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New insights into insulin action and resistance in the vasculature

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Abstract

Two-thirds of adults in the United States are overweight or obese, and another 26 million have type 2 diabetes. Decreased insulin sensitivity in cardiovascular tissue is an underlying abnormality in these individuals. Insulin metabolic signaling increases endothelial cell nitric oxide production. Impaired vascular insulin sensitivity is an early defect leading to impaired vascular relaxation. In overweight and obese persons, as well as in those with hypertension, systemic and vascular insulin resistance often occurs in conjunction with activation of the cardiovascular tissue renin–angiotensin–aldosterone system (RAAS). Activated angiotensin II type 1 receptor and mineralocorticoid receptor signaling promote the development of vascular insulin resistance and impaired endothelial nitric oxide–mediated relaxation. Research in this area has implicated excessive serine phosphorylation and proteasomal degradation of the docking protein insulin receptor substrate and enhanced signaling through hybrid insulin/insulin-like growth factor (IGF-1) receptor as important mechanisms underlying RAAS impediment of downstream vascular insulin metabolic signaling. This review will present recent evidence supporting the notion that RAAS signaling represents a potential pathway for the development of vascular insulin resistance and impaired endothelial-mediated vasodilation.

Keywords

insulin resistance; endothelium; cardiovascular disease; obesity; nitric oxide

Introduction

Type 2 diabetes mellitus (DM2) affects 26 million people in the United States and 347 million worldwide.[1] The prevalence of DM2 is closely associated with the alarming rates of physical inactivity and over-nutrition,[2] leading contributors to current pandemic rates of obesity. Resistance to the metabolic actions of insulin, termed *insulin resistance* for the

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purposes of this article, and consisting mainly of suppression of hepatic gluconeogenesis and skeletal muscle glucose disposal, is a key event in both DM2 and obesity.[3] Resistance to the vascular effects of insulin contributes to the pathogenesis of cardiovascular disease (CVD),[3] which accounts for the majority of the deaths in diabetic patients.[4] Thus, understanding the role of insulin in the vasculature in health and disease should help to elucidate new therapeutic strategies aimed at curbing the pandemic of CVD in diabetes. In this review, we will discuss the available evidence of normal and pathological actions of insulin in the vasculature, as well as its impact on glucose homeostasis and on the pathogenesis of CVD.

Distinct molecular effects of insulin in the vasculature

The net effects of insulin on the vasculature are determined by different cellular signaling pathways that are activated by stimulation of the insulin receptor (IR) (Fig. 1).[5] Classically, insulin metabolic signaling results in vasodilation via increased nitric oxide (NO) production and increases in bioavailable NO. However, in conditions of insulin resistance, it promotes vasoconstriction and vascular proliferation.[5]

Binding of insulin to IR triggers its phosphorylation and activation via an intrinsic kinase activity, leading to tyrosine phosphorylation of the insulin receptor substrate (IRS) proteins.[6] Phosphorylation of the IRS molecules creates Src homology 2 (SH2) domain binding motifs that serve as docking points for SH2-containing proteins like phosphatidylinositol 3-kinase (PI3K) and Grb-2.[6] The docking of PI3K to IRS-1 activates, via p85, the p110 catalytic subunit of PI3K, resulting in production of phosphatidylinositol 3,4,5-trisphosphate (PIP_3). PIP_3 promotes phosphorylation and activation of 3-phosphoinositide-dependent protein kinase-1 (PDK-1), which then activates different serine/threonine kinases such as Akt. (Fig. 1).[6] In turn, Akt activates the endothelial NO synthase (eNOS) by phosphorylation in serine residue 1177.[7,8] eNOS catalyzes the conversion of L-arginine and O_2 to L-citrulline and NO.[6] eNOS is expressed in caveola, where it is inhibited by caveolin-1, and its activity is modulated in a Ca^{+2}/calmodulin–sensitive manner.[9] Nevertheless, in endothelial cells, eNOS activation by insulin stimulation is only partially blunted by calmodulin inhibitors, suggesting a calcium-independent mechanism of insulin-mediated eNOS activation.[7]

Insulin also promotes eNOS phosphorylation of threonine 495 in human endothelial cells (threonine 497 bovine).[10] Dephosphorylation of this residue is involved in uncoupling eNOS and increasing production of reactive oxygen species (ROS).[10] Importantly, the PI3K–Akt cascade is not the only determinant of eNOS activity; heat-shock protein 90 modulates eNOS activity[11] and an inadequate supply of tetrahydrobiopterin (eNOS cofactor) limits the enzyme activity and results in eNOS uncoupling.[12] The NO produced by eNOS decreases vascular tone and vascular smooth muscle cell (VSMC) proliferation and diminishes adhesion of inflammatory cells and platelet aggregation to the endothelium.[5] Furthermore, insulin modulates production of prostaglandins and endothelium-derived hyperpolarizing factors.[5]

In addition to vasodilatation, insulin can promote vasoconstriction. Under some circumstances, insulin activates the mitogen-activated protein kinase (MAPK) cascade that coordinates insulin vasoconstriction and growth-promoting effects.[5] These effects of insulin

are mediated, in part, by the increased production of endothelin-1 (ET-1) and the activation of signaling through the vascular tissue RAAS.[5,6] ET-1 is produced in the endothelium and acts via the ET-1 receptor A to cause vasoconstriction, increase oxidative stress, and promote cell growth and mitogenesis in VSMC.[13] Supraphysiological levels of insulin have been shown to increase the production of ET-1 in cultured endothelial cells and in rats.[14] Similar deleterious effects are seen with the activation of vascular tissue RAAS.[5]

It has been recently demonstrated that insulin activates the insulin-like growth factor-1 (IGF-1) receptor (IGF1-R)[15] and stimulates NO production through this alternative mechanism. However, production of NO can be limited by the fact that insulin binds less avidly to IGF1-R than to its native receptor, and in conditions of insulin resistance, IRs are outnumbered by IGF1-Rs.[15]

In diabetic CVD, insulin resistance is generated by chronic low-grade inflammation, increased oxidative stress, lipotoxicity, and activation of the renin angiotensin aldosterone system (RAAS) (16). These conditions promote serine phosphorylation of different insulin signaling molecules such as IRS-1 and the impairment of the normal tyrosine phosphorylation cascade (17), thus impairing insulin metabolic signaling.

Actions of insulin in the skeletal muscle vasculature

Skeletal muscle is one of the main metabolic targets of insulin and promotes glucose uptake into skeletal muscle fibers.[18] Insulin plays a modulatory role on skeletal muscle vasculature by increasing blood flow and regulating its own delivery.[19] Skeletal muscle vasculature is extremely flexible.

Under basal, non-exercising conditions, the skeletal muscle receives 0.03–004 ml/min of blood flow per gram of tissue,[19] but upon initiation of exercise, blood flow increases up to 100-fold.[20] Capillary recruitment in the skeletal muscle is initially determined by vasomotor changes in the terminal arterioles.[19] Increased metabolic demands (such as exercise) result in a drop in tissue oxygen tension below the threshold necessary to maintain oxidative phosphorylation.[21,22] Once oxygen extraction is maximized, an ascending vasodilation response extends from the contracting skeletal muscle arterioles to the proximal feed arteries, resulting in increased blood flow to the contracting muscle.[22]

The mechanism behind localized ascending vasodilation is dependent on an intact endothelium and involves activation of the α adrenergic receptors to restrict the vasodilation to actively contracting areas of the muscle.[22] Once the terminal arterioles are maximally dilated, capillary perfusion is determined by tissue metabolic demands and the anatomic arrangement of the vessels.[19] Adequate skeletal muscle capillary recruitment is crucial for the normal metabolic effects of insulin,[23] and clinical conditions characterized by insulin resistance such as obesity and DM2 manifest with impaired capillary recruitment.[24]

Although insulin is critical for vasodilation in the vasculature, there are several additional factors involved in vascular reactivity that are independent of insulin, including catecholamine release, glucagon-like protein 1 (GLP-1) secretion, and RAAS activation, as will be discussed in this review.

The effects of insulin in the vasculature have been examined using different invasive and non-invasive approaches like the 1-methylxantnine (1-MX),[25,26] thermodilution method, laser-Doppler perfusion,[27] contrast-enhanced ultrasound (CEU), and positron-emission tomography.[28,29] The CEU is a non-invasive technique adapted from heart muscle imaging.[25] It uses lipid-coated microbubbles filled with perfluorocarbon that behave similarly to red blood cells and remain in the intravascular compartment.[25,28] 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 of the area studied.[19] This technique requires anesthesia when used in rodents and immobilization in humans, which may interfere with the behavior of the microvasculature.[25,28] CEU has been widely used by several authors to describe the response of the skeletal muscle vasculature to different stimuli.[19,25,28]

Whole-limb blood flow

Even though effects of insulin on the vasculature have been described for several decades,[18] controversy exists regarding the significance of insulin-mediated vasodilation in glucose uptake.[30] Earlier reports in humans studied the effect of insulin on whole-limb blood flow using the thermodilution method and the hyperinsulinemic–euglycemic clamp.[31–35] Thermodilution allows for uninterrupted monitoring of limb blood flow, which potentially gives a more accurate description of the hemodynamic changes produced by insulin.[19] Insulin increases whole-limb blood flow in a dose-dependent manner in lean humans, and it correlates with increased skeletal muscle glucose uptake.[36] The effect of insulin on leg blood flow is largely dependent on increased NO production and is blunted by eNOS inhibition.[37] Furthermore, eNOS inhibition results in blunting of insulin-mediated skeletal muscle glucose uptake via decreased skeletal muscle blood flow.[35]

Nevertheless, some investigators have challenged these concepts. Yki– Järvinen and colleagues showed that, in lean subjects, augmentation of limb blood flow through bradykinin infusion did not translate into increased insulin-mediated glucose uptake by skeletal muscle (measured by [^{18}F]-fluoro-deoxy-glucose and positron emission tomography).[30] Furthermore, the same group did not find any evidence that increased blood flow to skeletal muscle in response to insulin relates to the muscle areas where insulin stimulated glucose uptake is increased.[38] Even though the opposing findings can be partially explained by the different techniques used by the authors to assess blood flow and glucose uptake, the current available knowledge does not completely clarify the role of insulin-mediated vasodilation on whole-limb blood flow and its impact on insulin-stimulated glucose uptake.

Microvascular recruitment

Recruitment of under-perfused capillaries by ascending vasodilation is necessary at times of increased metabolic demand such as during exercise.[28,29]. Capillary recruitment increases the endothelial surface available for nutrient exchange, as well as for the delivery of insulin to the skeletal muscle.[28] Insulin has been proposed to have an exercise-like effect on the vasculature of skeletal muscle.[28] The evidence of capillary recruitment secondary to insulin is derived from using the indirect methods described above. Insulin infusion in healthy non-

obese adults (systemically and through the brachial artery) results in increased forearm blood volume without significant changes in whole-limb blood flow.[39] In addition, insulin-mediated increases in microvascular blood flow, which parallel microvascular recruitment, precede increases in total limb blood flow caused by insulin[40] and are dependent on NO production.[23] These increases are seen at lower insulin doses compared to those required to increase whole-limb blood flow.[23]

Finally, transport of insulin across the endothelial barrier has gained relevance as it has been postulated to be the rate-limiting factor for the action of insulin in skeletal muscle. One investigative group has proposed that the delivery of insulin to skeletal muscle depends on binding of insulin to IR in the caveloae, resulting in internalization of the vesicles and transendothelial transport of the hormone.[19]

RAAS modulation of skeletal muscle microvasculature

Overweight/obesity and DM2 are characterized by inappropriate activation of the RAAS, which plays an important role in the modulation of the skeletal muscle vasculature.[3,5,16] Both beneficial and deleterious effects have been ascribed to the activation of the system. The vascular effects of angiotensin II (Ang II) have been extensively characterized.[41] Ang II signals through G-coupled membrane-bound type 1 and type 2 receptors (AT1R and AT2R, respectively). In the vasculature, AT1R activation increases oxidative stress and promotes vasoconstriction and remodeling.[27] In endothelium, chronic AT1R activation leads to increased activity of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymatic complex, enhanced production of ROS, and uncoupling of eNOS.[41] Conversely, eNOS uncoupling decreases bioavailable NO and results in increased production of ROS.[42] Furthermore, in endothelial cells, Ang II induces impairment of insulin-stimulated eNOS activation (via decreased Ser1177 phosphorylation) and diminishes NO production via the mammalian target of rapamycin/p70S6 kinase 1 pathway (mTOR/S6K1).[43]

On the other hand, AT2R activation counteracts the deleterious effects of Ang II via AT1R signaling.[44] Vasodilation mediated by AT2R results from activation of the bradykinin/NO system.[45] AT2R increases the expression of prolylcarboxipeptidase,[46] which converts prekallikrein to kallikrein and cleaves high–molecular weight kininogen (HMWK), resulting in release of bradykinin.[47] Likewise, in a rodent model of diabetes and insulin resistance, increased ROS resulted in reduced AT2R-mediated dilatation.[48] In this regard, it has been reported that treatment for one year with an AT1R blocker in hypertensive diabetic persons resulted in increased AT2R expression and enhanced vasodilatory response in resistance arteries.[49]

Angiotensin II regulates skeletal muscle perfusion through AT2R and AT1R

The differential effects of Ang II receptor activation and its impact on glucose homeostasis have been extensively investigated. Acute infusion of pressor and non-pressor doses of Ang II in control rats resulted in a twofold increase in microvascular blood volume. Moreover, AT1R blockade with losartan results in a further increment in blood flow and glucose extraction, which was abolished by the NO inhibitor NG-nitro-L-arginine methyl ester (L-NAME). Not surprisingly, AT2R blockade decreased microvascular blood flow by 80%,

with a parallel drop in glucose extraction.[50] Additionally, this study examined the interaction between Ang II receptors and skeletal muscle insulin-stimulated vasodilation and glucose uptake. During continuous insulin infusion, AT1R blockade did not have an additive effect on these parameters. On the other hand, AT2R antagonism resulted in whole-body insulin resistance and attenuation of microvasculature recruitment. These changes paralleled a decrease in plasma NO and skeletal muscle eNOS activation (as evaluated by eNOS phosphorylation).[51]

Lastly, RAAS exerts direct effects on the skeletal muscle vasculature to impair endothelial-mediated vasorelaxation.[5,18] For example, Ang II, acting via AT1R signaling, has been shown to impair insulin metabolic signaling and glucose uptake in cultured myotubules through activation of the NAPDH oxidase and increased oxidative stress.[52] The resultant increase in oxidative stress promotes the activation of redox-sensitive serine kinases that, in turn, promote the serine phosphorylation of IRS and result in its proteasomal degradation and reduced downstream insulin metabolic degradation. This reduced insulin metabolic signaling also results in attenuated activation of eNOS, increased destruction of NO, and enhanced vascular smooth muscle intracellular calcium and calcium sensitization (Fig. 1).[3,5,16]

Insulin resistance in the vasculature

As previously noted, obesity and DM2 are associated with impaired endothelial-dependent vasodilation.[53] After a mixed meal, lean humans exhibit increased brachial blood flow and forearm microvascular recruitment, and obese subjects have a blunted response in the post-prandial state despite hyperinsulinemic conditions.[34,54] This blunted response is related to insulin resistance and associated endothelial dysfunction, which contributes to increased CVD.[55] Endothelial dysfunction manifests as decreased NO bioavailability, abnormal vasoreactivity, increased oxidative stress, and increased expression of inflammatory, immune and pro-thrombotic mediators.[55] The impaired endothelial-dependent vasodilation of skeletal muscle in insulin-resistant models correlates with arteriolar remodeling and increased stiffness of the vessel wall.[56] Resistance to the vascular effects of insulin has been shown to selectively involve the PI3K–Akt–NO pathway with intact activation of the MAPK pro-mitotic pathway.[55] Induction of insulin resistance in cultured endothelial cells, via blockade of the PI3K pathway, results in blunted production of NO with increased expression of pro-atherosclerotic molecules.[8] In the vasculature of obese fa/fa rats, tyrosine phosphorylation of IR and IRS-1/IRS-2, as well as activation of PI3K and Akt, are markedly decreased, while basal phosphorylation of MAPK is augmented.[57] The spontaneously hypertensive rats exhibit selective insulin resistance in the vasculature with decreased insulin-stimulated NO production but enhanced ET-1 secretion.[58] Finally, although they are not the focus of the present paper, factors other than impaired vasodilatation are important in the pathogenesis of obesity and insulin resistance–related vascular dysfunction, such as impaired nutrient and insulin delivery due to abnormalities in extracellular matrix.

Lipotoxicity

Lipotoxicity is a common finding in DM2 and obesity, and its effects have been extensively characterized in the vasculature. Rodents subjected to intravenous infusion of lipids and heparin demonstrated decreased muscle glucose uptake and blunted insulin-mediated microvascular recruitment in skeletal muscle.[31] Similarly, in healthy humans, lipid infusion causes systemic insulin resistance and decreased forearm microvascular recruitment.[59] Both lipotoxicity and glucotoxicity converge in augmented production of diacylglycerol (DAG) and ceramides.[60] In the vasculature, DAG activates protein kinase C (PKC) isoforms β1 and β2.[61] Protein kinase C (PKC) isoform β is known to inhibit insulin effects in the vasculature.[62] In insulin-resistant Zucker fatty rats, activation of PKC isoform β relates to decreased Akt-dependent eNOS activation.[62] Conversely, treatment with ruboxistaurin, a PKCβ inhibitor, restored the insulin-induced eNOS activation in fatty rats.[62] Overexpression of PKC-β2 and concomitant ApoE knockout (KO) in mice fed a high-fat diet resulted in a significant impairment in insulin-stimulated Akt/eNOS activation, with augmented leukocyte–endothelial binding and increased ET-1 production.[63] Aortic atherosclerosis was 70% greater in the KO rodents when compared to control mice.[63] Despite those findings, whole-body insulin sensitivity and blood pressure were not different.[63] Recently, Tabit and colleagues demonstrated that PKCβ expression is markedly increased in endothelial cells isolated from DM2 patients. Insulin-induced eNOS activation was severely depressed in cells from diabetic patients, with a parallel increased in oxidative stress and expression of inflammatory markers.[64] *Ex vivo* use of a PKCβ inhibitor restores eNOS activation by insulin.[64]

The deleterious effects of lipotoxicity also involve the activation of inflammatory pathways.[65] Jang and colleagues examined the effect of Toll-like receptor 2 (TLR2) activation by saturated fatty acids in vascular insulin resistance.[65] In vascular cultured endothelial cells, exposure to palmitate results in activation of pro-inflammatory molecules with impaired insulin-driven production of NO. This effect was ameliorated by the TLR2 knockdown. Furthermore, mice that lack TLR2 were protected from whole-body and endothelium insulin resistance upon high-fat feeding.[65]

Vascular insulin resistance: evidence from transgenic models

The molecular defects that lead to impairment of insulin signaling in the vasculature have been explored with different KO and overexpression models. Mice with vascular endothelial IR knockout have impaired ET-1 and eNOS expression.[66] Interestingly, using a low salt–diet feeding, mice lacking endothelial IR became insulin resistant relative to controls,[66] and the high salt diet–feeding impaired insulin sensitivity in the control group but not in the KO group.[66] Systemic insulin sensitivity, assessed via a hyperinsulinemic–euglycemic clamp, was not different under basal condition in KO mice.[66]

Investigators utilizing a transgenic mouse with endothelium overexpression of a dominant-negative mutant human IR containing a mutation in the tyrosine kinase domain (ESMIRO) demonstrated normal whole-body insulin sensitivity but decreased vasodilation in response to acetylcholine (Ach).[67] These changes occurred in concert with blunted vascular responses

to insulin stimulation and augmented production of superoxide derived from the Nox2 isoform of NAPDH oxidase. Interestingly, *ex vivo* and *in vivo* pharmacological inhibition of Nox2 under insulin-resistant conditions resulted in decreased production of ROS and improvement in Ach-mediated vasodilation.[68] In this study, genetic deletion of Nox2 in the setting of the ESMIRO model resulted in improved aortic response to Ach and decreased oxidative stress.

In the ApoE KO mice fed a high-fat diet, the classical model of atherosclerosis, additional KO of IR in endothelial cells resulted in accelerated atherosclerosis in areas of turbulent flow with a parallel increase in endothelial oxidative stress.[69] Intriguingly, this model did not exhibit whole-body insulin resistance. As noted earlier, the IGF1-R is also involved in the actions of insulin in the vasculature.[15] IGF1-Rs heterodimerize with the IR and form a hybrid with a 50-fold weaker affinity for insulin than for IGF-1.[70] The presence of this hybrid receptor has been correlated in humans with hyperinsulinemia and obesity.[71,72] Others used a rodent model of whole-body haploinsufficiency of IGF1-R and endothelium-specific deletion of IGF1-R to demonstrate that IGF1-R negatively regulates insulin sensitivity (evaluated by insulin stimulated eNOS phosphorylation and NO production).[73] On the other hand, targeted endothelial models of human IGF1-R overexpression resulted in an increased vasoconstrictor response to phenylephrine, endothelial insulin resistance, reduced NO bioavailability, and enhanced endothelium regeneration after denuding wire injury (Fig. 1).[74]

Insulin-resistant conditions coexist with hyperinsulinemia for as long as pancreatic β cells are able to increase production of insulin.[2] Compensatory hyperinsulinemia is not a benign process, and increasing data support its role in the pathogenesis of vascular disease.[75] To this point, investigators have explored the role of forkhead box, sub-group O (FoxO) transcription factors in the pathogenesis of vascular disease.[76] FoxO proteins are classically considered to impair insulin signaling, and are known to decrease eNOS transcription and promote oxidative stress. In physiologic conditions, FoxO proteins are excluded from the nuclei upon phosphorylation by Akt.[75] Earlier studies showed that mice lacking low-density lipoprotein (LDL) receptors, fed a high fat diet, exhibited insulin resistance and atherosclerosis with reduced aortic IRS-1, IRS-2, Akt, eNOS, FoxO1, and FoxO3a phosphorylation.[76] Mice lacking LDL receptors in addition to triple knockout of *Foxo1*, *Foxo3a*, and *Foxo4* had restored vascular endothelial-dependent vasodilation and decreased atherosclerotic lesions in the coronary artery by 80% without an effect on glucose homeostasis.[76] Endothelial cells derived from these mice exhibited increased NO production, decreased expression of adhesion molecules, and reduced oxidative stress.[76] Further work from this group described the metabolic and vascular phenotype of triple *Foxo* knockout mice under conditions of regular chow and LDL receptor expression. Surprisingly, the of triple *Foxo* knockout mice exhibited insulin resistance and impaired glucose homeostasis, with decreased insulin signaling in the liver but not in muscle or adipose tissue. Further examination demonstrated that insulin resistance in this model is secondary to hyperinsulinemia-driven increased NO production by the endothelium and correlates with nitration of insulin-signaling cascade proteins in the liver.[77]

RAAS-system activation and insulin resistance in the vasculature

RAAS activation mediated by signaling through AT1R, as well as through the mineralocorticoid receptor (MR) promote fibrosis, remodeling, proliferation, migration, and hypertrophy in vascular tissues.[42,78] Again, ROS production plays a critical role in this process, through Rac-mediated activation of NADPH oxidase,[42] and triggers several pro-inflammatory pathways, including Janus activated kinase (JAK), Rho/RhoK, and MAPK.[79,80] Mineralocorticoids are typically elevated in the setting of insulin resistance and obesity[78] and result in impaired insulin sensitivity in the vasculature in healthy humans.[81] Furthermore, mild increases in aldosterone levels, even within currently accepted normal ranges, may result in increased cardiovascular mortality in individuals with coronary artery disease.[16]

Aldosterone promotes insulin resistance by increasing the proteasomal degradation of IRS-1 in a c-Src- and ROS-mediated mechanism in vascular tissue.[82] Ang II also reduces IRS-1 levels in VSMC via a similar mechanism involving c-Src and ROS.[83] As a result, insulin signaling through the PI3K–Akt pathway is impaired, leading to insulin resistance. Importantly, aldosterone-induced vascular dysfunction is prevented by blockade of the MR as well as use of antioxidants and Src inhibition, thus underscoring the participation of signaling through MR. In turn, serine phosphorylation of IRS molecules can be the result of several pathways involved in insulin resistance, including the mTOR/S6K1, JNK, IκB kinase (IKK), tumor necrosis factor α (TNF-α) and PKCθ.[84] As previously mentioned, aldosterone also induces vascular insulin resistance and dysfunction through upregulation and activation of IGF1-R and its hybrid insulin/IGF1-R in VSMC and in rats, which was prevented by treatment via MR blockade and antioxidants.[85]

Therapeutic strategies

Diet and exercise

Increased physical activity on a regular basis has been shown to improve insulin sensitivity in the vasculature. In a spontaneously hypertensive insulin-resistant rat model, vascular insulin sensitivity, assessed as insulin-induced vasodilation of the mesenteric vasculature, improved significantly after 10 weeks of exercise and was correlated with a reduction in blood pressure.[86] Furthermore, exercise decreased the expression and activity of G protein–coupled kinase-2 (GRK-2).[86] GRK-2 is known to inhibit insulin signaling in classical insulin-sensitive tissues like liver and fat,[87,88] as well as in vascular endothelium.[89] Similarly, in the Otsuka Long–Evans Tokushima fatty (OLEFT) rat, a model of hyperphagia-induced obesity and insulin resistance, voluntary running resulted in improved insulin response in the microvasculature of skeletal muscle.[90]

Caloric restriction leading to weight loss, through decreased visceral fat, improves endothelial function in conduit and resistance arteries of overweight and obese adults. Changes are related to an increase in NO bioavailability.[91] The effects of lifestyle modifications are also seen once obese patients become diabetic.[92] Six months of caloric restriction (500 calorie negative balance) and 150 minutes of weekly exercise augmented brachial artery flow–mediated dilation along with an improvement in systemic insulin

sensitivity and decreased inflammatory markers.[92] Weight loss also decreases circulating levels of ET-1 in obese males.[93]

Insulin sensitizers: 5′-adenosine monophosphate–activated kinase activation/metformin

Metformin, a commonly used antidiabetic medication and insulin sensitizer, has been shown to improve endothelial-dependent vasodilation independently of NO production in a rodent model of insulin resistance.[94] In contrast, in cultured aortic endothelial cells, metformin activated eNOS and increased NO production in a PI3K-dependent manner. The vascular effects of metformin requires 5′-AMP–activated kinase (AMPK), as mice lacking AMPK do not obtain these beneficial effects.[95] In women with polycystic ovary syndrome, another condition characterized by insulin resistance, metformin therapy improves flow-mediated dilation and decreases levels of ET-1.[96] In DM2 patients with coronary artery disease, metformin, which improves insulin sensitivity and reduces glucose liver output, has been shown to significantly decrease cardiovascular events in comparison with glipizide, which enhances insulin secretion by β cells.[97]

RAAS blockade

Four 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 in subjects with metabolic syndrome.[52] The vascular effects were potentiated by the use of an α-lipoic acid (an antioxidant). A similar study with quinapril, an angiotensin-converting enzyme, and an α-lipoic acid showed improvement in endothelial function and albuminuria with 8 weeks of treatment in hypertensive patients.[98]

Antioxidants

As previously discussed, the use of antioxidants in experimental conditions improves markers of insulin resistance in the vasculature and positively influences its function. From a clinical standpoint, however, not all interventions aimed at improving endothelial function with antioxidants in insulin-resistant conditions have yielded positive results.[99] Chen and colleagues examined the effects of high-dose vitamin C in vascular and systemic insulin resistance. Sadly, no beneficial effect was attained with this intervention, which according to the authors might have been linked to failure to reach therapeutic levels of vitamin C in DM2 patients relative to control individuals.[99]

Another intervention in DM2 patients comparing lipoic acid and vitamin C (low and high dose) showed that high doses of vitamin C and lipoic acid improved NO-mediated dilation.[100] Use of vitamin C or vitamin E for six months did not have a beneficial effect in the endothelial mediated vasodilation in DM2 patients, whereas it did improve endothelial function in type 1 diabetics.[101]

Polyphenols

Epigallocatechin gallate (EGCG), a green tea polyphenol, has been explored as a potential therapeutic agent against vascular insulin resistance.[102] In human aortic endothelial cells, EGCG decreases expression and secretion of ET-1.[103] Similarly, in a rodent model of

insulin resistance induced by high fat–diet feeding, EGCG treatment resulted in decreased insulin resistance, improved insulin-mediated vasodilation of mesenteric arteries, and decreased macrophage vascular infiltration.[104] Furthermore, in humans, green tea consumption has been associated with decreased risk of cardiovascular events and improvement in blood glucose control.[105,106]

Another polyphenol, hesperidin, also increases NO production in endothelial cells and decreases the expression of inflammatory markers.[107] Oral administration of hesperidin to patients with metabolic syndrome improves endothelial function assessed by flow-mediated dilation and decreases circulating levels of inflammatory markers.[107] The mechanisms underlying the effects of polyphenols on insulin sensitivity are largely unknown, and it has even been suggested that green tea can actually impair the absorption of some medications. Thus, additional studies in this area are warranted before polyphenols can be added to currently accepted medications.[108]

Gut-derived peptides: incretin hormones

Incretin hormones are produced in the gut in response to nutrients and act as insulin secretagogues.[109] However, their effects seem to extend beyond being antihyperglycemic agents.[109] Incretins currently used clinically are the glucagon like peptide-1 (GLP-1) analogs and the dipeptidyl peptidase-4 (DDP-4) inhibitors (DDP-4 metabolizes GLP-1). The vascular effects of incretins have been explored in different models. Initially, the cardiovascular vascular effects of GLP-1 were described in models of cardiomyopathy and ischemic reconditioning.[110–112] More recently, the effect of GLP-1 and GLP-1 receptor activation of skeletal muscle vasculature and endothelial cells have been explored. Lie and colleagues demonstrated that GLP-1 treatment on bovine aortic endothelial cells results in increased Akt and eNOS phosphorylation, as well as cyclic AMP (cAMP)-dependent protein kinase (PKA) activity. *In vivo*, using adult Sprague–Dawley rats, GLP-1 infusion for 2 hours resulted in marked increments of skeletal muscle blood flow and glucose extraction. These effects were abolished by co-administration of L-NAME.[113] In hypertensive rodents, sitagliptin, a DDP-4 inhibitor, reduced blood pressure, improved renal endothelial vasodilation, and increased eNOS activation in an AMPK-dependent fashion. These effects were mediated via the GLP-1 receptor, as they were abolished by the use of an antagonist.[114] In contrast, treatment with alogliptin (another DDP-4 inhibitor), resulted in aortic relaxation in an endothelium-dependent manner, but a GLP-1 receptor antagonist did not blunt the effect.[115] As recently reviewed,[115] DPP-4 inhibitors may also exert beneficial cardiovascular and renal effects by reducing inflammation and maladaptive immune modulation.

A recent randomized, placebo-controlled study examined the role of saxagliptin, a DDP-4 inhibitor, in 16,492 patients with DM2 and increased cardiovascular risk. There was no cardiovascular protective effect (primary outcome), but the use of the DDP-4 inhibitor improved glycemic control and microalbuminuria while increasing the risk of heart failure hospitalization and hypoglycemia.[116] Another recent double-blinded trial explored the effect of alogliptin after an acute coronary event in 5380 patients with DM2. The DDP-4 inhibitor had no effect in the rate of cardiovascular events in this population.117

Summary

Insulin resistance and hyperinsulinemia play a major role in the pathophysiology of obesity and DM2, which are leading risk factors for hypertension and CVD. It has also become increasingly clear that insulin resistance not only affects tissues considered classic targets for insulin action, but also significantly affects cardiovascular tissue, leading to vascular dysfunction and contributing to atherosclerosis. Expanding knowledge about the mechanisms of vascular injury mediated by resistance to the metabolic actions of insulin has uncovered novel roles for inflammation, excessive oxidative stress, incretins, and inappropriate RAAS activation in the pathophysiology of CVD as it relates to obesity and DM2, which can to some extent be manipulated with therapeutic objectives. Among non-pharmacologic interventions, weight-loss strategies are of paramount importance. Moderate caloric restriction, as well as regular physical activity, has been demonstrated to improve parameters of insulin resistance and vascular function, and add to our already abundant pharmacologic alternatives. RAAS modulation focused on MR and AT1R blockade are established forms of treatment, whereas anti-inflammation, antioxidation, and insulin-sensitizing agents have an encouraging therapeutic potential. Further research is needed, however, in order to provide more effective and comprehensive management of CVD, which is beyond doubt the most important burden for public health systems worldwide.

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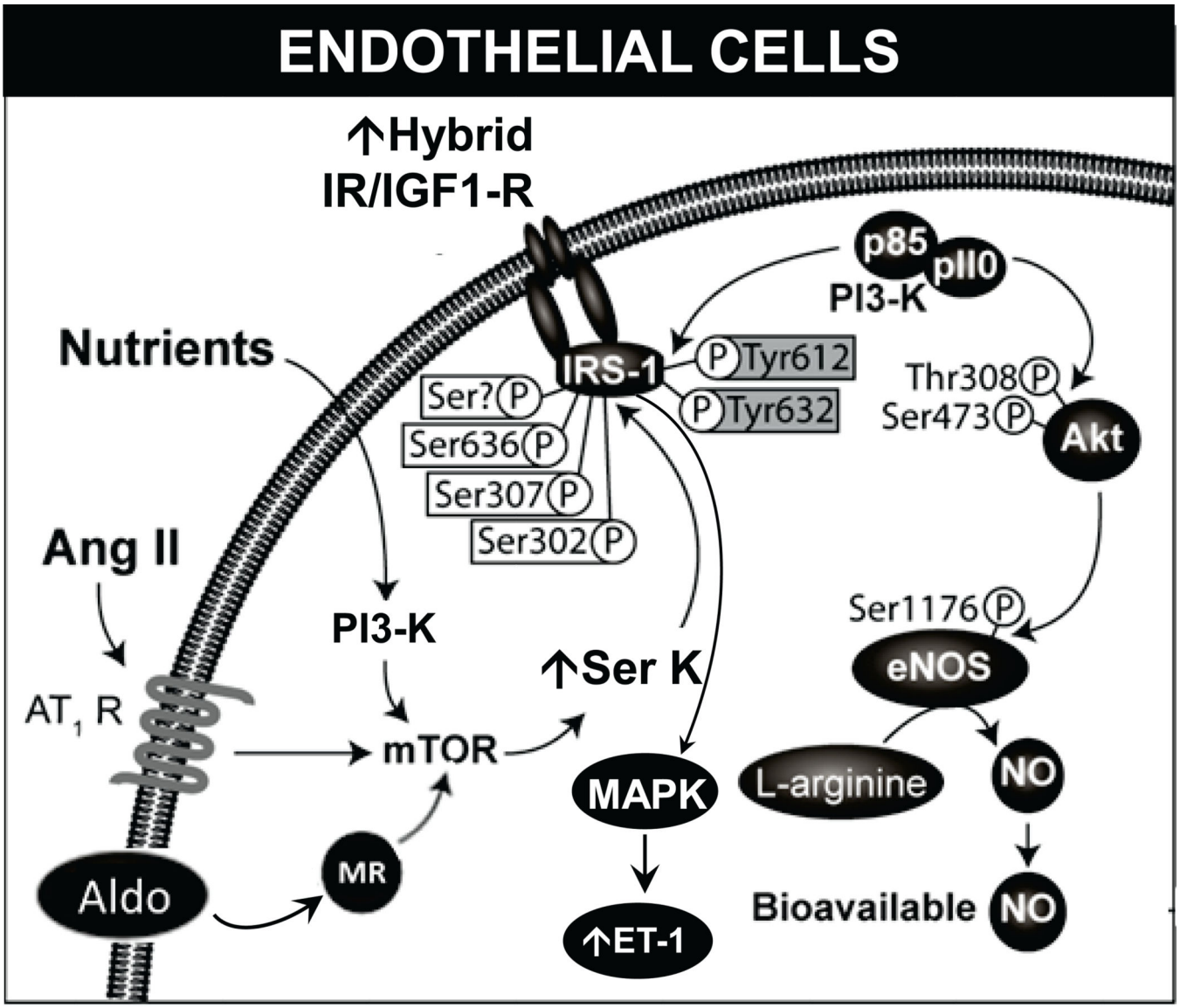

Figure 1.

Insulin effects on endothelial cells. Under normal conditions, stimulation of IR results in activation of the PI3K–Akt pathway, eNOS phosphorylation, and vasodilation. Insulin resistance induced by RAAS activation and excess nutrients causes increased serine phosphorylation of insulin receptor substrate and metabolic signaling with uninhibited activation of mitogenic and growth pathways. Aldo, aldosterone; Ang II, angiotensin II; AT1R, angiotensin II type 1 receptor; eNOS, endothelial NO synthase; ET-1,endothelin-1; IGF1-R, insulin-like growth factor-1 receptor; IR, insulin receptor; mTOR, mammalian target of rapamycin; MR, mineralocorticoid receptor; MAPK, mitogen activated protein kinase; NO, nitric oxide; PI3K, phosphatidylinositol 3-kinase; p, phosphorylation; Akt, protein kinase B; Ser, serine; Ser K, serine kinase; thr, threonine; Tyr, tyrosine.