

Review

Diabetes pathogenesis and management: the endothelium comes of age

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Endothelium, acting as a barrier, protects tissues against factors that provoke insulin resistance and type 2 diabetes and itself responds to the insult of insulin resistance inducers with altered function. Endothelial insulin resistance and vascular dysfunction occur early in the evolution of insulin resistance-related disease, can co-exist with and even contribute to the development of metabolic insulin resistance, and promote vascular complications in those affected. The impact of endothelial insulin resistance and vascular dysfunction varies depending on the blood vessel size and location, resulting in decreased arterial plasticity, increased atherosclerosis and vascular resistance, and decreased tissue perfusion. Women with insulin resistance and diabetes are disproportionately impacted by cardiovascular disease, likely related to differential sex-hormone endothelium effects. Thus, reducing endothelial insulin resistance and improving endothelial function in the conduit arteries may reduce atherosclerotic complications, in the resistance arteries lead to better blood pressure control, and in the microvasculature lead to less microvascular complications and more effective tissue perfusion. Multiple diabetes therapeutic modalities, including medications and exercise training, improve endothelial insulin action and vascular function. This action may delay the onset of type 2 diabetes and/or its complications, making the vascular endothelium an attractive therapeutic target for type 2 diabetes and potentially type 1 diabetes.

Keywords: diabetes, insulin resistance, endothelium, vascular function

Introduction

Drastic increases of obesity and type 2 diabetes (T2D) prevalence, stemming primarily from caloric surplus, have created a worldwide public health crisis. In the United States, the number of people with diagnosed diabetes rose from 11 million in 2000 to 34.2 million in 2018 ([Centers for Disease Control and Prevention, 2020](#)). Individuals with type 1 diabetes (T1D) are also increasingly impacted by weight gain and insulin resistance.

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Diabetes profoundly and adversely affects the vasculature, increasing the prevalence of atherosclerotic, arteriosclerotic, and microvascular diseases. Throughout the vascular tree, the vascular endothelium in particular serves as both a barrier and a facilitator of nutrients, oxygen, hormone, and peptide exchange between the circulation and the tissues being perfused. For example, it can protect somatic tissues from exposure to excess of lipid nutrients present in the circulation by regulated expression of fatty acid transfer proteins ([Yucel and Arany, 2019](#)). It is increasingly appreciated that the endothelium is a first-line body defense against excess nutrients, inflammatory cytokines, and other environmental and endogenous factors that contribute to the development of insulin resistance.

While diabetes pathophysiology varies amongst affected people, most eventually develop and many succumb to

cardiovascular complications. Mounting evidence confirms that endothelial insulin resistance and endothelial dysfunction coexist, mutually perpetuate, and underlie the pathogenesis of T2D and the associated macrovascular and microvascular complications of diabetes. As such, the vascular endothelium has emerged as a possible therapeutic target for diabetes prevention and management.

Insulin action and the endothelium

Endothelium actively regulates vascular health and function, and insulin is an important physiological factor in this process. Endothelium expresses abundant insulin, insulin-like growth factor 1 (IGF-1), as well as the hybrid insulin/IGF-1 receptors (King and Johnson, 1985; Li et al., 2005, 2009). Upon binding to these receptors, insulin initiates proximal components of the canonical downstream signaling pathways within the endothelial cells: (i) the phosphatidylinositol 3-kinase (PI3-K) signaling pathway, which regulates the activity of endothelial nitric oxide (NO) synthase (eNOS) and the production of NO, a potent vasodilatory and vasoprotective compound, and (ii) the mitogen-activated protein kinase (MAPK) pathway, which mediates cell proliferation, adhesion molecule production, and the expression and secretion of endothelin-1. It is the crosstalk between these two signaling pathways that dynamically modulates vascular tone, vascular cell proliferation, adhesion molecule production, and platelet aggregation to the endothelium (Muniyappa et al., 2007; Figure 1). It is important to note that endothelial cells exhibit different biochemical and physiologic properties depending on their tissue and vessel of origin (Aird, 2007) with remarkable endothelial cell transcriptome and transcriptome diversity at each vascular bed allowing for these phenotypic differences (Cleuren et al., 2019; Kalucka et al., 2020). These differences in gene expression based on environment present a unique challenge for endothelial cell culture

studies. The outcomes of insulin's endothelial actions depend on insulin's concentration, duration of exposure, and the size and location of the blood vessel.

Insulin at physiological concentrations only binds to and activates the insulin receptors, but at supra-physiological or pharmacological concentrations also stimulates the IGF-1 receptors and the hybrid insulin/IGF-1 receptors (Li et al., 2005). This is significant in that insulin at high concentrations is able to stimulate vascular cell adhesion molecule 1 (VCAM-1) production via the IGF-1 receptor (Li et al., 2009). High concentrations of insulin also downregulate cell surface insulin receptors, reducing subsequent insulin responses.

The endothelium serves unique functions in different vascular beds, yet insulin is a key regulator at all sites. Insulin in health generally has a vasodilatory action on vessels of varying size. The elastic conduit arteries expand in response to rising pressure from cardiac ejection to maintain a stable arterial pressure and insulin acts to regulate arterial compliance. Insulin infusion in healthy people acutely enhances muscular femoral artery vasodilation in response to methacholine (Steinberg et al., 1996) and reduces augmentation index (a measure of arterial stiffness) (Tamminen et al., 2001, 2002). In resistance arteries, which determine vascular resistance and blood flow distribution to end-organs (Intengan and Schiffrin, 2000), insulin acutely causes vasodilation, lowering vascular resistance and increasing tissue blood flow (Baron, 1994). The microvasculature, which includes small arterioles, capillaries, and small venules, maintains tissue health and function by providing adequate tissue perfusion. In the resting state, approximately only one-third of skeletal muscle capillaries (Honig et al., 1982) and ~50% of myocardial capillaries (Jayaweera et al., 1999) are perfused. Microvascular perfusion arises in response to increased metabolic demands. Numerous studies in humans and rodents have confirmed that insulin enhances microvascular perfusion in the skeletal muscle (Barrett et al., 2009, 2011), cardiac muscle (Liu, 2007, 2011; Chai et al., 2011; Tan et al., 2018), adipose tissue (Karpe et al., 2002; Sjøberg et al., 2011), brain (Fu et al., 2017), and skin (Meijer et al., 2012; de Boer et al., 2014). In the muscle, insulin-mediated microvascular recruitment occurs rapidly, within 5–10 min, and precedes insulin-stimulated glucose disposal (Vincent et al., 2003, 2004).

Muscle perfusion is important for insulin's metabolic action. In humans, insulin-mediated changes in leg blood flow parallel those of leg glucose disposal, and inhibiting NO synthase activity reduces insulin-mediated glucose uptake in the leg by ~25% (Steinberg et al., 1994, 2000). In rodents, abolishing insulin's vascular action reduces insulin-stimulated muscle glucose uptake by ~40% (Vincent et al., 2002, 2003). This is not surprising as insulin must initially be delivered to the capillaries supplying the myocytes and then transported through the capillary endothelium to enter the interstitial space to act on its receptors in the myocyte membrane. Studies have confirmed that insulin delivery to the muscle is rate-limiting for insulin action in the muscle (Chiu et al., 2008) and it is the interstitial, not the plasma, insulin concentrations that correlate

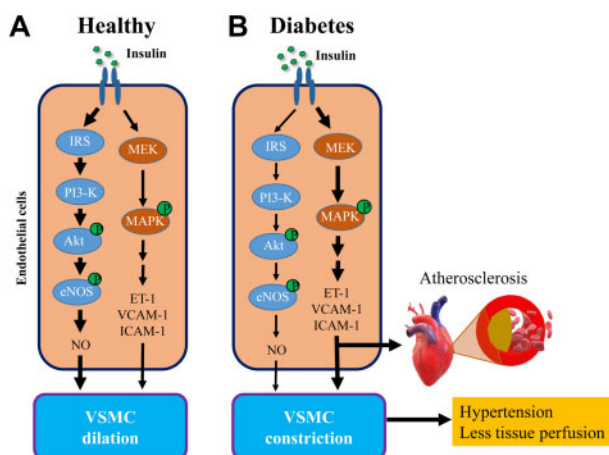


Figure 1 Endothelial insulin signaling and the associated consequences in health and diabetes. ET-1, endothelin-1; ICAM-1, intercellular adhesion molecule 1; MEK, MAPK kinase.

with insulin's metabolic action (Castillo et al., 1994). Insulin enhances its own delivery to the muscle by increasing muscle microvascular perfusion (Vincent et al., 2004) and facilitating its own trans-endothelial transport (Wang et al., 2008).

Insulin resistance and the endothelium

To clarify the attributes and impact of 'endothelial insulin resistance' from both biochemical and physiologic perspectives, several questions need to be addressed. Does endothelial insulin resistance arise passively as collateral damage propagated by insulin resistance in the metabolically dominant host tissue? Alternatively, does antecedent endothelial insulin resistance initiate or exacerbate insulin resistance in metabolically dominant tissues hosting specific vascular beds? Finally, in response to the same environmental provocateurs that affect the muscle, liver, and adipose, does insulin resistance develop sooner within the endothelium than in somatic cells of the host tissue, and is it a necessary link?

It has been well-documented that endothelial insulin resistance is present in arteries and arterioles and that it co-exists with metabolic insulin resistance. While the outcomes of endothelial insulin resistance vary depending on the vessel size and location, the fundamental pathophysiology resides in a selective insulin resistance in the PI3-K pathway with the MAPK pathway being spared, resulting in a disordered insulin response between the two pathways and predisposing affected people to atherosclerosis, hypertension, and/or microvascular complications. Inasmuch as NO generation is critical for insulin's physiologic actions on the vasculature, and inhibiting its production negatively impacts both vascular relaxation and integrity. Countering insulin's stimulation of NO production is increased generation of endothelin-1, a potent vasoconstrictor, via the MAPK pathway (Oliver et al., 1991; Eringa et al., 2002). Insulin stimulates endothelial cells to produce other vasodilatory and vasoconstrictive compounds, but currently insulin's overall vasoactive actions have been explained principally based on the differential activity stimulation of these two downstream pathways (Jiang et al., 1999; Mather et al., 2002; Muniyappa et al., 2007) with endothelial insulin resistance characterized by selective decrease of insulin action on the PI3-K→protein kinase B (Akt)→eNOS→NO pathway while endothelin production is preserved or enhanced (Figure 1).

How does this biochemistry play out physiologically? In response to injected insulin, mice with endothelial specific insulin receptor deletion (VE-cadherin-cre:IR^{flox/flox}) have delayed activation of insulin signaling in the skeletal muscle, brown fat, and brain (Konishi et al., 2017), suggesting that normal endothelial cell insulin receptor function is required for plasma insulin to reach these target tissues. Over time, these mice develop obesity and hyperinsulinemia with increased leptin and impaired glucose tolerance, i.e. they develop an 'insulin-resistance' phenotype. Other investigators generated endothelium-specific knockout of insulin receptor substrate 1 (IRS1), IRS2,

or IRS1&2 using a Tie2-Cre promoter (Kubota et al., 2011). The Tie2-Cre:IRS2^{flox/flox} mice have decreased insulin signaling to eNOS in endothelial cells, impaired insulin-mediated glucose uptake by the muscle, and systemic insulin resistance. Importantly, insulin's vascular action to expand capillary volume in the skeletal muscle was also diminished as was insulin delivered to muscle interstitium. This collectively illustrates that impairing endothelial insulin signaling selectively in the PI3-K pathway is sufficient to produce metabolic insulin resistance. Interestingly, endothelium-selective knockout of IRS1 did not alter these variables but knockout of IRS1&2 caused even more pronounced metabolic and vascular abnormalities that could be reversed by restoring NO availability (Kubota et al., 2011).

Findings like these suggest a close interdependence between insulin's metabolic with its vascular actions and that lesioning vascular insulin action can clearly provoke metabolic insulin resistance. Interestingly, much of this effect seems to arise from impaired access of insulin to target tissues, particularly those with more restrictive endothelia (the brain and muscle) with less impact on the liver.

It is also true that tissue-specific null lesions of the insulin receptor in metabolically dominant tissues such as the muscle (Brüning et al., 1998) and liver (Michael et al., 2000) produce mice with a metabolic syndrome-like phenotype. Adipose tissue is an exception, with tissue-specific knockout of the insulin receptor in adipose tissue protecting against obesity and glucose intolerance (Blüher et al., 2002). Vascular insulin action has not been well studied in these murine models.

High-fat diet (HFD) provokes both metabolic and vascular insulin resistance in rodents. Several studies have carefully examined the time course for the development of both metabolic and vascular insulin resistance in response to a HFD. In mice initiating a HFD, impaired vascular insulin signaling appears within one week, and then diminished insulin signaling in the muscle and liver in 4–8 weeks and in adipose tissue in 14 weeks (Kim et al., 2008). In rats, microvascular insulin resistance was observable three days after switching animals to a HFD, while impaired insulin-mediated glucose disposal was seen between one and two weeks of HFD (Zhao et al., 2015). These and other observations led us to suggest that the endothelium could be an 'early responder' to environmental changes, perhaps in part due to its privileged position interposed between the circulation and insulin-responsive tissues (Barrett and Liu, 2013). Using the skeletal muscle as an example, all circulating insulin resistance inducers such as pro-inflammatory cytokines secreted and free fatty acids released from the adipose tissue would have to meet with endothelial cells first prior to acting on the myocytes (Figure 2).

T2D and vascular dysfunction

Endothelial insulin resistance and dysfunction are present in all arterial beds in people with T2D, result in vascular

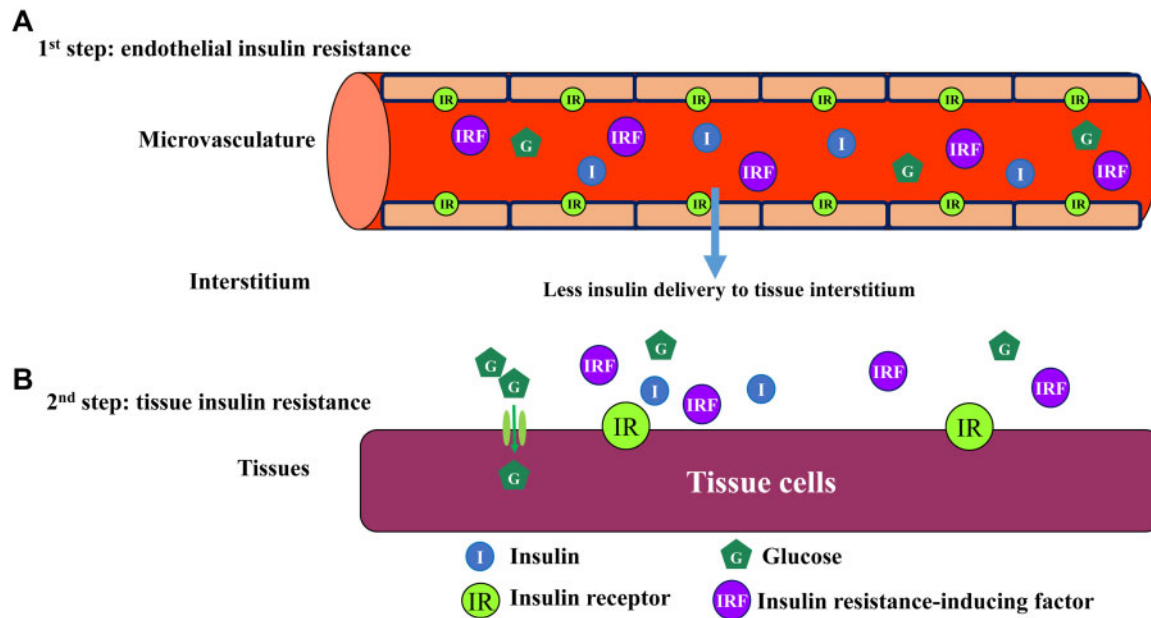


Figure 2 Insulin resistance in endothelium and tissue: a two-stepped process.

dysfunction, and predispose people to atherosclerosis, hypertension, heart failure, and metabolic disarray. While metabolic control clearly predicts coronary artery disease (CAD) in people with T2D, the importance of insulin resistance has been convincingly demonstrated in the Botnia study where people with metabolic syndrome had a 3- to 6-fold increase in the risk for CAD, stroke, and cardiovascular mortality (Isomaa et al., 2001).

T2D is associated with impaired endothelium-dependent, flow-mediated dilation (FMD) (Henry et al., 2004) and insulin-mediated NO-dependent vasodilation (Steinberg et al., 1994), particularly in those with longer diabetes duration (Naka et al., 2012). It is important to note that vascular dysfunction usually occurs early during the development of T2D and the outcomes vary based on the segment and location of the blood vessel affected. In a population-based prospective study, endothelium-dependent vasodilation in resistance arteries, but not in the brachial conduit artery, was associated with 5-year risk of a composite end-point of death, myocardial infarction, or stroke independently of major cardiovascular disease (CVD) risk factors (Lind et al., 2011). Arterial stiffness, an independent predictor of atherosclerotic CVD diseases, occurs early during diabetes development such as in the impaired glucose metabolism state (Stehouwer et al., 2008), and there is a marked resistance of the ability of insulin to decrease arterial stiffness in humans with obesity or metabolic syndrome (Westerbacka et al., 1999, 2001). Even in apparently healthy humans, such as those included in the Rotterdam study, arterial stiffness (aortic pulse wave velocity) independently predicted CAD and stroke (Mattace-Raso et al., 2006).

Endothelial insulin resistance and dysfunction are also present in the microvasculature in T2D. The Maastricht Study provided evidence of generalized microvascular dysfunction in

people with prediabetes and T2D (Sørensen et al., 2016). Furthermore, insulin-mediated microvascular perfusion is clearly impaired in the skeletal muscle of obese and diabetic animals (Wallis et al., 2002; Clerk et al., 2007) and in the cardiac muscle of humans with T2D (Jagasia et al., 2001). Even in people with class 1 obesity (body mass index between 30 and 35 kg/m²), insulin-mediated microvascular perfusion is absent in the skeletal as well as cardiac muscle (Wang et al., 2019). The abdominal subcutaneous adipose tissue microvascular recruitment response to glucose ingestion is present in healthy individuals but not in people with T2D (Hu et al., 2018). Importantly, in response to a mixed meal, myocardial blood flow increased by 70% in healthy humans but decreased by almost 40% in patients with T2D (Scognamiglio et al., 2005b, 2006).

Vascular dysfunction of the resistance arteries and the microvasculature reduces tissue blood flow and perfusion. Adding to the insult is a reduction of the capillary density in T2D, a phenomenon called capillary rarefaction. The degree of capillary rarefaction correlates with the severity of insulin resistance (Lillioja et al., 1987; Solomon et al., 2011), likely due to aberrant expression and action of the vascular endothelial growth factor family of proteins, which trigger muscle capillary regression. The combination of microvascular dysfunction and capillary rarefaction further decreases tissue perfusion and the endothelial surface area availability for substrate exchanges. Several important areas require further investigation in T2D including mechanisms for initial endothelial dysfunction and whether this varies at each vascular bed, noninvasive biomarkers to determine increased risk of endothelial dysfunction, and mechanisms of CVD benefits for medications with favorable cardioprotective outcomes.

The vascular aspect of non-insulin glycaemic agents

Most medications used in T2D management have been shown to impact vascular function, either directly acting on the endothelium or indirectly via improving glycemia, oxidative stress, and insulin resistance. While the UGDP study in 1970 suggested that the sulfonylurea class might increase cardiovascular mortality, more recent UKPDS showed no such evidence in T2D. Most recently, the CAROLINA study demonstrated no deleterious impact on cardiovascular events with glimepiride (Rosenstock et al., 2019). Overall, sulfonylureas exert a neutral to modest effect on the vasculature, likely via improving glycemia.

Metformin

Metformin's pleiotropic effects at multiple tissues result in several mechanisms of glucose lowering and improved endothelial function, which remain to be fully elucidated (Foretz et al., 2019). While it is widely accepted that metformin inhibits mitochondrial respiratory chain complex 1 and thereby inhibits hepatic gluconeogenesis, the role of AMP-activated protein kinase (AMPK), initially believed to be a key mediator of these glucose-lowering effects, has become controversial over the last 10 years (Foretz et al., 2014) and the mechanism of vascular benefits are not completely defined. As the first-line therapeutic agent in T2D owing to its cardiovascular benefits, tolerability, and low cost, metformin improves endothelium-dependent vasodilation in patients with T2D (Mather et al., 2001) and their first degree relatives with metabolic syndrome (de Aguiar et al., 2006), independent of glucose-lowering effects. In HFD-fed rats, metformin restores femoral artery blood flow and insulin-mediated microvascular perfusion (Bradley et al., 2019). In people with T2D, metformin improves post-prandial muscle and adipose microvascular responses (Jansson et al., 1996). Although the underlying mechanisms for improved conduit artery and microvascular function remain to be fully elucidated, increased eNOS activation, reduced oxidative stress, and inhibited inflammatory signaling may in part explain them by mechanisms both dependent (Kukidome et al., 2006) and independent of AMPK (Kelly et al., 2015).

Thiazolidinediones

Thiazolidinediones activate the peroxisome proliferator-activated receptor gamma (PPAR γ), may provide vascular protection (Mukohda et al., 2016), and improve vascular insulin sensitivity. Indeed, PPAR γ overexpression in human umbilical vein endothelial cells (HUVECs) increases eNOS protein expression and NO production while reducing endothelin-1 and inflammatory cytokines (Kong et al., 2019). Additionally, this class of drugs has been shown to reduce oxidative stress (Lu et al., 2010; Mukohda et al., 2016). Pioglitazone improves endothelial function in nondiabetic patients with major CVD risks (Campia et al., 2006) and in patients with newly diagnosed T2D and CAD (Sourij et al., 2006), independent of the observed

benefits of insulin sensitivity and β -cell function (Sourij et al., 2006). However, concerns regarding weight gain, bone fractures, and heart failure warrant consideration when using these agents. In an intriguing mouse model mimicking the deacetylated state of PPAR γ , rosiglitazone enhanced NO-dependent endothelial function and atherosclerosis protection while preventing edema and bone loss in these animals (Liu et al., 2020). This may have important drug-development implications if selective PPAR γ agents can retain the beneficial endothelial benefits while avoiding concerning adverse effects.

Glucagon-like peptide-1 receptor (GLP-1R) agonists

The discovery of abundant GLP-1R expression throughout the vasculature hinted an important role for GLP-1 in the regulation of vascular function (Ban et al., 2008; Drucker, 2016). We have shown that GLP-1 infusion vasodilates conduit arteries and increases muscle microvascular perfusion in healthy (Chai et al., 2012) and insulin-resistant (Chai et al., 2014) rats, likely through a protein kinase A–NO-mediated pathway (Chai et al., 2012; Dong et al., 2013). Consistent with these findings, we and others have shown that GLP-1 enhances acetylcholine-induced vasodilation, dilates conduit arteries, and increases skeletal and cardiac muscle microvascular perfusion in healthy humans (Subaran et al., 2014; Tan et al., 2018). Importantly, the vasodilatory action of GLP-1 is preserved in the microvasculature but not in the conduit arteries in people with obesity (Wang et al., 2019). In individuals with T1D, GLP-1 infusion attenuates endothelial dysfunction, inflammation, and oxidative stress induced by hypoglycemia and hyperglycemia (Ceriello et al., 2013b). However, the impact of GLP-1R agonists on FMD has shown mixed results even while attenuation of reactive oxygen species (ROS) is a more consistent finding (Wu et al., 2011; Hopkins et al., 2013; Lambadiari et al., 2018). Both liraglutide and exenatide have been shown to improve (Irace et al., 2013; Lambadiari et al., 2018) or have no effect on brachial artery FMD (Hopkins et al., 2013; Nomoto et al., 2015) in participants with T2D, despite a significantly decreased oxidative stress. Nonetheless, large-scale clinical studies have demonstrated the cardiovascular safety of GLP-1R agonists, and some are able to reduce major adverse cardiovascular events in T2D. How oxidative stress and inflammation contribute to the cardiovascular actions of GLP-1R agonists remains to be defined.

Dipeptidyl peptidase-4 (DPP4) inhibitors

DPP-4 inhibitors slow the degradation of GLP-1 and glucose-dependent insulinotropic polypeptide and raise their plasma concentrations 2-fold. Thus, one would expect considerable overlap between the endothelial effects of DPP4 inhibitors and GLP-1R agonists. Indeed, DPP4 inhibitors reduce ROS and inflammatory cytokine expression (Hu et al., 2013; Ishibashi et al., 2013). Some (Liu et al., 2012) but not all of these effects depend on GLP-1R activation (Hu et al., 2013). Despite

consistent benefits in cultured cells, DPP4 inhibitor clinical studies in T2D have shown inconsistent NO-dependent vasodilation outcomes, running the gamut from worsening [sitagliptin and alogliptin (Ayaori et al., 2013)], no change [sitagliptin (Nomoto et al., 2016)], to improving [vildagliptin (van Poppel et al., 2011) and sitagliptin (Nakamura et al., 2014)]. More study is needed to reconcile the myriad vascular benefits seen in pre-clinical studies with the basically cardiovascularly neutral large-scale clinical outcomes with this drug class.

Sodium glucose cotransporter (SGLT-2) inhibitors

This class consistently reduces major cardiovascular events and hospitalizations for heart failure in high CVD risk patients with T2D. Despite an absence of SGLT-2 on endothelial cells, SGLT-2 inhibitors may provide indirect or off-target endothelium benefits. Dapagliflozin acutely improves endothelial dysfunction and reduces aortic stiffness and oxidative stress in T2D patients (Solini et al., 2017). Even in humans with T1D, empagliflozin for 12 weeks improved FMD (Lunder et al., 2018). However, the DEFENCE study showed a non-significant trend toward improved FMD with 16 weeks of dapagliflozin when compared with metformin in participants with uncomplicated T2D, despite similar HbA1c benefit and a reduced oxidative stress marker (Shigiyama et al., 2017). SGLT-2 inhibitors have demonstrated early promising results at the microvasculature as well. In a cross-over study, 6 weeks of dapagliflozin significantly improved retinal capillary flow and arteriole remodeling in patients with T2D (Ott et al., 2017). These findings are aligned with data from insulin-resistant rodents demonstrating that empagliflozin improves endothelium-dependent vasorelaxation and cardiac microvascular perfusion (Zhou et al., 2018). In cultured human coronary arterial endothelial cells, empagliflozin and dapagliflozin restored NO bioavailability and abolished increased ROS generation due to TNF- α without a change in eNOS expression or signaling (Uthman et al., 2019). Validation of this model and mechanistic information for improved NO bioavailability and diminished ROS by SGLT-2 inhibitors, which appear partially independent of glucose-lowering effects, are clearly needed.

T1D and vascular dysfunction

Since individuals with T1D have a lower burden of traditional CVD risk factors compared to people with T2D, T1D offers a case study on the deleterious impact of glucose excursions and moderate insulin resistance on the vasculature. Individuals with T1D achieving target glycemic control have a nearly 3-fold higher cardiovascular mortality risk compared to the general population (Lind et al., 2014). There is a clear evidence of micro- and macrovascular dysfunction (Irace et al., 2017) and microvascular insulin resistance (Chan et al., 2012) in T1D. Even prior to microvascular complications, a substantial percentage of individuals with T1D fail to appropriately

increase cardiac microvascular perfusion in response to exercise (Scognamiglio et al., 2005a), and sublingual capillaries are diminished with loss of glycocalyx in children with T1D (Nussbaum et al., 2014). Dermal microvessel density changes are a less consistent finding (Adamska et al., 2019), but dysfunction in post-occlusive reactive hyperemia skin microvascular perfusion precedes retinal complications by at least 3 years (Santesson et al., 2017).

Insulin clamp studies have confirmed insulin resistance in individuals with T1D (DeFronzo et al., 1982; Bergman et al., 2012; Donga et al., 2015), with likely contributors including systemic hyperinsulinemia from exogenous insulin administration (Bergman et al., 2012; Gregory et al., 2020) and reduced muscle glucose and insulin delivery due to impaired insulin-mediated vasodilation (Baron et al., 1991). This has clear clinical consequences, as insulin resistance independently predicts microvascular complications and coronary artery calcification (Bjornstad et al., 2016) as well as mortality in people with T1D (Olson et al., 2002). Similar to T2D, endothelial dysfunction occurs early in the disease course, precedes the microvascular complications, and is a harbinger of future CAD in T1D (Costacou et al., 2005). More than a third of children (Järvisalo et al., 2004) or adolescents (Cé et al., 2011) with T1D exhibit evidence of endothelial dysfunction within 5 years of diagnosis.

Mechanistically, oxidative stress provides a link for the onset of endothelial dysfunction and atherosclerotic CVD in T1D (Ceriello, 2003). Among factors implicated, hyperglycemia is thought a major driver of vascular oxidative stress (Brownlee, 2001) with diminishing antioxidant capacity in concert (Marra et al., 2002). Hyperglycemia clearly impairs acetylcholine-induced vasodilation (Brouwers et al., 2010). On the opposite end of the spectrum, hypoglycemia also leads to ROS generation and impairs endothelium-dependent vasodilation. Exposure of human subcutaneous or mesenteric adipose arterioles to low concentrations of glucose resulted in increased ROS and blunted acetylcholine-induced vasodilation (Wang et al., 2012). Similarly in healthy humans, moderate hypoglycemia (mean glucose 52 mg/dl) diminished NO-dependent vasodilation as assessed by brachial artery FMD (Joy et al., 2015). Further, in individuals with T1D, hypoglycemic clamp increased plasma markers of oxidative stress and inflammation while reducing FMD (Ceriello et al., 2013a). Additionally, glycemic variability *per se* may contribute to increased oxidative stress and endothelial dysfunction in T1D, but this remains to be elucidated. Many major questions remain in the arena of T1D endothelial dysfunction and warrant our attention: what are the underlying mechanisms and are they unique at each vascular bed? How do hyperinsulinemia and glycemic variability *per se* contribute to microvascular dysfunction? Does microvascular dysfunction predict microvascular complications or cardiac diastolic dysfunction? Do biomarkers exist that could foretell these complications in T1D before they occur? Do medications known to provide cardiovascular benefits in T2D improve endothelial dysfunction and cardiovascular outcomes in T1D?

Exercise and vascular dysfunction in diabetes

Exercise delays T2D development, slows the progression of vascular diseases, and reduces cardiovascular morbidity and mortality associated with insulin resistance (Diabetes Prevention Program Research Group, 2002). Regular physical activity engenders myriad physiologically important hemodynamic changes (Padilla et al., 2011), and the exercise-induced improvement in vasodilatory function accompanies increased insulin sensitivity in obesity and T2D (De Filippis et al., 2006). Moderate daily exercise greatly improves insulin sensitivity, and even a single bout of low-intensity exercise is sufficient to temporarily enhance insulin sensitivity in people with obesity (Newsom et al., 2013). While it is known that physical fitness and endothelial function are independent determinants of insulin-stimulated blood flow in normal subjects (Utriainen et al., 1996), the amount of physical activity required to favorably elevate vascular insulin sensitivity has received less attention. Recent work suggests that interval exercise, alternating between brief periods of high- and low-intensity exercise, improves brachial artery FMD more than continuous exercise in sedentary people (Sawyer et al., 2016). However, not all studies agree with this notion (Shenouda et al., 2017). Our group recently compared the effects of interval vs. continuous exercise training for 2 weeks in older adults with prediabetes and found a similar lowering of post-prandial arterial stiffness but no effect on brachial artery FMD (Eichner et al., 2019; Malin et al., 2019).

At the microvascular level, exercise potently induces muscle microvascular recruitment. A gentle hand grip (at 25% of maximal strength) (Vincent et al., 2006) or low-frequency electric stimulation (Inyard et al., 2007, 2009) that does not increase conduit artery blood flow significantly increases muscle microvascular perfusion, which is associated with increased muscle insulin delivery and action (Holmäng et al., 1997; Inyard et al., 2007, 2009). A mathematic model projects higher interstitial insulin concentrations after exercise in people with or without diabetes (Derouich and Boutayeb, 2002).

Aerobic fitness directly correlates with insulin-stimulated microcirculatory function in healthy young adults following lipid infusion (Eggleston et al., 2007), suggesting that increasing exercise capacity induces adaptations and sensitizes the microvasculature to insulin. In obese Zucker rats, muscle contraction-mediated capillary recruitment and glucose uptake in the muscle are preserved (Wheatley et al., 2004). Somewhat surprisingly, 8 weeks of low-to-moderate intensity walking exercise (to simulate 150 min/week of moderate intensity exercise) in adults with T2D did not improve insulin-mediated muscle microvascular response despite raising popliteal artery FMD (Park et al., 2020). Moreover, there was no effect on pulse wave velocity (arterial stiffness). These results suggest that ‘brisk walking’ may not be adequate to rescue microvascular dysfunction in people with T2D. In contrast, resistance exercise training at moderate to high intensity for 6 weeks increases muscle microcirculatory blood flow during an oral glucose tolerance test in adults with T2D (Russell et al., 2017). Others have reported

that single legged exercise increased microvascular perfusion in response to insulin during the immediate post-exercise period in lean individuals and this was reversed by co-infusion of NO synthesis inhibitor (Sjøberg et al., 2017). This is important as NO is key for insulin-mediated capillary perfusion and exercise training improves microvascular responses to insulin. How exercise dose affects endothelial insulin sensitivity and function awaits further studies. The impact of diabetes medications on exercise tolerance deserves further attention in order to improve adherence to exercise interventions in both T2D and T1D.

Sex differences in vascular function

Sex differences in vascular function in people with T2D have garnered notable attention recently. In general, reports suggest that men are more affected by the microvascular, while women are more affected by the macrovascular consequences of T2D (Maric-Bilkan, 2017). Clearly, T2D contributes significantly to morbidity and mortality in both sexes, but existing evidence suggests that the effects of diabetes on vascular/cardiovascular health are worse in women than in men. Women with T2D tend to have worse cardiovascular consequences compared to nondiabetic female counterparts or men with and without diabetes (Mauvais-Jarvis et al., 2020).

In nondiabetic men and women, women typically develop CVD at an older age than men (Regensteiner et al., 2015b; Huebschmann et al., 2019). However, women with T2D develop CVD at an earlier age than nondiabetic women and have greater risk of death from myocardial infarction than men with T2D (Sprafka et al., 1991; Kannel, 2002; Regensteiner et al., 2015b). Similarly, women with T1D have a greater risk of coronary heart disease, stroke, and end-stage renal disease than men with T1D (Peters and Woodward, 2018). In addition, although early evidence of cardiovascular abnormalities is present in both sexes with uncomplicated T2D, women are more affected in this regard than men (Regensteiner et al., 2015a; Huebschmann et al., 2019). While both men and women with T2D have impaired peak oxygen consumption, endothelial function, and tissue blood flow, these impairments were more pronounced in premenopausal women with T2D than age-similar men with T2D (Regensteiner et al., 2015a; Huebschmann et al., 2019).

The reasons for sex (biological) and gender (psychosocial) differences in vascular health and disease in healthy women and men are not well known, and the additional effects of diabetes further complicate the (patho)physiology. Both the female and male hormones are implicated as contributing factors to sex differences in vascular health in people without diabetes. Estrogen enhances vasodilation through its effects on NO via multiple mechanisms, and it is thought that nondiabetic women are protected from CVD by the presence of estrogen until menopause. In the endothelial cell, estrogen binds to one of the three receptors (ER α , ER β , or G-coupled protein estrogen receptor-1 [GPER]), with ER α identified as particularly important

for vasodilatory endothelial function (Adlanmerini et al., 2014). 17 β -estradiol activates the same canonical signaling pathway, which mediates insulin-stimulated NO production, the PI3-K/Akt/eNOS pathway, but via Src kinase activation (Haynes et al., 2003). GPER agonism similarly allows a dose-dependent increase in eNOS phosphorylation in HUVECs by c-Src, EGFR, PI3-K, and ERK pathways (Fredette et al., 2018). Further in mice, estradiol via ER α binding activates cyclo-oxygenase to produce prostacyclin, a potent vasodilator and inhibitor of platelet aggregation (Egan et al., 2004). Estrogen also has antioxidant properties, reducing the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) enzyme and superoxide ROS (Khalil, 2013). Conversely, estradiol inhibits production of the vasoconstrictor endothelin-1 (David et al., 2001). While these estrogen actions produce beneficial vascular responses, these effects remain only partially understood given that the diminished study after the Women's Health Initiative revealed detrimental effects of estrogen on breast cancer and CVD in postmenopausal nondiabetic women. Moreover, it has also been proposed that androgens are detrimental in part because nondiabetic men have a higher prevalence of CVD than premenopausal women (Maric-Bilkan, 2017). Overall, the effects of the sex hormones on vascular health are complex and not well understood.

Not surprisingly, given the incomplete understanding of the effects of sex hormones on vascular health in people without diabetes, the effects of sex hormones on vascular health are even less understood in diabetes. The age-related estrogen benefits appear to be negated by diabetes as evidenced by a substantial increase in CVD in premenopausal women with diabetes. Both endothelial function and tissue perfusion are impaired in T2D (White et al., 2010; Huebschmann et al., 2019) and T1D (Cé et al., 2011). We have previously observed a clear difference in FMD, blood flow, and peak exercise capacity, despite a lack of significant difference in estradiol levels between women with or without T2D (Regensteiner et al., 2015a). However, the impairments in endothelial function may partially be explained by differences in the vascular effects of estradiol in women with and without diabetes. Although much remains to be defined at the endothelial cell level, hyperinsulinemia reduces vascular smooth muscle cell (VSMC) ER α expression through ER α hypermethylation, leading to VSMC proliferation and atherosclerosis in rodents, which was rescued by exogenous ER α administration via lentivirus infection (Min et al., 2016).

In summary, sex differences in vascular health in the setting of diabetes are a relatively recent area of focus, and women with T2D and T1D seem to fare worse than men with regard to CVD. The underlying reasons remain to be established, and the contributory roles of sex hormones, estrogen receptors, genetic, epigenetic, and other sex-specific factors warrant investigation.

Racial/ethnic disparities in vascular outcomes and function

Racial and ethnic disparities in microvascular and macrovascular diabetes complications clearly exist. In the United States,

black and Hispanic populations have a higher risk of retinopathy, nephropathy, cerebrovascular disease, and diabetes-related mortality compared to non-Hispanic white individuals (Haw et al., 2021). Many factors contribute to these differences and clear causality remains obscure, but reduced preventative care access (Clements et al., 2020) and diminished treatment to appropriate glycemic, blood pressure, and lipid targets are well documented in black and Hispanic populations (Stark Casagrande et al., 2013). Racial differences exist in endothelial function as well and studies have demonstrated impaired endothelial function across the lifespan of black individuals. Healthy young black, compared to white, adults have impaired cutaneous microvascular responses to local heating (Patik et al., 2018). In healthy middle-aged participants, black participants had diminished acetylcholine-induced forearm vasodilation compared to their white counterparts, on par with individuals with multiple vascular risk factors (Ozkor et al., 2014). The underlying mechanisms remain to be clarified. A race-specific discrepancy in endothelial cell oxidative stress was first reported in 2004 (Kalinowski et al., 2004). This study compared HUVECs donated by healthy black and white women with similar blood pressure, cholesterol, nonsmoking, and normoglycemic status, yet the HUVECs donated by the black participants generated less NO due to increased ROS produced by NOX and uncoupled eNOS. The finding of upregulated NOX was confirmed in a later study, which further demonstrated lower antioxidant superoxide dismutase and increased interleukin-6 production from HUVECs donated by black women (Fairheller et al., 2011). This has led to a call for examination of race by exposure interactions in gene expression, analysis of transcriptome from various endothelial vascular beds across races and ethnicities, and interrogation of modifiable lifestyle factors to explain the epigenetics of racial differences in vascular pathology (Robinson et al., 2020). While these studies have focused on people without diabetes, racial and ethnic diversities in both *in vitro* and *in vivo* diabetes research are desperately needed to define mechanisms of impaired vascular outcomes related to race/ethnicity, raise awareness of these disparities, and target these disparities with appropriate drug and lifestyle interventions.

Conclusions and future directions

Endothelium is a target of insulin action, and insulin is an important physiological mediator of vascular health. It is important to note that, while endothelial insulin resistance and dysfunction co-exist with metabolic insulin resistance in T2D, they occur early in the disease course and likely contribute to the development of metabolic insulin resistance and the microvascular and macrovascular complications of diabetes. The impact of endothelial insulin resistance and dysfunction varies depending on the size and location of the blood vessel, causing a decreased arterial plasticity, increased atherosclerotic tendency and vascular resistance, and decreased tissue

<u>Blood vessels</u>	<u>Conduit artery</u>	<u>Resistance artery</u>	<u>Microvasculature</u>
<u>Function</u>	Absorbing pressure increase from cardiac ejection	Maintaining tissue blood flow	Tissue perfusion and substrate exchange
<u>Endothelial insulin action</u>	↑ Compliance	↑ Tissue blood flow	↑ Tissue perfusion and substrate exchanges
<u>Endothelial insulin resistance and dysfunction</u>	Atherosclerosis Stiffness	↑ Vessel resistance ↓ Tissue blood flow	↓ Tissue blood flow ↓ Endothelial substrate exchange surface area
<u>Clinical consequences</u>	Atherosclerotic diseases	Hypertension Tissue ischemia	Dysglycemia Microangiopathies Tissue ischemia

Figure 3 Arterial functions and the impact of endothelial insulin resistance and dysfunction.

perfusion. Thus, reducing endothelial insulin resistance and improving endothelial function in the conduit arteries may reduce atherosclerotic complications, in the resistance arteries lead to better blood pressure control, and in the microvasculature lead to less microvascular complications and more tissue perfusion (Liu, 2013). Given that vascular endothelium serves as the first line of defense against insulin resistance inducers and microvascular insulin resistance contributes to the development of metabolic insulin resistance (Figure 2), attenuation of microvascular insulin resistance could potentially delay the pathogenesis of metabolic insulin resistance thus diabetes and its associated cardiovascular complications. All these make the vascular endothelium a viable and attractive therapeutic target for the prevention and management of T2D (Figure 3) and warrant further investigation for the prevention of complications related to T1D. Indeed, multiple T2D treatment modalities such as exercise, metformin, GLP-1R agonists, and SGLT2 inhibitors have demonstrated salutary effects on endothelial insulin action and function and evidence of delaying disease and/or complication progression in humans. More studies are needed to better understand the molecular machinery and signaling pathways underpinning the pathogenesis of endothelial insulin resistance and dysfunction in diabetes.

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