tissue and skeletal muscle is impaired in obese women with postprandial hyperglycemia (Sandqvist et al., 2011) (Fig. 2). Overall, irrespective of the discrepancies regarding the predominant mechanism of insulin extravasation (transcellular vs. paracellular), consistent evidence supports the notion that obesity impacts insulin egress from capillaries, thereby feeding the vicious circle of insulin resistance.

Extracellular matrix

Whereas earlier studies have focused predominantly on transendothelial transport as a mechanism of impaired insulin delivery to its target tissues, more recent work has outlined the role of extracellular matrix (ECM) changes in reducing insulin action. Changes of perivascular ECM occurring in obese AT contribute to its maladaptive remodeling by reducing new capillary formation and insulin delivery to adipocytes (Williams et al., 2015). Similarly, in the skeletal muscle, ECM remodeling associated with obesity has been shown to reduce insulin and glucose delivery to the myofibers by multiple mechanisms. These include the formation of a physical (spatial) barrier for access to the myofibers, the reduction in capillary number, and changes in cell signaling through interaction with cell surface receptors that sense the extracellular microenvironment, in particular the integrin receptor family (Wasserman et al., 2018). In fact, increase in the glycosaminoglycan hyaluronan, a major component of capillary glycocalyx, is associated with reduced insulin access to tissues in animal models of obesity; use of hyaluronidase or of an antibody against the main hyaluronan cell surface receptor (CD44), by contrast, improves insulin sensitivity in high-fat fed or obese mice (Kang et al., 2013; Kodama et al., 2015). Expansion of ECM is also associated with decreased muscle capillary density (Kang et al., 2014), whereas treatments that reduce ECM collagen levels result in both capillary growth and improved insulin sensitivity (Kang et al., 2014; Bonner et al., 2013). Moreover, obesity-related expansion of ECM may result in altered expression or activation of integrins, the main receptors for cell adhesion to the ECM proteins. This notion lends support from studies in animals with muscle-specific deletion of part of the integrin receptors or their downstream signaling molecules (such as the integrin-linked kinase (ILK) or the focal adhesion kinase (FAK)), showing that ILK is necessary for the development of insulin resistance in diet-induced obesity (Kang et al., 2016), whereas inhibition of FAK causes insulin resistance (Bisht et al., 2008). Abnormal accumulation of lumican (a small leucine-rich proteoglycan that modifies collagen I organization, impairs angiogenesis and activates adipocyte oxidative stress) was observed in subcutaneous fat of insulin resistant individuals; in omental fat, instead, insulin resistance was associated with increased levels of a small protein, GDI2, that alters lipid storage in adipocytes. Collectively, these findings lend support to the notion that obesity-related changes in ECM concur with defective insulin vascular signaling and extravasation to reduce the availability of the hormone at its target tissues.

Mechanisms of obesity-related vascular insulin resistance

Free fatty acids (FFAs) and other lipid species

A mechanism contributing to downregulation of the vascular insulin signaling in obesity relates to increased release of lipids from adipose

tissue and/or oversupply of lipids to tissues not suited for their storage. The changes brought about by excess circulating lipids are broadly termed lipotoxicity and may lead to modification of intracellular signaling mechanisms activated by insulin in the vasculature, in particular to changes in eNOS gene expression and catalytic activity that ultimately result in endothelial dysfunction (Imrie et al., 2010). Among countless lipid metabolites that may accrue in obesity, free fatty acids (FFAs), diacylglycerol (DAG) and ceramides seem to be the most deleterious in terms of disruption of insulin signaling and vascular reactivity. Early data indicating that FFA interfere with insulin signaling on PI 3-K in rat skeletal muscle sparked investigation on the possibility that higher amounts of circulating FFAs could contribute to the abnormal vascular reactivity to insulin in humans (Dresner et al., 1999). This possibility was tested by Steinberg et al., who exposed lean subjects to higher circulating FFAs; during euglycemic hyperinsulinemic clamp, infusion of FFAs for several hours resulted in a marked reduction of glucose uptake, associated with blunted insulin-mediated increase in leg blood flow and severely inhibited NO activity (Steinberg et al., 2000). Subsequent work by Kim et al. showed that palmitic, oleic and linoleic acids, the most abundant circulating FFAs in humans, impair basal and insulin-stimulated NO production in bovine aortic endothelial cell cultures (Kim et al., 2005). On a molecular standpoint, evidence indicates that increased intracellular concentration of long-chain fatty acids and DAG in insulin resistant states leads to chronic activation of protein kinase C (PKC), an enzyme that may phosphorylate serine or threonine residues on target proteins. In particular, the PKC isomers β and θ are serine kinases that phosphorylate the insulin receptor and IRS-1 on specific residues, thus preventing insulin-induced tyrosine phosphorylation and activation of the downstream signaling cascade. PKC β has been found chronically activated in the muscle of obese subjects (Itani et al., 2000), whereas PKC θ activation has been observed in association with FFA-induced skeletal muscle insulin resistance (Griffin et al., 1999). PKC- β can also be switched on in the muscle of healthy subjects by infusions of intralipid plus heparin for 6 hours (Itani et al., 2002). Moreover, in the vascular endothelium, PKC- β activation leads to attenuation of insulin-induced NO production and reduced recruitment of muscle capillaries (Naruse et al., 2006). Other work has demonstrated that, in isolated muscle resistance arteries of mice, activation of PKC θ by palmitic acid results in insulin-induced vasoconstriction by inhibition of Akt and stimulation of ERK1/2; this phenomenon shifts the balance of insulin-dependent vasoreactivity toward ET-1-mediated vasoconstriction and may provide a mechanistic link between PKC θ activation and insulin resistance (Bakker et al., 2008).

When tissues not suited for lipid storage are exposed to increased circulating levels of FFAs, accumulation of toxic bioactive lipid metabolites, such as the sphingolipid ceramide, occurs at various sites, including the endothelium of large arteries (Chun et al., 2011). Studies in animal models of diet-induced obesity have demonstrated that ceramide reduces insulin-stimulated eNOS phosphorylation and activity, hence leading to decreased NO production; these effects are activated by protein phosphatase 2A (PP2A), the primary phosphatase that dephosphorylates and inactivates Akt, and may be prevented in isolated arteries by inhibition of ceramide biosynthesis using genetic approaches (Zhang et al., 2012). Subsequent studies have also demonstrated that a small molecule inhibitor of PP2A is able to prevent the disruption of the PP2A/Akt/eNOS axis in the vasculature, thus preserving arterial function in obese mice (Bharath et al., 2015). More recently, Li et al. have shown that down-regulation of acid sphingomyelinase, a key enzyme in the formation of ceramide following exposure to palmitic acid, improves endothelial insulin resistance and NO production, concurrently enhancing glucose transporter-4 (GLUT-4) expression and glucose uptake (Li et al., 2018).

Systemic inflammation and oxidative stress

Over the past couple of decades, a close relationship has clearly emerged between nutrient excess and activation of the immune system,

leading to a state of chronic, low-grade inflammation in obesity. This inflammatory state, in turn, seems to play a mechanistic role in disrupting insulin signaling at various places, including the vasculature, whereby it may contribute to vascular dysfunction and ensuing damage. An important source of inflammation in obesity is the adipose tissue (AT), in particular the visceral fat, where activated intracellular inflammatory signals, such as the IKK β /NF- κ B and JNK pathways, result in increased content of resident macrophages and their polarization toward a M₁ pro-inflammatory profile, with overproduction of cytokines and other bioactive molecules (Shoelson et al., 2006). Among numerous cytokines, tumor necrosis factor (TNF)- α has been well characterized with regard to its mechanistic role in obesity-related insulin resistance (Hotamisligil, 1999). Thus, TNF- α is able to inhibit insulin-stimulated phosphorylation of IRS-1 and activation of the downstream PI 3-K/Akt signaling pathway that increase eNOS activity and NO production in cultured endothelial cells (Kim et al., 2001). Similarly, Li et al. have reported that TNF- α induces endothelial insulin resistance in the PI 3-K/Akt/eNOS pathway via a MAPK-dependent mechanism, while concurrently enhancing the ERK 1/2 pathway (Li et al., 2007). Additionally, TNF- α may induce vascular insulin resistance via positive modulation of the enzyme phosphatase and tension homologue (PTEN), leading to decreased Akt/eNOS/NO signaling in high fat fed mice (da Costa et al., 2016). In humans, Rask-Madsen et al. have reported that infusion of TNF- α in healthy individuals inhibits the stimulatory effects of insulin on glucose uptake and reduces endothelium-dependent vasodilation (Rask-Madsen et al., 2003). On the other hand, a study performed by us has demonstrated that infusion of the TNF- α neutralizing antibody infliximab into the brachial artery of patients with the metabolic syndrome enhances the stimulatory effects of insulin on NO-mediated vasodilator reactivity (Tesouro et al., 2008).

Heightened oxidative stress correlates well with accumulation of fat in mice and humans, and is one main determinant of the obesity-associated metabolic syndrome (Furukawa et al., 2004). Increased inflammatory cytokines (De Keulenaer et al., 1998), higher levels of FFAs (Du et al., 2006), activation of the renin-angiotensin system (Ramalingam et al., 2017), as well as decreased expression of antioxidant enzymes, are among the factors that accompany the elevation of reactive oxygen species (ROS), which, in turn, are potent inducers of maladaptive intravascular insulin signaling in insulin resistant states (Paneni et al., 2015). In a model of metabolic insulin resistance and endothelial dysfunction mediated by superoxide anion (O₂⁻) linked to local generation of angiotensin (Ang) II, Zhou et al. have shown impaired insulin activation of eNOS and impaired NO-mediated vasodilation (Zhou et al., 2010). Similarly, endothelium-specific overexpression of PKC β_2 , a key molecular event eliciting ROS production, suppresses insulin-dependent pathways with respect to Akt/eNOS activation in vessels isolated from APOE^{-/-} mice, while concurrently expressing the potent vasoconstrictor ET-1 (Li et al., 2013). Also, transgenic rats with elevated Ang II levels (TG(mRen2)27), exhibiting higher aortic TNF- α expression, greater nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) activity and increased ROS levels, also display substantially diminished Akt activation and eNOS activity in response to in vivo insulin stimulation compared to control animals (Wei et al., 2007). On the other hand, pharmacological tools reducing vascular oxidative stress through NADPH oxidase inhibition have proven effective at improving insulin-mediated vasodilation in obese animal models (Katakam et al., 2005). Similarly, obese mice with genetic disruption of Nox2 are protected against ROS accumulation and endothelial insulin resistance, suggesting that targeting Nox2 could represent a valuable therapeutic strategy against vascular dysfunction induced by high-fat diet (Du et al., 2013). Also, transgenic mice with endothelium overexpression of a dominant-negative mutant human insulin receptor (ESMIRO) exhibit blunted vasodilator responses to ACh and insulin; these abnormalities are related to augmented production of superoxide anions (Duncan et al., 2008) and are ameliorated by the genetic deletion of the Nox2 isoform of NADPH oxidase (Sukumar et al., 2013). In line with these findings, we have observed that, in the human fore-

arm circulation, infusion of the antioxidant vitamin C is able to improve NO-mediated vasodilator responsiveness during hyperinsulinemia in patients with the metabolic syndrome (Tesouro et al., 2008), thereby suggesting that increased oxidative stress has a role in the pathophysiology of insulin resistance not only in animal models, but also in human disease.

Perivascular adipose tissue changes

Perivascular adipose tissue (PVAT) is now recognized as more than a structural support around large, medium-sized and small blood vessels, being instead a source of countless mediators with profound abilities to influence vascular homeostasis. A number of studies have clearly demonstrated that, in healthy conditions, PVAT predominantly releases factors that exert an “anti-contractile”, hence beneficial, paracrine action on its neighbor vessels (Xia and Li, 2017). More specifically, very recent work in experimental models has elegantly shown defective insulin-induced vasodilation and microvascular recruitment in muscle resistance arteries in the absence of PVAT, associated with markedly decreased local glucose uptake, without changes in whole body insulin sensitivity (Turaihi et al., 2020). The enlargement of PVAT occurring in obesity, by contrast, results in maladaptive changes that translate into harmful effects on the entire cardiovascular system, from the heart (epicardial fat), through the aorta and the major branches of the arterial tree, up to the smaller nutritional arterioles (Xia and Li, 2017). With regard to microvessels, a study performed *ex vivo* (in pressure myograph) in human arterioles obtained from gluteal biopsies has shown that PVAT taken from lean women enhances insulin-induced vasodilation, whereas PVAT from obese woman stimulates insulin-induced vasoconstriction; not surprisingly the size of perivascular adipocyte was bigger in obese than in lean women and was directly related to the impairment of capillary recruitment assessed *in vivo* by contrast-enhanced ultrasound (Meijer et al., 2015). Interestingly, the same mediators responsible for the adverse cardiovascular actions of excessive visceral fat, such as lipid metabolites, inflammatory cytokines and ROS, are also released by obese PVAT and play a role in the disruption of insulin-mediated balance between NO and endothelin release in the nearby vessels (Yudkin et al., 2005).

In addition to inflammatory mechanisms, obese PVAT undergoes changes in the secretory profile of adipokines, previously believed to be produced only in visceral fat stores, a phenomenon contributing to its unfavorable influence on insulin signaling (Huang Cao et al., 2017). While adipokines secreted by intra-abdominal AT have an established role in liver insulin resistance and impaired pancreatic secretion of insulin, paracrine signaling by PVAT-secreted adipokines seem to have a greater impact on vascular insulin signaling and muscle insulin sensitivity. Among different adipokines, adiponectin has been well characterized with regard to its effects on insulin-mediated vasoreactivity. Thus, administration of exogenous adiponectin has been shown to mimic, at least in part, the effect of lean PVAT on insulin-mediated vasodilation in isolated mouse gracilis muscle resistance vessels, an effect that is prevented by an adiponectin receptor antagonist or by a 5' adenosine monophosphate-activated kinase (AMPK) inhibitor; the latter finding suggests an involvement of AMPK activation, resulting in Akt phosphorylation and inhibition of ERK1/2, with subsequent reduction in ET-1-mediated constriction (Meijer et al., 2013). Similarly, Zhao et al. have shown that pre-incubation with globular adiponectin improves the vasodilator effect of insulin in resistance arteries of rats fed high-fat diet via AMPK activation, an effect abolished by AMPK inhibition with compound C (Zhao et al., 2015). Other studies have demonstrated a role of the multidomain adaptor protein APPL1, an essential signaling component of the insulin sensitizer effect of adiponectin, in modulating the vascular actions of insulin in mice. Thus, transgenic expression of APPL1 prevents obesity-induced impairment in insulin-mediated vasodilation and reverses augmentation in insulin-evoked ET-1-dependent vasoconstriction; genetic disruption of APPL1, by contrast, shifts the effects of insulin from vasodilation to vasoconstriction via extracellular signal-

related kinase (ERK) 1/2 phosphorylation and ET-1 production (Wang et al., 2011). Importantly, recent work has clearly shown that the presence of locally generated adiponectin is needed for insulin-mediated vasodilation to occur in the skeletal muscle, whereas blood-borne adiponectin present in the vascular lumen is insufficient (Turaihi et al., 2020).

Microbiome and gut permeability

In addition to the adipose tissue and the skeletal muscle, the intestine is emerging as an important site for changes affecting the immune system, metabolism, and insulin resistance in obesity. Thus, accumulating proof now suggests that gut microbiome, defined as the community of microorganisms that share the gut lumen space, plays a role in the body response to changes in caloric intake and diet composition (Dabke et al., 2019). A number of studies have concordantly demonstrated that obesity, both experimental and human, is associated with substantial changes in the composition of gut microbiome, with great increase in the bacteria from the phylum *Firmicutes* over *Bacteroidetes* (Saad et al., 2016). These changes are, in turn, associated with disruption of tight junction integrity of the intestinal epithelium, leading to increased permeability to a number of substances that may impact insulin signaling. Among the gut-derived substances increased in the circulation of obese patients is lipopolysaccharides (LPS), which may initiate an inflammatory cascade via activation of pattern recognition receptors (PRRs), such as the toll-like receptor (TLR)-4, on target cells, hence leading to nuclear translocation of NF- κ B and expression of inflammatory cytokines, while concurrently inhibiting insulin signaling (Saad et al., 2016). Another consequence of gut microbiome changes in obesity is the reduction in intraluminal content of short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate, and the increase in branched-chain aminoacids (BCAAs) that may contribute to insulin resistance through complex, and still controversial, mechanisms (Neis et al., 2015; Newgard, 2012). It is conceivable, therefore, that obesity-associated microbiome changes, in addition to their contribution to systemic inflammation, might affect vascular insulin signaling through systemic spillover of some gut-derived molecules.

Unresolved issues and perspectives

As obesity-related complications, such as diabetes and cardiovascular disease, remain leading causes of morbidity and mortality worldwide, important goals of preventive medicine are better understanding of their underlying mechanisms and effective interventions to correct them. Achieving success in these strategies include enhanced characterization of the relative importance of some emerging pathophysiological factors involved in vascular insulin resistance. For example, recent work highlights the role of micro RNAs (miRNAs), noncoding RNAs that regulate gene expression and signaling pathways, in insulin responsive cells that are important for the maintenance of metabolic and vascular homeostasis, raising the possibility that miRNA inhibition or “replacement therapy” may eventually result in improved vascular insulin sensitivity (Sun et al., 2016). Also, novel findings suggest that exosomes, extracellular vesicles released from various tissues, carry countless mediators that might impact the vascular actions of insulin (Kita et al., 2019; Saez et al., 2019). Another current knowledge gap relates to a possible role of the vascular insulin signaling in maintaining a normal AT function, by preserving proper interactions between adipocytes and their surrounding vasculature. Given that adequate perfusion and new vessel formation are key determinants of healthy AT expansion (Saltiel and Olefsky, 2017), obesity-related changes in the vascular actions of insulin could result not only in impaired vasodilation but also in defective angiogenesis. The latter abnormality, in turn, could negatively impact the delivery of nutrients and oxygen required for lipid storage and mobilization in the expanding AT (Ferrannini et al., 2018). Finally, there is paucity of data regarding effective measures to improve vascular insulin resistance and hence ameliorate the metabolic and vascular

profile of obese patients. Some evidence demonstrates that lifestyle interventions are able to improve vascular insulin sensitivity in human obesity (Vinet et al., 2015); other clinical studies suggest some effectiveness of various pharmacological approaches (de Jongh et al., 2004; Wang et al., 2020). There is, however, strong need of further studies to find effective treatments that could translate into clinical benefits for these patients.

Fundings

This work was partially supported by the Beyond Borders 2019 grant from University of Tor Vergata to M.T. and by Fondi d'Ateneo funds from Università Cattolica to C.C.

Declaration of Competing Interest

The authors have no conflict of interests to disclose.

Peer Review Summary

Peer Review Summary associated with this article can be found in the online version, at doi:10.1016/j.endmts.2020.100075.

References

- Abe, H., Yamada, N., Kamata, K., Kuwaki, T., Shimada, M., Osuga, J., Shionoiri, F., Yahagi, N., Kadowaki, T., Tamemoto, H., Ishibashi, S., Yazaki, Y., Makuuchi, M., 1998. Hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation in mice lacking insulin receptor substrate-1. *J. Clin. Invest.* 101 (8), 1784–1788.
- Artunc, F., Schleicher, E., Weigert, C., Fritsche, A., Stefan, N., Haring, H.U., 2016. The impact of insulin resistance on the kidney and vasculature. *Nat. Rev. Nephrol.* 12 (12), 721–737.
- Azizi, P.M., Zyla, R.E., Guan, S., Wang, C., Liu, J., Bolz, S.S., Heit, B., Klip, A., Lee, W.L., 2015. Clathrin-dependent entry and vesicle-mediated exocytosis define insulin transcytosis across microvascular endothelial cells. *Mol. Biol. Cell* 26 (4), 740–750.
- Bakker, W., Sipkema, P., Stehouwer, C.D., Serne, E.H., Smulders, Y.M., van Hinsbergh, V.W., Eringa, E.C., 2008. Protein kinase C theta activation induces insulin-mediated constriction of muscle resistance arteries. *Diabetes* 57 (3), 706–713.
- Baron, A.D., Steinberg, H.O., Chaker, H., Leaming, R., Johnson, A., Brechtel, G., 1995. Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J. Clin. Invest.* 96 (2), 786–792.
- Barrett, E.J., Eringa, E.C., 2012. The vascular contribution to insulin resistance: promise, proof, and pitfalls. *Diabetes* 61 (12), 3063–3065.
- Barrett, E.J., Eggleston, E.M., Inyard, A.C., Wang, H., Li, G., Chai, W., Liu, Z., 2009. The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. *Diabetologia* 52 (5), 752–764.
- Barrett, E.J., Wang, H., Upchurch, C.T., Liu, Z., 2011. Insulin regulates its own delivery to skeletal muscle by feed-forward actions on the vasculature. *Am. J. Physiol. Endocrinol. Metab.* 301 (2), E252–E263.
- Bharath, L.P., Ruan, T., Li, Y., Ravindran, A., Wan, X., Nhan, J.K., Walker, M.L., Deeter, L., Goodrich, R., Johnson, E., Munday, D., Mueller, R., Kunz, D., Jones, D., Reese, V., Summers, S.A., Babu, P.V., Holland, W.L., Zhang, Q.J., Abel, E.D., Symons, J.D., 2015. Ceramide-initiated protein phosphatase 2A activation contributes to arterial dysfunction in vivo. *Diabetes* 64 (11), 3914–3926.
- Bisht, B., Srinivasan, K., Dey, C.S., 2008. In vivo inhibition of focal adhesion kinase causes insulin resistance. *J. Physiol.* 586 (16), 3825–3837.
- Bonner, J.S., Lantier, L., Hocking, K.M., Kang, L., Owolabi, M., James, F.D., Bracy, D.P., Brophy, C.M., Wasserman, D.H., 2013. Relaxin treatment reverses insulin resistance in mice fed a high-fat diet. *Diabetes* 62 (9), 3251–3260.
- Bornfeldt, K.E., Tabas, I., 2011. Insulin resistance, hyperglycemia, and atherosclerosis. *Cell Metab.* 14 (5), 575–585.
- Broussard, J.L., Castro, A.V., Iyer, M., Paszkiewicz, R.L., Bediako, I.A., Szczepaniak, L.S., Szczepaniak, E.W., Bergman, R.N., Kolka, C.M., 2016. Insulin access to skeletal muscle is impaired during the early stages of diet-induced obesity. *Obesity (Silver Spring)* 24 (9), 1922–1928.
- Cardillo, C., Kilcoyne, C.M., Nambi, S.S., Cannon 3rd, R.O., Quon, M.J., Panza, J.A., 1998. Vasodilator response to systemic but not to local hyperinsulinemia in the human forearm. *Hypertension* 32 (4), 740–745.
- Cardillo, C., Nambi, S.S., Kilcoyne, C.M., Choucair, W.K., Katz, A., Quon, M.J., Panza, J.A., 1999. Insulin stimulates both endothelin and nitric oxide activity in the human forearm. *Circulation* 100 (8), 820–825.
- Christou, D.D., Pierce, G.L., Walker, A.E., Hwang, M.H., Yoo, J.K., Luttrell, M., Meade, T.H., English, M., Seals, D.R., 2012. Vascular smooth muscle responsiveness to nitric oxide is reduced in healthy adults with increased adiposity. *Am. J. Physiol. Heart Circ. Physiol.* 303 (6), H743–H750.
- Chun, L., Junlin, Z., Aimin, W., Niansheng, L., Benmei, C., Minxiang, L., 2011. Inhibition of ceramide synthesis reverses endothelial dysfunction and atherosclerosis in streptozotocin-induced diabetic rats. *Diabetes Res. Clin. Pract.* 93 (1), 77–85.
- Clark, M.G., Wallis, M.G., Barrett, E.J., Vincent, M.A., Richards, S.M., Clerk, L.H., Rattigan, S., 2003. Blood flow and muscle metabolism: a focus on insulin action. *Am. J. Physiol. Endocrinol. Metab.* 284 (2), E241–E258.
- Clark, M.G., 2008. Impaired microvascular perfusion: a consequence of vascular dysfunction and a potential cause of insulin resistance in muscle. *Am. J. Physiol. Endocrinol. Metab.* 295 (4), E732–E750.
- Cusi, K., Maezono, K., Osman, A., Pendergrass, M., Patti, M.E., Pratipanawatr, T., DeFronzo, R.A., Kahn, C.R., Mandarino, L.J., 2000. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J. Clin. Invest.* 105 (3), 311–320.
- da Costa, R.M., Neves, K.B., Mestriner, F.L., Louzada-Junior, P., Bruder-Nascimento, T., Tostes, R.C., 2016. TNF-alpha induces vascular insulin resistance via positive modulation of PTEN and decreased Akt/eNOS/NO signaling in high fat diet-fed mice. *Cardiovasc. Diabetol.* 15 (1), 119.
- Dabke, K., Hendrick, G., Devkota, S., 2019. The gut microbiome and metabolic syndrome. *J. Clin. Invest.* 129 (10), 4050–4057.
- de Jongh, R.T., Serne, E.H., Ijzerman, R.G., de Vries, G., Stehouwer, C.D., 2004. Free fatty acid levels modulate microvascular function: relevance for obesity-associated insulin resistance, hypertension, and microangiopathy. *Diabetes* 53 (11), 2873–2882.
- De Keulenaer, G.W., Alexander, R.W., Ushio-Fukai, M., Ishizaka, N., Griendling, K.K., 1998. Tumour necrosis factor alpha activates a p22phox-based NADH oxidase in vascular smooth muscle. *Biochem. J.* 329 (Pt 3), 653–657.
- Dresner, A., Laurent, D., Marcucci, M., Griffin, M.E., Dufour, S., Cline, G.W., Slezak, L.A., Andersen, D.K., Hundal, R.S., Rothman, D.L., Petersen, K.F., Shulman, G.I., 1999. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J. Clin. Invest.* 103 (2), 253–259.
- Du, X., Edelstein, D., Obici, S., Higham, N., Zou, M.H., Brownlee, M., 2006. Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation. *J. Clin. Invest.* 116 (4), 1071–1080.
- Du, J., Fan, L.M., Mai, A., Li, J.M., 2013. Crucial roles of Nox2-derived oxidative stress in deteriorating the function of insulin receptors and endothelium in dietary obesity of middle-aged mice. *Br. J. Pharmacol.* 170 (5), 1064–1077.
- Duncan, E.R., Crossey, P.A., Walker, S., Anilkumar, N., Poston, L., Douglas, G., Ez-zat, V.A., Wheatcroft, S.B., Shah, A.M., Kearney, M.T., 2008. Effect of endothelium-specific insulin resistance on endothelial function in vivo. *Diabetes* 57 (12), 3307–3314.
- Eringa, E.C., Stehouwer, C.D., van Nieuw Amerongen, G.P., Ouwehand, L., Westerhof, N., Sipkema, P., 2004. Vasoconstrictor effects of insulin in skeletal muscle arterioles are mediated by ERK1/2 activation in endothelium. *Am. J. Physiol. Heart Circ. Physiol.* 287 (5), H2043–H2048.
- Ferrannini, E., Iozzo, P., Virtanen, K.A., Honka, M.J., Bucci, M., Nuutila, P., 2018. Adipose tissue and skeletal muscle insulin-mediated glucose uptake in insulin resistance: role of blood flow and diabetes. *Am. J. Clin. Nutr.* 108 (4), 749–758.
- Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., Shimomura, I., 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J. Clin. Invest.* 114 (12), 1752–1761.
- Griffin, M.E., Marcucci, M.J., Cline, G.W., Bell, K., Barucci, N., Lee, D., Goodyear, L.J., Kraegen, E.W., White, M.F., Shulman, G.I., 1999. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* 48 (6), 1270–1274.
- Hotamisligil, G.S., 1999. The role of TNF-alpha and TNF receptors in obesity and insulin resistance. *J. Intern. Med.* 245 (6), 621–625.
- Huang Cao, Z.F., Stoffel, E., Cohen, P., 2017. Role of perivascular adipose tissue in vascular physiology and pathology. *Hypertension* 69 (5), 770–777.
- Imrie, H., Abbas, A., Kearney, M., 2010. Insulin resistance, lipotoxicity and endothelial dysfunction. *Biochim. Biophys. Acta* 1801 (3), 320–326.
- Itani, S.I., Zhou, Q., Pories, W.J., MacDonald, K.G., Dohm, G.L., 2000. Involvement of protein kinase C in human skeletal muscle insulin resistance and obesity. *Diabetes* 49 (8), 1353–1358.
- Itani, S.I., Ruderman, N.B., Schmieder, F., Boden, G., 2002. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes* 51 (7), 2005–2011.
- Jahn, L.A., Hartline, L., Rao, N., Logan, B., Kim, J.J., Aylor, K., Gan, L.M., Westergren, H.U., Barrett, E.J., 2016. Insulin enhances endothelial function throughout the arterial tree in healthy but not metabolic syndrome subjects. *J. Clin. Endocrinol. Metab.* 101 (3), 1198–1206.
- Jialal, I., Crettaz, M., Hachiyi, H.L., Kahn, C.R., Moses, A.C., Buzney, S.M., King, G.L., 1985. Characterization of the receptors for insulin and the insulin-like growth factors on micro- and macrovascular tissues. *Endocrinology* 117 (3), 1222–1229.
- Kang, L., Lantier, L., Kennedy, A., Bonner, J.S., Mayes, W.H., Bracy, D.P., Bookbinder, L.H., Hasty, A.H., Thompson, C.B., Wasserman, D.H., 2013. Hyaluronan accumulates with high-fat feeding and contributes to insulin resistance. *Diabetes* 62 (6), 1888–1896.
- Kang, L., Mayes, W.H., James, F.D., Bracy, D.P., Wasserman, D.H., 2014. Matrix metalloproteinase 9 opposes diet-induced muscle insulin resistance in mice. *Diabetologia* 57 (3), 603–613.
- Kang, L., Mokshagundam, S., Reuter, B., Lark, D.S., Sneddon, C.C., Hennayake, C., Williams, A.S., Bracy, D.P., James, F.D., Pozzi, A., Zent, R., Wasserman, D.H., 2016. Integrin-linked kinase in muscle is necessary for the development of insulin resistance in diet-induced obese mice. *Diabetes* 65 (6), 1590–1600.
- Katakam, P.V., Tulbert, C.D., Snipes, J.A., Erdos, B., Miller, A.W., Busija, D.W., 2005. Impaired insulin-induced vasodilation in small coronary arteries of Zucker obese rats is mediated by reactive oxygen species. *Am. J. Physiol. Heart Circ. Physiol.* 288 (2), H854–H860.

- Kim, F., Gallis, B., Corson, M.A., 2001. TNF-alpha inhibits flow and insulin signaling leading to NO production in aortic endothelial cells. *Am. J. Physiol. Cell Physiol.* 280 (5), C1057–C1065.
- Kim, F., Tysseling, K.A., Rice, J., Pham, M., Haji, L., Gallis, B.M., Baas, A.S., Paramsothy, P., Giachelli, C.M., Corson, M.A., Raines, E.W., 2005. Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta. *Arterioscler. Thromb. Vasc. Biol.* 25 (5), 989–994.
- Kim, J.A., Montagnani, M., Koh, K.K., Quon, M.J., 2006. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 113 (15), 1888–1904.
- Kita, S., Maeda, N., Shimomura, I., 2019. Interorgan communication by exosomes, adipose tissue, and adiponectin in metabolic syndrome. *J. Clin. Invest.* 129 (10), 4041–4049.
- Kodama, K., Toda, K., Morinaga, S., Yamada, S., Butte, A.J., 2015. Anti-CD44 antibody treatment lowers hyperglycemia and improves insulin resistance, adipose inflammation, and hepatic steatosis in diet-induced obese mice. *Diabetes* 64 (3), 867–875.
- Kubota, T., Kubota, N., Kumagai, H., Yamaguchi, S., Kozono, H., Takahashi, T., Inoue, M., Itoh, S., Takamoto, I., Sasaki, T., Kumagai, K., Kawai, T., Hashimoto, S., Kobayashi, T., Sato, M., Tokuyama, K., Nishimura, S., Tsunoda, M., Ide, T., Murakami, K., Yamazaki, T., Ezaki, O., Kawamura, K., Masuda, H., Moroi, M., Sugi, K., Oike, Y., Shimokawa, H., Yanagihara, N., Tsutsui, M., Terauchi, Y., Tobe, K., Nagai, R., Kamata, K., Inoue, K., Kodama, T., Ueki, K., Kadowaki, T., 2011. Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell Metab.* 13 (3), 294–307.
- Laakso, M., Edelman, S.V., Brechtel, G., Baron, A.D., 1990. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J. Clin. Invest.* 85 (6), 1844–1852.
- Laakso, M., Edelman, S.V., Olefsky, J.M., Brechtel, G., Wallace, P., Baron, A.D., 1990. Kinetics of in vivo muscle insulin-mediated glucose uptake in human obesity. *Diabetes* 39 (8), 965–974.
- Li, G., Barrett, E.J., Barrett, M.O., Cao, W., Liu, Z., 2007. Tumor necrosis factor-alpha induces insulin resistance in endothelial cells via a p38 mitogen-activated protein kinase-dependent pathway. *Endocrinology* 148 (7), 3356–3363.
- Li, Q., Park, K., Li, C., Rask-Madsen, C., Mima, A., Qi, W., Mizutani, K., Huang, P., King, G.L., 2013. Induction of vascular insulin resistance and endothelin-1 expression and acceleration of atherosclerosis by the overexpression of protein kinase C-beta isoform in the endothelium. *Circ. Res.* 113 (4), 418–427.
- Li, X., Jin, S.J., Su, J., Li, X.X., Xu, M., 2018. Acid sphingomyelinase down-regulation alleviates vascular endothelial insulin resistance in diabetic rats. *Basic Clin. Pharmacol. Toxicol.* 123 (6), 645–659.
- Lteif, A., Vaishnav, P., Baron, A.D., Mather, K.J., 2007. Endothelin limits insulin action in obese/insulin-resistant humans. *Diabetes* 56 (3), 728–734.
- Meijer, R.I., Bakker, W., Alta, C.L., Sipkema, P., Yudkin, J.S., Viollet, B., Richter, E.A., Smulders, Y.M., van Hinsbergh, V.W., Serne, E.H., Eringa, E.C., 2013. Perivascular adipose tissue control of insulin-induced vasoreactivity in muscle is impaired in db/db mice. *Diabetes* 62 (2), 590–598.
- Meijer, R.I., Serne, E.H., Korkmaz, H.I., van der Peet, D.L., de Boer, M.P., Niessen, H.W., van Hinsbergh, V.W., Yudkin, J.S., Smulders, Y.M., Eringa, E.C., 2015. Insulin-induced changes in skeletal muscle microvascular perfusion are dependent upon perivascular adipose tissue in women. *Diabetologia* 58 (8), 1907–1915.
- Mozaffarian, D., Benjamin, E.J., Go, A.S., Arnett, D.K., Blaha, M.J., Cushman, M., Das, S.R., de Ferranti, S., Despres, J.P., Fullerton, H.J., Howard, V.J., Huffman, M.D., Isasi, C.R., Jimenez, M.C., Judd, S.E., Kissela, B.M., Lichtman, J.H., Lisabeth, L.D., Liu, S., Mackey, R.H., Magid, D.J., McGuire, D.K., Mohler, E.R., 3rd, M.o.y.C.S., Muntner, P., Mussolino, M.E., Nasir, K., Neumar, R.W., Nichol, G., Palaniappan, L., Pandey, D.K., Reeves, M.J., Rodriguez, C.J., Rosamond, W., Sorlie, P.D., Stein, J., Towfighi, A., Turan, T.N., Virani, S.S., Woo, D., Yeh, R.W., Turner, M.B., 2016. Heart disease and stroke statistics - 2016 update: a report from the American Heart Association. *Circulation* 133 (4), 447–454.
- Naruse, K., Rask-Madsen, C., Takahara, N., Ha, S.W., Suzuma, K., Way, K.J., Jacobs, J.R., Clermont, A.C., Ueki, K., Ohshiro, Y., Zhang, J., Goldfine, A.B., King, G.L., 2006. Activation of vascular protein kinase C-beta inhibits Akt-dependent endothelial nitric oxide synthase function in obesity-associated insulin resistance. *Diabetes* 55 (3), 691–698.
- Neis, E.P., Dejong, C.H., Rensen, S.S., 2015. The role of microbial amino acid metabolism in host metabolism. *Nutrients* 7 (4), 2930–2946.
- Newgard, C.B., 2012. Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab.* 15 (5), 606–614.
- Paneni, F., Costantino, S., Cosentino, F., 2015. Role of oxidative stress in endothelial insulin resistance. *World J. Diabetes* 6 (2), 326–332.
- Potenza, M.A., Marasciulo, F.L., Chieppa, D.M., Brigiani, G.S., Formoso, G., Quon, M.J., Montagnani, M., 2005. 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.* 289 (2), H813–H822.
- Ramalingam, L., Menikdiwala, K., LeMieux, M., Dufour, J.M., Kaur, G., Kalupahana, N., Moustaid-Moussa, N., 2017. The renin angiotensin system, oxidative stress and mitochondrial function in obesity and insulin resistance. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863 (5), 1106–1114.
- Rask-Madsen, C., Dominguez, H., Ihlemann, N., Hermann, T., Kober, L., Torp-Pedersen, C., 2003. Tumor necrosis factor-alpha inhibits insulin's stimulating effect on glucose uptake and endothelium-dependent vasodilation in humans. *Circulation* 108 (15), 1815–1821.
- Reaven, G.M., 1988. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 37 (12), 1595–1607.
- Reaven, G.M., 2008. Insulin resistance: the link between obesity and cardiovascular disease. *Endocrinol. Metab. Clin. North Am.* 37 (3), 581–601 vii–viii.
- Saad, M.J., Santos, A., Prada, P.O., 2016. Linking gut microbiota and inflammation to obesity and insulin resistance. *Physiology (Bethesda)* 31 (4), 283–293.
- Saez, T., Toledo, F., Sobrevia, L., 2019. Extracellular vesicles and insulin resistance: a potential interaction in vascular dysfunction. *Curr. Vasc. Pharmacol.* 17 (5), 491–497.
- Saltiel, A.R., Kahn, C.R., 2001. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414 (6865), 799–806.
- Saltiel, A.R., Olefsky, J.M., 2017. Inflammatory mechanisms linking obesity and metabolic disease. *J. Clin. Invest.* 127 (1), 1–4.
- Sandqvist, M., Strindberg, L., Schmelz, M., Lonnroth, P., Jansson, P.A., 2011. Impaired delivery of insulin to adipose tissue and skeletal muscle in obese women with postprandial hyperglycemia. *J. Clin. Endocrinol. Metab.* 96 (8), E1320–E1324.
- Schinzari, F., Tesouro, M., Rovella, V., Galli, A., Mores, N., Porzio, O., Lauro, D., Cardillo, C., 2010. Generalized impairment of vasodilator reactivity during hyperinsulinemia in patients with obesity-related metabolic syndrome. *Am. J. Physiol. Endocrinol. Metab.* 299 (6), E947–E952.
- Schulman, I.H., Zhou, M.S., 2009. Vascular insulin resistance: a potential link between cardiovascular and metabolic diseases. *Curr. Hypertens. Rep.* 11 (1), 48–55.
- Shoelson, S.E., Lee, J., Goldfine, A.B., 2006. Inflammation and insulin resistance. *J. Clin. Invest.* 116 (7), 1793–1801.
- Sjostrand, M., Gudbjornsdottir, S., Holmang, A., Lonn, L., Strindberg, L., Lonnroth, P., 2002. Delayed transcapillary transport of insulin to muscle interstitial fluid in obese subjects. *Diabetes* 51 (9), 2742–2748.
- Steinberg, H.O., Brechtel, G., Johnson, A., Fineberg, N., Baron, A.D., 1994. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J. Clin. Invest.* 94 (3), 1172–1179.
- Steinberg, H.O., Chaker, H., Leaming, R., Johnson, A., Brechtel, G., Baron, A.D., 1996. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J. Clin. Invest.* 97 (11), 2601–2610.
- Steinberg, H.O., Paradisi, G., Hook, G., Crowder, K., Cronin, J., Baron, A.D., 2000. Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. *Diabetes* 49 (7), 1231–1238.
- Sukumar, P., Viswambharan, H., Imrie, H., Cubbon, R.M., Yuldasheva, N., Gage, M., Galloway, S., Skromma, A., Kandavelu, P., Santos, C.X., Gatenby, V.K., Smith, J., Beech, D.J., Wheatcroft, S.B., Channon, K.M., Shah, A.M., Kearney, M.T., 2013. Nox2 NADPH oxidase has a critical role in insulin resistance-related endothelial cell dysfunction. *Diabetes* 62 (6), 2130–2134.
- Sun, X., Lin, J., Zhang, Y., Kang, S., Belkin, N., Wara, A.K., Icli, B., Hamburg, N.M., Li, D., Feinberg, M.W., 2016. MicroRNA-181b improves glucose homeostasis and insulin sensitivity by regulating endothelial function in white adipose tissue. *Circ. Res.* 118 (5), 810–821.
- Tesouro, M., Schinzari, F., Rovella, V., Melina, D., Mores, N., Barini, A., Mettimano, M., Lauro, D., Iantorno, M., Quon, M.J., Cardillo, C., 2008. Tumor necrosis factor-alpha antagonism improves vasodilation during hyperinsulinemia in metabolic syndrome. *Diabetes Care* 31 (7), 1439–1441.
- Turaihi, A.H., Serne, E.H., Moltzoff, C.F.M., Koning, J.J., Knol, J., Niessen, H.W., Goumans, M., van Poelgeest, E.M., Yudkin, J.S., Smulders, Y.M., Jimenez, C.R., van Hinsbergh, V.W.M., Eringa, E.C., 2020. Perivascular adipose tissue controls insulin-stimulated perfusion, mitochondrial protein expression, and glucose uptake in muscle through adipomuscular arterioles. *Diabetes* 69 (4), 603–613.
- Utriainen, T., Malmstrom, R., Makimattila, S., Yki-Jarvinen, H., 1995. Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. *Diabetologia* 38 (5), 555–564.
- Vicent, D., Ilany, J., Kondo, T., Naruse, K., Fisher, S.J., Kisanuki, Y.Y., Bursell, S., Yanagisawa, M., King, G.L., Kahn, C.R., 2003. The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *J. Clin. Invest.* 111 (9), 1373–1380.
- Vincent, M.A., Barrett, E.J., Lindner, J.R., Clark, M.G., Rattigan, S., 2003. Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am. J. Physiol. Endocrinol. Metab.* 285 (1), E123–E129.
- Vinet, A., Obert, P., Duthel, F., Diagne, L., Chapier, R., Lesourd, B., Courteix, D., Walther, G., 2015. Impact of a lifestyle program on vascular insulin resistance in metabolic syndrome subjects: the RESOLVE study. *J. Clin. Endocrinol. Metab.* 100 (2), 442–450.
- Vollenweider, P., Randin, D., Tappy, L., Jequier, E., Nicod, P., Scherrer, U., 1994. Impaired insulin-induced sympathetic neural activation and vasodilation in skeletal muscle in obese humans. *J. Clin. Invest.* 93 (6), 2365–2371.
- Wang, H., Wang, A.X., Barrett, E.J., 2011. Caveolin-1 is required for vascular endothelial insulin uptake. *Am. J. Physiol. Endocrinol. Metab.* 300 (1), E134–E144.
- Wang, Y., Cheng, K.K., Lam, K.S., Wu, D., Wang, Y., Huang, Y., Vanhoutte, P.M., Sweeney, G., Li, Y., Xu, A., 2011. APPL1 counteracts obesity-induced vascular insulin resistance and endothelial dysfunction by modulating the endothelial production of nitric oxide and endothelin-1 in mice. *Diabetes* 60 (11), 3044–3054.
- Wang, N., Tan, A.W.K., Jahn, L.A., Hartline, L., Patrie, J.T., Lin, S., Barrett, E.J., Aylor, K.W., Liu, Z., 2020. Vasodilatory actions of glucagon-like peptide 1 are preserved in skeletal and cardiac muscle microvasculature but not in conduit artery in obese humans with vascular insulin resistance. *Diabetes Care* 43 (3), 634–642.
- Wasserman, D.H., Wang, T.J., Brown, N.J., 2018. The vasculature in prediabetes. *Circ. Res.* 122 (8), 1135–1150.
- Wei, Y., Whaley-Connell, A.T., Chen, K., Habibi, J., Uptergrove, G.M., Clark, S.E., Stump, C.S., Ferrario, C.M., Sowers, J.R., 2007. NADPH oxidase contributes to vascular inflammation, insulin resistance, and remodeling in the transgenic (mRen2) rat. *Hypertension* 50 (2), 384–391.
- Williams, A.S., Kang, L., Wasserman, D.H., 2015. The extracellular matrix and insulin resistance. *Trends Endocrinol. Metab.* 26 (7), 357–366.

- Williams, I.M., Valenzuela, F.A., Kahl, S.D., Ramkrishna, D., Mezo, A.R., Young, J.D., Wells, K.S., Wasserman, D.H., 2018. Insulin exits skeletal muscle capillaries by fluid-phase transport. *J. Clin. Invest.* 128 (2), 699–714.
- Xia, N., Li, H., 2017. The role of perivascular adipose tissue in obesity-induced vascular dysfunction. *Br. J. Pharmacol.* 174 (20), 3425–3442.
- Yki-Jarvinen, H., Utriainen, T., 1998. Insulin-induced vasodilatation: physiology or pharmacology? *Diabetologia* 41 (4), 369–379.
- Yudkin, J.S., Eringa, E., Stehouwer, C.D., 2005. Vasocrine" signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. *Lancet* 365 (9473), 1817–1820.
- Zeng, G., Quon, M.J., 1996. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J. Clin. Invest.* 98 (4), 894–898.
- Zhang, Q.J., Holland, W.L., Wilson, L., Tanner, J.M., Kearns, D., Cahoon, J.M., Pettey, D., Losee, J., Duncan, B., Gale, D., Kowalski, C.A., Deeter, N., Nichols, A., Deesing, M., Arrant, C., Ruan, T., Boehme, C., McCamey, D.R., Rou, J., Ambal, K., Narra, K.K., Summers, S.A., Abel, E.D., Symons, J.D., 2012. Ceramide mediates vascular dysfunction in diet-induced obesity by PP2A-mediated dephosphorylation of the eNOS-Akt complex. *Diabetes* 61 (7), 1848–1859.
- Zhao, L., Fu, Z., Wu, J., Aylor, K.W., Barrett, E.J., Cao, W., Liu, Z., 2015. Globular adiponectin ameliorates metabolic insulin resistance via AMPK-mediated restoration of microvascular insulin responses. *J. Physiol.* 593 (17), 4067–4079.
- Zhou, M.S., Schulman, I.H., Raji, L., 2010. Vascular inflammation, insulin resistance, and endothelial dysfunction in salt-sensitive hypertension: role of nuclear factor kappa B activation. *J. Hypertens.* 28 (3), 527–535.