



Published in final edited form as:

*Circ Res.* 2018 April 13; 122(8): 1135–1150. doi:10.1161/CIRCRESAHA.118.311912.

## The Vasculature in Prediabetes

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### Abstract

The frequency of prediabetes is increasing as the prevalence of obesity rises worldwide. In prediabetes, hyperglycemia, insulin resistance, and inflammation and metabolic derangements associated with concomitant obesity cause endothelial vasodilator and fibrinolytic dysfunction, leading to increased risk of cardiovascular and renal disease. Importantly, the microvasculature impacts on insulin sensitivity by affecting the delivery of insulin and glucose to skeletal muscle; thus, endothelial dysfunction and extracellular matrix remodeling promote the progression from prediabetes to diabetes. Weight loss is the mainstay of treatment in prediabetes, but therapies that improved endothelial function and vasodilation may not only prevent cardiovascular disease but also slow progression to diabetes.

### Subject Codes

Endothelium/Vascular Type/Nitric Oxide; Metabolism

### Keywords

Insulin resistance; endothelial function; obesity; metabolic syndrome

### Introduction

Prediabetes is characterized by hyperglycemia that falls below the level used to define diabetes. As the prevalence of obesity grows worldwide, the prevalence of prediabetes is also increasing. In the United States, more than 38% of adults have prediabetes.<sup>1</sup> In China, the prevalence of prediabetes among adults reaches 50%.<sup>2</sup> Without intervention, prediabetes often progresses to diabetes and prediabetes is associated with increased risk of cardiovascular disease, cancer, renal disease, and dementia. While indices of hyperglycemia define clinical criteria for prediabetes, vascular dysfunction results not only from effects of hyperglycemia, but also from vascular insulin resistance and from pro-inflammatory and

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#### Disclosures

Dr. Brown is a member of the Scientific Advisory Board for Alnylam Pharmaceuticals and serves as a consultant for Novartis Pharmaceuticals, Shire Pharmaceuticals, and Viamet Pharmaceuticals. Dr. Brown receives research support from Novo Nordisk. Dr. Wang has served as a consultant to Takeda Pharmaceuticals and Ultragenyx and has received research support from Diasorin.

metabolic consequences of attendant obesity. In insulin resistance, muscle, fat, liver, and vascular cells do not respond normally to insulin. This may result from abnormal insulin signaling, but insulin resistance can also result from vascular endothelial dysfunction which leads to decreased delivery of insulin and glucose to insulin-sensitive tissues, as detailed below. Increased inflammation associated with obesity promotes endothelial dysfunction, extracellular matrix (ECM) formation and changes in capillary density and glucose uptake, further promoting insulin resistance. Hyperglycemia ensues when insulin production cannot overcome insulin resistance.

## Definition of Prediabetes

The American Diabetes Association (ADA) defines prediabetes based on any one of three criteria: fasting plasma glucose (FPG) of 5.6 mmol/L or higher but less than 7.0 mmol/L (100-125 mg/dL) [impaired fasting glucose (IFG)], a two-hour glucose of 7.8 mmol/L or higher but less than 11.1 mmol/L during a 75 gram oral glucose tolerance test (OGTT) (140-200 mg/dL) [impaired glucose tolerance (IGT)], or a plasma hemoglobin (Hb) A1c of 5.6% or greater but less than 6.5%.<sup>3</sup> The World Health Organization (WHO) uses a higher cutoff for FPG (6.1 mmol/L or 110 mg/dL), and does not include the HbA1c criterion.<sup>4</sup> Clinicians do not typically test for insulin resistance, but insulin resistance may be estimated from fasting insulin and glucose, by the homeostasis model assessment of insulin resistance (HOMA-IR) [(insulin × glucose)/22.5].

## Heterogeneity of Prediabetes

Based on the classification criteria, prediabetes is a heterogeneous disorder. Hyperglycemia and insulin resistance are the common threads of this condition, however. Glucose clamps and intravenous (IV) GTT have been used to elucidate the causes of IFG and insulin resistance in patients and pre-clinical models. The glucose clamp technique tests beta cell insulin secretory function by clamping at elevated glucose concentrations (~10 mM) or insulin sensitivity by clamping glucose at fasting levels during an insulin infusion (e.g. insulin dose of 40 mU·m<sup>-2</sup>·min<sup>-1</sup>).<sup>5</sup> Glucose is clamped using a glucose infusion, which is varied based on feedback from frequent blood glucose measurements. If insulin is infused, the glucose infusion rate required to maintain euglycemia reflects insulin sensitivity. Subjects who are insulin-resistant require less glucose at a fixed insulin dose than subjects with normal insulin sensitivity,<sup>6</sup> and subjects with prediabetes require more glucose than a subject with overt type 2 diabetes mellitus (T2DM).<sup>6, 7</sup> Stable or radioactive isotopes of glucose can be given during a hyperinsulinemic, glucose clamp to distinguish between insulin's effect on glucose appearance in the blood (liver effect) and glucose disappearance (primarily muscle).<sup>8</sup> The liver responds more rapidly and to lower insulin concentrations than skeletal muscle. The insulin dose during a clamp should target insulin concentrations obtained during a meal high in carbohydrates (~500 pM). Insulin effects on vascular endothelium, adipocytes, and other tissues can also be examined during an insulin clamp. IVGTT with frequent blood insulin and glucose sampling when combined with the Minimal Model described by Bergman and colleagues<sup>9, 10</sup> can be used to estimate the roles of the insulin secretory response, insulin-dependent actions, and insulin-independent effects in response. As with any model, results must be considered in light of the assumptions and

parsimony of the model. Detailed phenotyping studies using clamps or IVGTT suggest that IFG is characterized primarily by impaired hepatic insulin sensitivity and a decrease in the first-phase insulin response, whereas IGT is characterized by impaired muscle insulin sensitivity as well as early and late phase insulin responses.<sup>11-14</sup> In addition, many patients with prediabetes also have metabolic syndrome, characterized by IFG, hypertension, abdominal obesity, dyslipidemia (hypertriglyceridemia and low high-density lipoprotein cholesterol).<sup>15</sup>

## Risk of Progression to Type 2 Diabetes Mellitus

Insulin resistance precedes the development of hyperglycemia and T2DM and results in compensatory hyperinsulinemia. IGT and T2DM ensue when beta cell function fails to compensate.<sup>16</sup>

In a meta-analysis of prospective studies examining rates of progression to diabetes published prior to 2004, the incidence of diabetes was 4-6% in individuals with IGT alone, 6-9% in individuals with isolated IFG, and 15-19% in individuals with both IGT and IFG.<sup>17</sup> In general, HbA1c measurement has less predictive value. In the Diabetes Prevention Program (DPP), the annual incidence of diabetes in patients with IGT was 11% in the control group.<sup>18</sup> In the Multi-Ethnic Study of Atherosclerosis the incidence of diabetes was 4% in individuals with IFG.<sup>19</sup> In the Toranomon Hospital Health Management Center Study 7% of individuals who met HbA1c criteria and 9% of individuals with IFG progressed to diabetes.<sup>20</sup> In a population-based Rotterdam Study of adults aged 45 years, 75% of patients with IFG progressed to T2DM over their lifetime.<sup>21</sup> In the China Da Qing Diabetes Prevention Study 90% of participants with IGT developed T2DM over 20 years.<sup>22</sup>

## Risk of Cardiovascular and Renal Disease in Prediabetes

Even before the development of diabetes, prediabetes is associated with increased risks of macrovascular disease, nephropathy, neuropathy, cancer and dementia. We focus here on macrovascular complications and later on microvascular disease. In the Emerging Risk Factors Collaboration study, there was a linear relationship between glucose and the hazard ratio for coronary heart disease in non-diabetics with a fasting glucose above 5.5 mmol/L (100 mg/dL).<sup>23</sup> In a meta-analysis of observational studies, Ford et al estimated a relative risk of cardiovascular disease of 1.20 (95% CI 1.12-1.28) when 6.1 mmol/L (110 mg/dL) was used to define IFG (IFG-WHO) and a relative risk of 1.18 (95% CI 1.09-1.28) when 5.5 mmol/L (IFG-ADA) was used.<sup>24</sup> The relative risk associated with IGT was similar. In a more recent meta-analysis, Huang et al reported a relative risk of cardiovascular disease of 1.13, 1.26, and 1.30 in IFG-ADA, IFG-WHO, and IGT, respectively.<sup>25</sup> The relative risk of all-cause mortality was 1.13, 1.13, and 1.32 in the same three groups. Increases in HbA<sub>1C</sub> that met the ADA definition of prediabetes were associated with increased risk of cardiovascular disease and coronary heart disease, but not with stroke or all-cause mortality. In the Atherosclerosis Risk in Communities (ARIC) study, patients with prediabetes (HbA1c 5.7% to 6.5%) were thirty percent more likely to be hospitalized than non-diabetics.<sup>26</sup>

Among patients with prediabetes, factors associated with insulin resistance including increased oxidative stress, inflammation, and dyslipidemia contribute to microvascular and macrovascular disease. Lowering glucose without addressing the vascular effects of insulin resistance may be insufficient to reduce risk of cardiovascular events.<sup>27</sup> Conversely, phenotypes of insulin resistance such as the presence of non-alcoholic fatty liver disease or visceral obesity predict atherosclerosis.<sup>28</sup>

## Endothelial Vasodilator Dysfunction in Prediabetes

The vascular endothelium regulates vascular permeability and tone and protects against intravascular thrombosis. Endothelial dysfunction is characterized by a loss of vasodilation in response to shear stress induced by release of an occlusive cuff (flow-mediated dilation or FMD) or pharmacological stimuli causing nitric oxide synthase (NOS) activation such as acetylcholine or bradykinin.<sup>29</sup> Methods for measuring endothelial vasodilator function are reviewed elsewhere.<sup>29</sup> Endothelial dysfunction occurs early in the pathogenesis of atherosclerosis, and predicts future cardiovascular events.<sup>30-32</sup> In hyperglycemia, endothelial vasodilator dysfunction precedes the development of T2DM and is seen in both IFG and IGT.<sup>33-36</sup>

Insulin vasodilates skeletal muscle<sup>6, 37</sup> and coronary<sup>38, 39</sup> vasculature. Insulin resistance contributes to endothelial dysfunction in prediabetes (Figure 1). Insulin induces nitric oxide (NO)-dependent vasodilation in skeletal muscle via stimulation of the insulin receptor, activation of insulin receptor substrate 1 (IRS1), phosphatidylinositol 3 kinase (PI3-kinase), phosphorylation of Akt and subsequent phosphorylation of endothelial NOS (eNOS).<sup>40-44</sup> Counteracting this, insulin stimulates endothelin-1 (ET-1) production via raf-1, p38 mitogen activated protein kinase (MAPK)/ERK kinase (MEK), and extracellular signal-regulated kinase 1/2 (ERK1/2).<sup>44-47</sup> Vascular insulin resistance leads to downregulation of IRS-1 and 2 and decreased phosphorylation of Akt and eNOS, whereas the ET-1 pathway remains unaffected.<sup>48, 49</sup> Insulin also increases adipose tissue blood flow,<sup>50</sup> but the mechanism appears to be quite different than for other insulin-sensitive tissues. Local insulin administration has no effect on adipose tissue blood flow<sup>51</sup> and glucose-stimulated vasodilation is  $\beta$ -adrenergic receptor-dependent and independent of eNOS.<sup>52</sup>

Hyperglycemia *per se* influences endothelial function in prediabetes. Because endothelial cells take up glucose through the insulin-independent carrier GLUT-1,<sup>53</sup> intra-cellular glucose concentrations reflect plasma glucose concentrations. Excess glucose is metabolized through the polyol pathway to fructose and its metabolites, which are potent glycation agents.<sup>54</sup> In glycation, the aldehyde group of reducing sugars bind non-enzymatically to proteins to form a Schiff base which can rearrange into a stable ketoamine or Amadori product.<sup>55</sup> Increased glycated hemoglobin or HbA1c is one criterion used to define prediabetes by the ADA. Glycation is less in prediabetes than that defining diabetes. A high hemoglobin glycation index, defined as the measured HbA1c minus a predicted HbA1c calculated from blood glucose, has been associated with cardiovascular disease, in treatment-naïve patients with prediabetes and diabetes.<sup>56</sup> This suggests that the negative effects of glycation pertain to prediabetes as well as diabetes.

Early-stage glycation products undergo a series of reactions including oxidation, dehydration, and condensation to form advanced glycation products (AGEs).<sup>55</sup> AGEs activate the ERK, c-Jun N-terminal kinase (JNK), p38, and PI3K pathways and promote activation of NF- $\kappa$ B.<sup>57-59</sup> Binding of AGEs to the receptor for AGE (RAGE) enhances the expression of adhesion molecules on endothelial cells to promote the migration and differentiation of monocytes into macrophages.<sup>60</sup> AGEs also stimulate monocytes to secrete inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6.<sup>61</sup> Glycated low density lipoprotein (LDL) cholesterol, once oxidized or AGE-modified, is recognized by scavenger receptors on macrophages and lead to the formation of foam cells.<sup>62</sup> Glycation of complex III proteins enhances mitochondrial superoxide production.<sup>63</sup> In addition, activation of the polyol pathway leads to NADPH depletion and reduced concentration of the anti-oxidant glutathione.<sup>64</sup> Increased intracellular NADH leads to an increase in glycerol-3-phosphate and activation of protein kinase C (PKC).<sup>65</sup> Activated PKC in turn promotes oxidative stress by activating NADPH oxidase.<sup>66, 67</sup> Activated PKC contributes to endothelial dysfunction,<sup>68, 69</sup> increased endothelial permeability,<sup>70</sup> and ECM expansion.<sup>71</sup> During hyperglycemia, excess shunting of glucose through the hexosamine pathway results in the O-GlcNAcylation of serine and threonine residues on proteins; O-GlcNAcylation of eNOS at Ser 1177 decreases its activity.<sup>72</sup>

Increased free fatty acids also contribute to insulin resistance and endothelial dysfunction in obese patients with prediabetes. High fat intake induces endothelial dysfunction in mice,<sup>73</sup> and consumption of a meal high in fat acutely decreases brachial artery reactivity in humans.<sup>74</sup> FFAs reduce tyrosine phosphorylation of IRS-1/2 and inhibit the PI3K/Akt pathway, leading to decreased glucose transport and decreased phosphorylation of eNOS.<sup>75-77</sup> FFAs also reduce ATP-induced mobilization and influx of calcium in endothelial cells.<sup>78</sup> FFAs activate NADPH oxidase via PKC to generate ROS.<sup>66</sup> The generation of ROS in turn promotes inflammation by activating NF- $\kappa$ B. FFAs also induce production of high mobility group box 1 (HMGB1) resulting in activation of the nucleotide-binding domain and leucine-rich repeat-containing (NLR) family and the pyrin and HIN domain (PYHIN) (NLRP3) inflammasome.<sup>79</sup> Interestingly, dipeptidyl peptidase-4 (DPP4) inhibition prevents the amino-terminus degradation of HMGB1.<sup>80</sup>

Inflammation associated with visceral obesity contributes to insulin resistance and endothelial dysfunction in many patients with prediabetes. Visceral adipose tissue has an increased number of adipose resident macrophages.<sup>81, 82</sup> The total number of T cells, B cells, neutrophils and mast cells is also increased, whereas the number of T-helper (Th2) and regulatory T cells (Treg) is unchanged or decreased. Under stimulation by B cells, effector T cells and Th1 cells release cytokines such as interferon- $\gamma$  to further stimulate activation of macrophages and induce a shift in macrophages from an M2-like phenotype to an M1-like phenotype.<sup>83</sup> M1-like macrophages secrete inflammatory cytokines such as TNF- $\alpha$  and IL-6, and studies in M1-like macrophage knockout mice suggest they also promote insulin resistance.<sup>84</sup> Whereas macrophages are distributed throughout lean adipose tissue, lipid-laden macrophages accumulate in crown-like structures around dead adipocytes.<sup>85, 86</sup> The presence of these crown-like structures is associated with inflammation and insulin resistance.<sup>87</sup>

Adipose tissue also influences systemic endothelial function through secretion of inflammatory cytokines such as TNF- $\alpha$ , IL-6, leptin, and resistin.<sup>88</sup> While leptin suppresses appetite and enhances energy expenditure, it also stimulates pro-inflammatory immune cells, increases ROS production by endothelial cells, and facilitates formation of foam cells.<sup>89-91</sup> In humans, resistin concentrations are increased in prediabetes and elevated resistin concentrations are associated with cardiovascular events.<sup>92-94</sup> The best studied anti-inflammatory adipokine is adiponectin. Adiponectin concentrations decrease with increased visceral obesity,<sup>95, 96</sup> and reduced adiponectin concentrations are associated with increased inflammation and risks of diabetes and cardiovascular disease.<sup>88, 97, 98</sup> In patients with IGT in the DPP, intensive lifestyle modification increased adiponectin and decreased CRP, IL-6, fibrinogen, and adhesion molecules e-selectin and intercellular adhesion molecule-1 (ICAM-1), as well as insulin resistance.<sup>99</sup> Increases in adiponectin were also associated with increases in HDL-cholesterol particle size.<sup>99</sup> In the Nurses' Health Study, increased e-selectin and ICAM-1 concentrations independently predicted incident diabetes.<sup>100</sup>

Activation of the renin-angiotensin-aldosterone system in obesity further accentuates vascular insulin resistance. Angiotensin II (Ang II) causes serine (Ser307) phosphorylation and degradation of IRS-1 via the proto-oncogene tyrosine-protein kinase Src.<sup>101</sup> Aldosterone induces degradation of IRS-1 in vascular smooth muscle cells via a mineralocorticoid receptor-, ROS- and Src-dependent mechanism.<sup>102</sup> Aldosterone also increases insulin-like growth factor (IGF)-1 receptor expression and hybridization with IRS-1 leading to insulin resistance.<sup>103</sup> Aldosterone enhances Ang II-stimulated ERK1/2 phosphorylation in vascular smooth muscle cells.<sup>104</sup>

Impaired endothelium-dependent vasodilation results not only in impaired macrovascular function, but also in microvascular dysfunction, as measured by retinal artery vasodilation and heat-induced skin hyperemia.<sup>105-107</sup> Insulin-induced capillary recruitment and microvascular dilation, measured by laser Doppler flowmetry during iontophoresis of acetylcholine, correlate with insulin sensitivity and are reduced in obese compared to lean women.<sup>108</sup> In the Maastricht Study, microvascular function was impaired in prediabetes and there was a linear relationship between microvascular dysfunction and HbA1c and fasting glucose.<sup>109</sup> In addition, the prevalence of microalbuminuria, a marker of microvascular dysfunction, is increased in prediabetes and predicts cardiovascular morbidity.

## Endothelial Fibrinolytic Dysfunction in Prediabetes

Impaired vascular fibrinolytic function contributes to the risk of cardiovascular disease in individuals with insulin resistance and prediabetes. Tissue-type plasminogen activator (t-PA) is stored in the endothelium and protects against local thrombosis.<sup>110, 111</sup> Plasminogen activator inhibitor-1 (PAI-1) is the primary inhibitor of t-PA *in vivo*.<sup>112</sup> Increased circulating PAI-1 activity or antigen is associated with increased risk of thrombotic events including myocardial infarction and stroke.<sup>113</sup> PAI-1 levels also predict the development of diabetes in at-risk patients.<sup>114</sup> Glucose increases PAI-1 expression via Sp1 sites.<sup>115</sup> Insulin and insulin-like growth factor also increase PAI-1 expression; several forkhead proteins are involved in the regulation of PAI-1 expression by insulin.<sup>116-118</sup> Very-low-density lipoprotein (VLDL) increases PAI-1 expression via a response element localized from 672 to 657 of the promoter

region.<sup>119</sup> Thus, PAI-1 concentrations correlate with body mass and markers of insulin resistance. Ang II, aldosterone, and inflammatory cytokines such as IL-6 and TNF- $\alpha$ , all of which are increased in visceral obesity, also stimulate PAI-1 expression.<sup>120, 121</sup> Conversely, cGMP decreases PAI-1 expression and impaired endothelial NO production may contribute to impaired fibrinolytic balance in prediabetes.<sup>122, 123</sup>

T-PA antigen levels are also associated with increased risk of thrombotic events, but this is largely because 80% of t-PA antigen circulates complexed to PAI-1.<sup>124</sup> T-PA is released both constitutively and in response to receptor stimulation by bradykinin, acetylcholine or methacholine, substance P, and norepinephrine.<sup>125-127</sup> Increased shear force also stimulates t-PA release. Stimulated t-PA release in the human forearm vasculature correlates with t-PA release in the coronary vasculature and diminished forearm endothelial t-PA release is associated with an increased risk of cardiovascular events.<sup>128, 129</sup> Endothelial t-PA release is diminished in obesity and in conditions associated with prediabetes, such as hypertension, but the effect of hyperglycemia is not well-defined.<sup>130, 131</sup> Acute glucose and insulin administration decrease t-PA activity in the rat.<sup>132</sup>

## microRNAs in Insulin resistance, Prediabetes and Diabetes

The transition from normoglycemia to prediabetes and diabetes involves changes in the expression of many genes involved in fat and glucose metabolism.<sup>133</sup> MicroRNAs are small, non-coding RNAs, 18 to 22 nucleotides in length, that contribute to gene regulation by altering translation and/or mRNA stability. microRNAs can alter the expression of genes in insulin-producing and insulin-sensitive tissues, including the pancreas, liver, and vasculature.<sup>134, 135</sup>

For instance, a variety of microRNAs have been shown to influence pancreatic beta cell development and function.<sup>136, 137</sup> One of the best studied is miR-375, an islet-specific microRNA that regulates both insulin secretion and beta cell mass.<sup>138, 139</sup> Overexpression of miR-375 is associated with reduced insulin secretion, thought to be mediated by inhibition of myotrophin, a protein involved in insulin exocytosis.<sup>138</sup> miR-375 also regulates many genes involved in cellular proliferation, which likely underlies its effect on the maintenance of beta cell mass.<sup>139</sup>

The most abundant microRNA in the liver is miR-122.<sup>134, 140</sup> Inhibition of miR-122 reduces plasma cholesterol and hepatic steatosis, though the specific gene regulatory pathways involved are unclear.<sup>140</sup> Another well-studied microRNA is miR-33, which has two isoforms, miR-33a and miR-33b. These microRNAs participate in the regulation of cholesterol synthesis, fatty acid metabolism, and insulin signaling.<sup>141-143</sup> They are co-transcribed and act in conjunction with their host genes, *Srebp1* and *Srebp2*, to regulate cholesterol synthesis.<sup>143</sup>

microRNAs may also link the metabolic dysregulation seen in prediabetes with vascular dysfunction.<sup>135</sup> Hyperglycemia increases the expression of several microRNAs found in the endothelium, including miR-320, miR-221, miR-503, and miR-125b.<sup>135, 144-147</sup> These

microRNAs may promote endothelial dysfunction by inhibiting genes involved in angiogenesis, vascular repair, and inflammatory suppression.

## The Vasculature in Glucose and Insulin Delivery

Skeletal muscle represents the bulk of insulin-sensitive tissue and is a major determinant of glucose tolerance. The muscle vasculature has, therefore, been the focus of studies on endothelial dysfunction and insulin resistance. Attenuated increases in insulin-stimulated limb blood flow result in decreased limb glucose uptake.<sup>6, 7</sup> Indeed, rats and mice made insulin-resistant by high fat intake have such severe impairment of microvascular blood flow that the delivery of glucose to skeletal muscle limits insulin-stimulated glucose uptake during an insulin clamp.<sup>148-150</sup> Impaired adipose tissue blood flow does not contribute substantially to glucose disposal in prediabetes but may contribute to impaired lipid storage in adipocytes and increased ectopic lipid stores.<sup>151</sup>

Several lines of evidence suggest that microvascular function (determined by capillary blood flow, the number of perfused capillaries, and capillary insulin permeability) is key to skeletal muscle insulin action in health and prediabetes. First, the gradient in insulin concentration from plasma to interstitial fluid is increased in humans with prediabetes and in mouse models of prediabetes during an insulin clamp,<sup>152-154</sup> suggesting impaired microvascular insulin delivery. The effect of the impairment in skeletal muscle access is illustrated in Figure 2.

Insulin-induced blood flow increases its own tissue delivery, as well as the delivery of nutrient substrates.<sup>155</sup> Access to perfused tissues requires not only increased blood flow, but also capillary egress. Glucose can easily traverse narrow paracellular gaps. The endothelium poses a major barrier for insulin as the diameter of insulin approaches the size of the paracellular space. Insulin movement across the muscle capillary endothelium has been postulated to be regulated and rate-limiting.<sup>156, 157</sup> Still, little is known about mechanism(s) underlying this process.

Direct visualization of fluorescent insulin across the endothelium of capillaries perfusing mouse muscle using confocal microscopy shows that insulin moves across the capillary endothelium by a mechanism that is unsaturable, insulin receptor-independent, and conforms to the properties of fluid phase transport.<sup>158</sup> Fluid-phase transport is determined by the balance between hydrostatic and oncotic pressures. An increase in microvascular blood flow could facilitate insulin efflux should it result in increased capillary hydrostatic pressure. Fluid-phase transport can occur through paracellular junctions or by transcytosis through vesicles formed in endothelial cells. Insulin access to skeletal muscle is illustrated in Figure 3.

## Impaired Microvascular Blood Flow in the Progression of Prediabetes to Diabetes

The contribution of impaired blood flow to the progression of prediabetes to diabetes is evidenced by the demonstration that vasodilators can partially prevent<sup>159, 160</sup> or reverse<sup>161</sup>



diet-induced insulin resistance in rodents. Administration of the phosphodiesterase (PDE5) inhibitor sildenafil to obese mice prevents inflammation<sup>162</sup> and insulin resistance.<sup>159</sup> Liraglutide treatment increases NO, decreases ET-1, normalizes endothelial function, and increases microvasculature blood flow and glucose disposal in obese rats.<sup>160</sup>

Relaxin acts on RXFP1 receptors expressed on vascular endothelial and smooth muscle cells. Activation of these receptors leads to vasodilation due to actions on both endothelium<sup>163</sup> and smooth muscle.<sup>164</sup> Relaxin administration caused endothelial relaxation and reversed insulin resistance in obese mice.<sup>161</sup> Relaxin did not improve insulin action in isolated muscle, demonstrating the reliance on the microvascular for its insulin-sensitizing effect.

If increased microvascular blood flow improves insulin action in prediabetes, a decrease in microvascular function could create insulin resistance in healthy mice. This hypothesis was tested in mice in which skeletal and cardiac muscle VEGF-A was deleted and tissue capillarity was reduced by ~50%.<sup>165</sup> Capillary skeletal and cardiac muscle rarefaction resulted in glucose intolerance and impairments in skeletal and cardiac muscle insulin-stimulated glucose uptake *in vivo*.<sup>165</sup> There was no impairment in insulin action in muscle isolated from lean VEGF-A knockout mice, demonstrating the requirement for intact microvasculature for this effect.

## Extracellular Matrix and Vascular Glucose and Insulin Delivery

The ECM is a dynamic structure comprised of proteins and proteoglycans that remodels in responses to injury and inflammation.<sup>166</sup> Changes in ECM may affect insulin and glucose delivery either by causing capillary regression or neo-vascular growth (Figure 4). Increased recruitment of pro-inflammatory macrophages to muscle<sup>167</sup> and adipose<sup>81</sup> results in transforming growth factor  $\beta$  (TGF $\beta$ )-mediated fibrosis.<sup>168</sup> Increased PAI-1 in prediabetes contributes to fibrosis, as does elevated concentrations of the adipokine thrombospondin-1.<sup>169</sup> Thrombospondin-1 contributes to the skeletal muscle meta-fibrosis and insulin resistance in high fat-fed mice.<sup>170</sup>

The pro-inflammatory prediabetic state results in an increase in skeletal muscle collagens and other ECM proteins<sup>162, 171</sup> and ECM remodeling.<sup>172</sup> The glycosaminoglycan hyaluronan is increased in tissues of prediabetic animals,<sup>173, 174</sup> and serum hyaluronan is increased in T2DM.<sup>175</sup> Hyaluronan is a major component of the glycocalyx of capillary lumens, where it may affect the access of insulin to tissues. A reduction of hyaluronan using PEGylated hyaluronidase in high fat-fed mice improves insulin action.<sup>173</sup> CD44, the main hyaluronan cell surface receptor is associated with T2DM, as shown by expression-based genome-wide association studies.<sup>176</sup> Anti-CD44 antibody treatment reduces inflammation, and improves insulin sensitivity in obese mice.<sup>177</sup>

ECM remodeling<sup>170, 172, 173, 178</sup> causes changes in signaling via cell surface receptors that sense the extracellular microenvironment.<sup>162, 179-181</sup> ECM remodeling is necessary for increased capillary growth and proliferation.<sup>165</sup> Treatments that improve insulin action in rodent models of prediabetes normalize collagen levels and increase muscle capillaries.

161, 162, 173, 179 Expansion of the muscle ECM and decreased muscle capillaries are associated with insulin resistance.<sup>178</sup> Collectively, these data suggest endothelial dysfunction and capillary rarefaction are mechanisms by which ECM remodeling may mediate muscle insulin resistance.

The expanded ECM in prediabetes produces a distinct signal that alters activation of integrin and other cell surface receptors. The integrin  $\alpha 2$  subunit is highly expressed in endothelial cells, fibroblasts and other cell types. The  $\alpha 2\beta 1$  integrin receptor regulates cell migration, proliferation and survival.<sup>182</sup> Genetic deletion of the  $\alpha 2$  subunit has no effect on insulin action in lean mice but increases capillaries and insulin action in obese mice.

Studies in mice with a muscle-specific deletion of the integrin downstream protein, the integrin-linked kinase (ILK), support the importance of muscle ECM in insulin action. Skeletal muscle ILK knockout has no effect on insulin action in lean mice but increases capillaries and insulin action in obese mice.<sup>179</sup> The downstream integrin signaling molecule, focal adhesion kinase (FAK), has also been implicated in the regulation of insulin action in the muscle.<sup>183, 184</sup> FAK phosphorylation is decreased in muscle from obese rats<sup>183</sup> and siRNA knockdown of FAK impairs glucose tolerance and insulin action in lean mice.<sup>184</sup>

ECM expansion occurs in adipose tissue of obese subjects and limits adipocyte fat storage,<sup>185</sup> leading to ectopic fat stores in liver and skeletal muscle, lipotoxicity, oxidative stress, and inflammation.<sup>186</sup>

## Treatment of Prediabetes – Weight Loss and Lifestyle Modification

Weight loss is the mainstay of reducing the risk of progression to diabetes in patients with prediabetes. In addition to reducing hyperglycemia, weight loss leads to significant improvements in other cardiovascular risk factors including blood pressure, lipids and biomarkers of inflammation.

In patients with IGT followed for six years in the Da Qing Study, diet, exercise, and combined diet and exercise reduced incident T2DM by 31%, 46% and 42% respectively compared to control.<sup>187</sup> In the Finnish Diabetes Prevention Program, weight loss counseling targeted to 5% total body weight, decreased total and saturated fat consumption, increased fiber consumption and increased activity to 30 min/day resulted in a 58% reduction in incident T2DM in individuals with IGT and BMI greater than 25 kg/m<sup>2</sup>.<sup>188</sup>

In the DPP study of 3234 patients with IGT and BMI  $\geq 24$  kg/m<sup>2</sup> ( $\geq 22$  kg/m<sup>2</sup> for Asian-Americans), intensive lifestyle modification led to a loss of 5.6 kg over an average follow-up of 2.8 years, and this was associated with a 58% reduction in incident diabetes.<sup>189</sup> At ten years, the sustained risk reduction was 34% in the lifestyle arm compared to placebo even though weights were no longer different between groups.<sup>18</sup> Regression to normal glucose tolerance results in an approximately 6% reduction in ten-year risk of cardiovascular risk by Framingham criteria.<sup>190</sup>

Similar effects of diet and exercise were seen in men in the Japanese IGT study and in the Indian Diabetes Prevention Programme in patients with IGT.<sup>20, 191</sup> The reduction of incident

diabetes appears to be proportionate to the amount of weight loss, but plateaus at a loss of 10% of body mass.

Long-term follow-up of participants in lifestyle intervention trials demonstrate that weight loss is difficult to maintain. Pharmacological weight loss therapy also reduces the risk of progression to diabetes. In the XENDOS study, orlistat therapy resulted in a 5.8 kg weight loss and reduced the progression of IGT to T2DM by 37%.<sup>192</sup> Phentermine/topiramate ER reduced incident T2DM in patients with prediabetes or metabolic syndrome by 79%.<sup>193</sup>

Bariatric surgery reduces incident diabetes not only through weight loss but through effects on incretin hormones, bile acids, and/or gut microflora. In the Swedish Obese Subjects (SOS), a prospective, non-randomized study of bariatric surgery (gastric banding, vertical-banded gastroplasty, and Roux-en-Y gastric bypass) patients and obese controls, among patients without diabetes at baseline, incident diabetes was reduced by 76%.<sup>194</sup> Retrospective and cohort studies have showed similar reductions in T2DM in patients with impaired fasting glucose or obesity.<sup>195</sup>

### Treatment of Prediabetes with Anti-diabetic Agents

In the DPP, treatment with metformin 850 mg twice a day reduced incident diabetes by 31% compared to placebo in patients with IGT over 2.8 years of follow-up.<sup>189</sup> The effect was greater in more obese patients and those with a higher fasting glucose. The DPP Outcomes Study followed participants for up to 15 years and found a persistent reduction in incident diabetes of 18%.<sup>196</sup> Weight loss explained 64% of the beneficial effect. Despite an initial effect on lipoprotein subfractions, C-reactive protein and t-PA, with long-term follow-up there was no effect of metformin on cardiovascular risk factors. Coronary artery calcification was decreased in men but not in women. There was no effect of metformin on microvascular complications independent of effects of diabetes. In the Indian Diabetes Prevention Programme metformin 250 mg twice a day reduced incident diabetes by 28% in IGT.<sup>191</sup> There was no added benefit of combined metformin and lifestyle modification. Despite the lack of persistent effect of metformin on cardiovascular risk factors such as lipids and inflammation in the DPP Outcomes Study, metformin may improve insulin sensitivity through effects on endothelial function. Metformin improves endothelial function in insulin-resistant rodents.<sup>197</sup> In humans, the effect of metformin on endothelial function has been studied most in patients with T2DM or polycystic ovarian syndrome,<sup>198</sup> but metformin improves endothelial function in patients with metabolic syndrome and improvement in FMD correlates with improvement in insulin resistance.<sup>199</sup>

Alpha-glucosidase inhibitors have also been studied in prediabetes. In the STOP-NIDDM trial acarbose reduced the incidence of diabetes by 25% in individuals with IGT and a FPG from 5.6 to 7.7 mmol/L.<sup>200</sup> Despite a 25% dropout rate, acarbose treatment reduced cardiovascular events by 49%.<sup>201</sup> Voglibose (0.2 mg tid) treatment reduced incident diabetes 41% compared to placebo in a trial in Japan.<sup>202</sup>

Thiazolidinediones prevent the development of diabetes in individuals with IFG or IGT. In Actos Now for Prevention of Diabetes (ACT NOW), treatment with pioglitazone 45 mg per

day reduced incident diabetes 72% over 2.4 years in patients with IGT and at least one other risk factor for T2DM and BMI  $\geq 25$  kg/m<sup>2</sup>.<sup>203</sup> Pioglitazone also reduced PAI-1 and slowed the rate of progression of carotid intima-media thickening.<sup>204</sup> In the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) trial, rosiglitazone 8 mg/d reduced incident diabetes 60% in patients with IFG or IGT older than 29 years.<sup>205</sup> In the CANadian Normoglycemia Outcomes Evaluation (CANOE) trial, treatment with rosiglitazone 2 mg per day and metformin 500 mg twice a day combined, reduced incident diabetes 66%, an effect size larger than that observed in trials of metformin alone; however, there was no single-drug comparator arm.<sup>206</sup> The thiazolidinediones increase the risk of heart failure in patients with diabetes.<sup>207</sup>

Treatment with the GLP-1 receptor agonist, liraglutide, also reduces incident diabetes in obese patients with prediabetes.<sup>208</sup> In the SCALE Obesity and Prediabetes study, patients with prediabetes with BMI  $\geq 30$  kg/m<sup>2</sup> or a BMI  $\geq 27$  kg/m<sup>2</sup> and hyperlipidemia or hypertension were randomized to liraglutide 3.0 mg or placebo. Patients randomized to liraglutide lost more weight (6.5 $\pm$ 8.1 kg versus 2.0 $\pm$ 7.3 kg) and were less likely to progress to T2DM (2% versus 6%) over three years. Whether the effect of GLP-1 receptor antagonism exceeds the effect of equivalent weight loss is not known.

Importantly, long-acting GLP-1 agonists reduce mortality in patients with T2DM. In the Liraglutide Effect and Action in Diabetes of Cardiovascular Outcomes Results (LEADER) trial, liraglutide 1.8 mg/d reduced the primary composite outcome of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke in patients with T2DM and high cardiovascular risk compared to placebo (from 14.9% to 13%, HR 0.87, 95% CI 0.78-0.97, P=0.01).<sup>209</sup> Patients in the liraglutide group lost 2.3 kg more than the placebo treatment group. SBP was also significantly lower in the liraglutide group. Semaglutide (0.5 or 1 mg/week) reduced the primary composite endpoint of first occurrence of death from cardiovascular causes, nonfatal myocardial infarction or nonfatal stroke in patients with T2DM and a HbA1c  $\geq 7\%$  or more compared to placebo (HR 0.74, 95% CI 0.58 to 0.94, P<0.001).<sup>210</sup> Body weight decreased 3.6 kg and 4.9 kg in the 0.5 and 1.0 mg groups. Again, whether the effect of GLP-1 receptor antagonism exceeds the effect of equivalent weight loss on cardiovascular risk is not known. In mice, GLP-1 causes vasodilation through both GLP-1 receptor-dependent and -independent mechanisms.<sup>211</sup> The latter requires degradation of GLP-1 to GLP-1 (9-36) by DPP-4 and involves NO. Human studies provide conflicting information about the effect of GLP-1 or stable analogues on endothelial function.<sup>212-217</sup>

## Effect of Drugs that Improve Vascular (Endothelial) Function on Insulin Resistance

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB)s improve endothelium-dependent vasodilation and reduce morbidity and mortality in heart failure and nephropathy, including diabetic nephropathy.<sup>218-221</sup> ACE inhibitors also improve endothelial fibrinolytic function and reduce the risk of thrombotic events such as myocardial infarction.<sup>222-226</sup> Given the aforementioned evidence that Ang II and aldosterone alter

insulin sensitivity, there has been significant interest in the possibility that treatment with an ACE inhibitor or ARB could prevent progression of prediabetes to diabetes while reducing cardiovascular risk. In the DREAM study, ramipril did not reduce incident diabetes in patients with IFG.<sup>227</sup> A greater proportion of patients treated with ramipril regressed to fasting normoglycemia compared to placebo treated patients (42.5% versus 38.2%,  $p < 0.001$ ), however. The median two-hour glucose following OGTT was also lower in the ramipril group. In the Nateglinide and Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) study, treatment with valsartan reduced the risk of incident diabetes compared to placebo (31% versus 33%,  $p < 0.001$ ) in patients with IGT and cardiovascular risk factors, although there was no effect on cardiovascular mortality.<sup>228</sup> In a meta-analysis of randomized, placebo-controlled clinical trials of ACE inhibitors or ARBs in patients without heart failure (i.e. not specifically prediabetes), ACE inhibitors reduced the risk of incident DM 13.7% ( $p = 0.02$ ) and ARBs reduced the risk of incident diabetes by 10% ( $p < 0.001$ ).<sup>229</sup>

Studies in obese, insulin-resistant rodents indicate that ACE inhibitors improve insulin sensitivity through bradykinin B2 receptor- and NOS-dependent effects, and NO contributes to bradykinin-stimulated muscle glucose uptake in humans.<sup>230, 231</sup> An alternative pharmacological strategy for increasing vascular cGMP without increasing NO include increasing the production of cGMP by soluble guanylate cyclase or decreasing the degradation of cGMP via PDE5. As noted earlier, administration of sildenafil prevents insulin resistance in high fat fed mice.<sup>159</sup> In a proof-of-concept study, three-week treatment with tadalafil improved  $\beta$ -cell function measured using the frequently-sampled IVGTT in individuals with metabolic syndrome, and reduced disposition index (DI, a composite measure of insulin sensitivity and secretion) in the women studied.<sup>232</sup> Ho and colleagues reported that twelve-week treatment with tadalafil improved DI and oral DI, and tended to improve insulinogenic index in insulin-resistant subjects undergoing OGTT; tadalafil also improved insulin sensitivity among severely obese participants.<sup>233</sup> Ramirez et al reported that three-month treatment with sildenafil increased the insulin sensitivity index calculated from hyperglycemic clamps in individuals with prediabetes without affecting early or late insulin secretion.<sup>234</sup> Sildenafil reduced urine microalbuminuria, a marker of endothelial function that predicts adverse cardiovascular outcomes. Sildenafil treatment also improved fibrinolytic balance, decreasing PAI-1 concentrations without altering t-PA concentrations. Several studies have reported that PDE5 inhibition reduces the urine albumin-to-creatinine ratio and HbA1c in patients with T2DM and microalbuminuria.<sup>235, 236</sup>

## Acknowledgments

### Sources of Funding

The authors are funded by grants HL125426, DK054902, DK050277, DK059637, DK108159, HL128983, HL132320, DK081572 from the National Institutes of Health and by 17SFRN33520059 from the American Heart Association.

## Nonstandard Abbreviations and Acronyms

**FPG**            fasting plasma glucose

<b>IFG</b>	impaired fasting glucose
<b>IGT</b>	impaired glucose tolerance
<b>HOMA-IR</b>	homeostasis model assessment of insulin resistance
<b>T2DM</b>	type 2 diabetes mellitus

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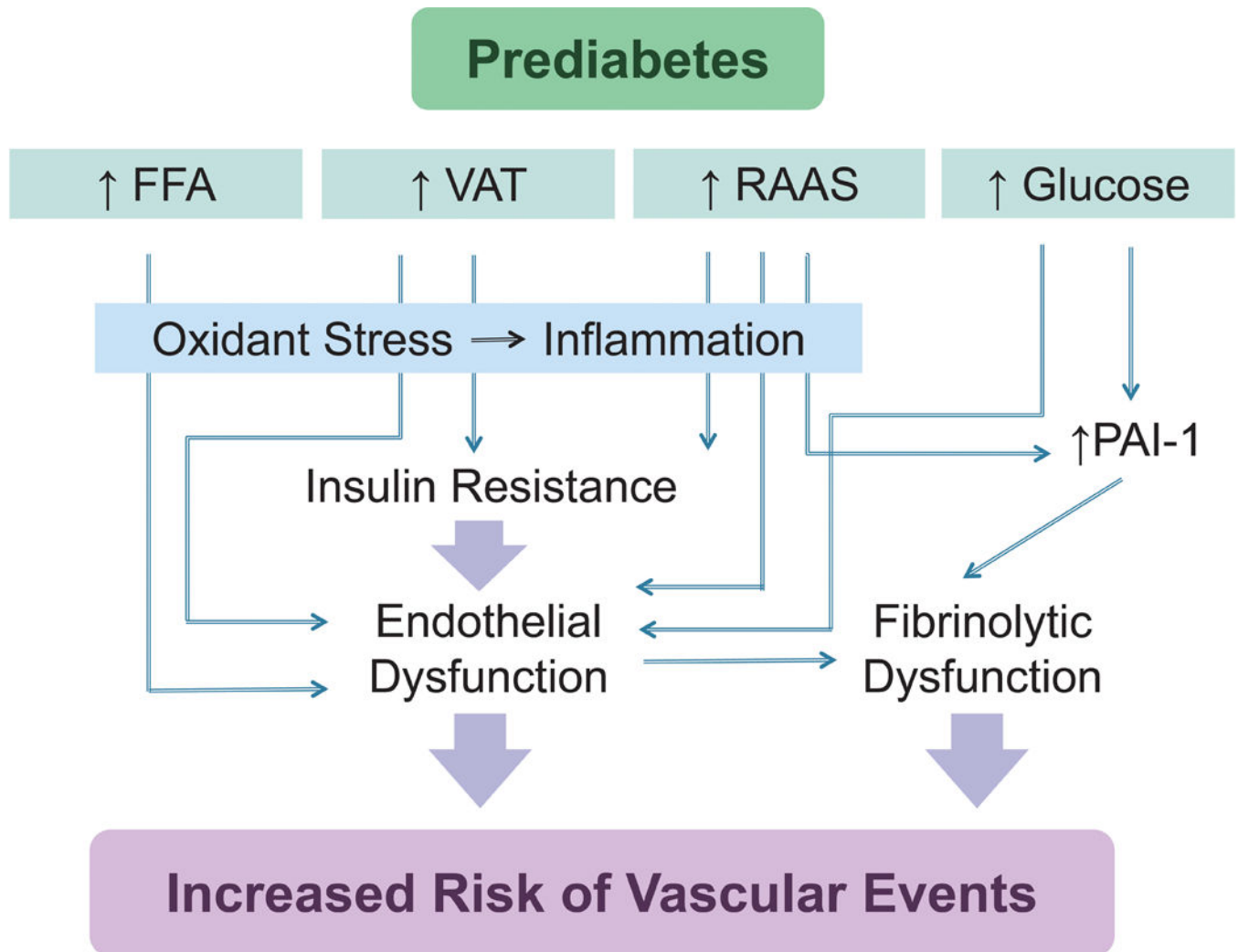
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### Summary and Future Directions

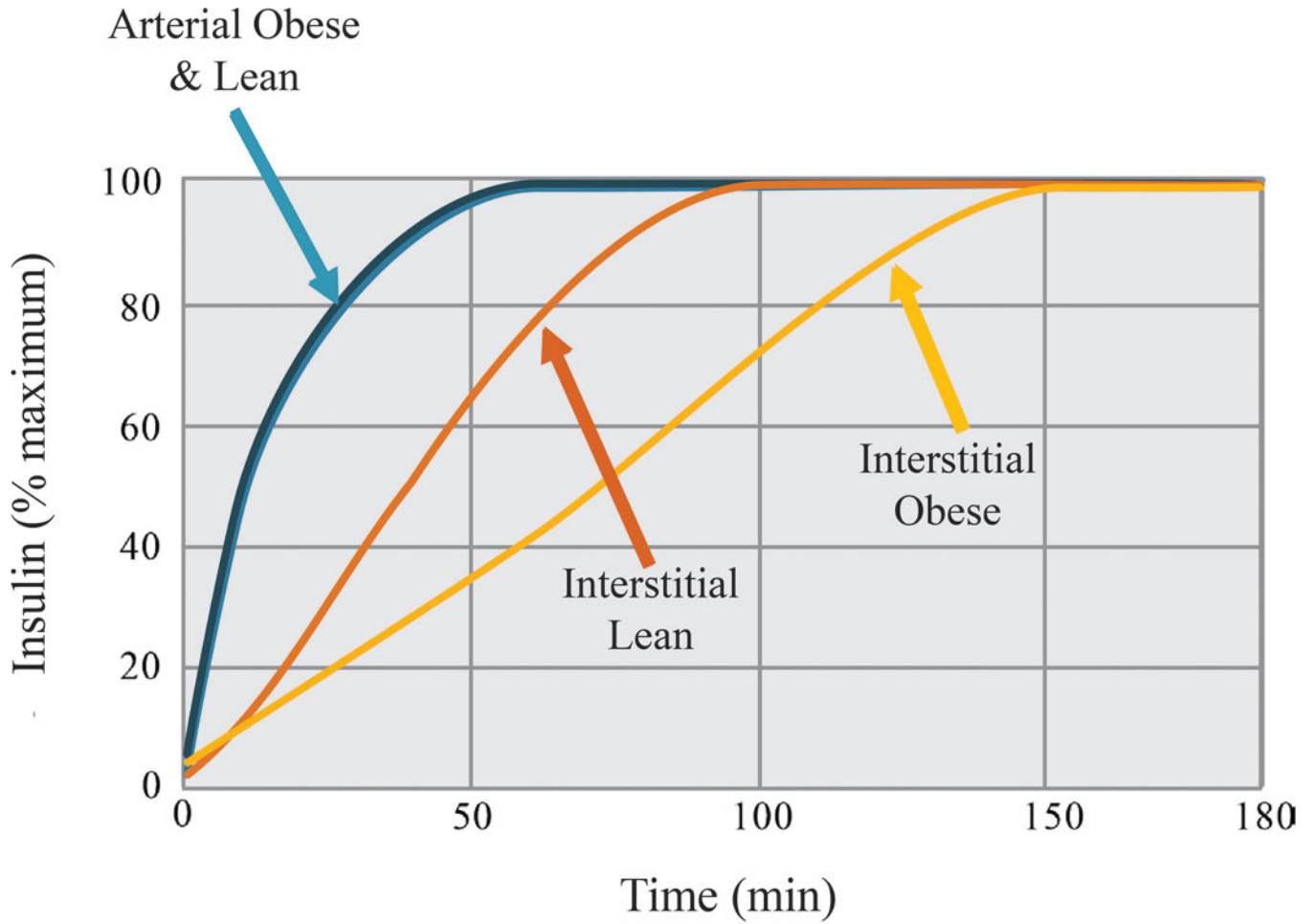
Prediabetes is defined clinically by hyperglycemia. Pathophysiologic features include vascular insulin resistance, oxidative stress, and inflammation, which collectively promote endothelial vasodilator and fibrinolytic dysfunction. Concomitant obesity increases inflammation and free fatty acids to exacerbate these processes. Weight loss is the most effective treatment of prediabetes accompanied by obesity as it interrupts these pathophysiological processes.

Considering the challenges of sustained weight loss, identification of pharmaceutical targets is needed. Preclinical studies demonstrate that the inflammatory response of prediabetes and obesity leads to ECM expansion, which accompanies endothelial dysfunction. ECM deposition is associated with capillary rarefaction, decreased insulin delivery and insulin resistance in liver and muscle. It is increasingly apparent that microcirculatory events are closely intertwined with insulin action. Therapies that improve endothelial function, such as metformin, also reduce risk of incident diabetes and cardiovascular events. Moreover, vasodilatory agents prevent or reverse insulin resistance in preclinical models.

Future studies are needed to elucidate the mechanism of the beneficial effect of GLP-1 analogues on cardiovascular mortality, to determine whether weight loss is requisite, and to develop more convenient and less-expensive GLP-1 delivery methods. Based on preclinical and mechanistic human studies, trials of PDE5 inhibitors and other drugs that increase cGMP, such as guanylate cyclase activators, on endothelial function and insulin sensitivity in prediabetes are warranted. While the role of microRNAs in the pathogenesis of prediabetes has not been fully elucidated, promising preclinical data suggest these should be explored as therapeutic targets.



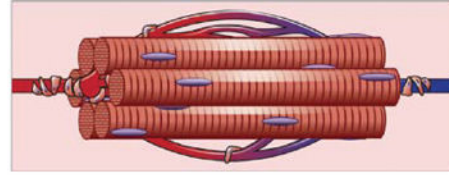
**Figure 1.** Endothelial insulin resistance, hyperglycemia and the formation of advanced glycation products, and increased free fatty acids give rise to oxidative stress, inflammation and endothelial vasodilator and fibrinolytic dysfunction in prediabetes.



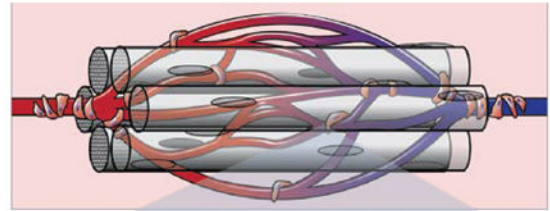
**Figure 2.**

The microcirculation is a barrier to skeletal muscle insulin access which is delayed in obesity. Interstitial insulin concentrations rise more slowly than arterial insulin concentrations due to capillary insulin egress. This time is prolonged in obesity. Derived from Sjosgrand et al.<sup>152</sup>

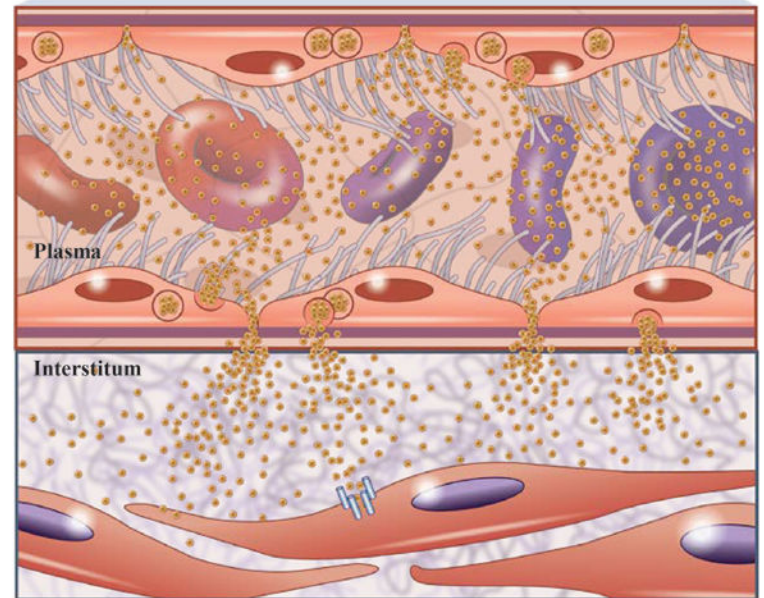
Prediabetes associates with reduced microvascular flow due to impaired vascular reactivity resulting from endothelial dysfunction.



Prediabetes associates with capillary rarefaction and therefore decreased surface area for exchange.



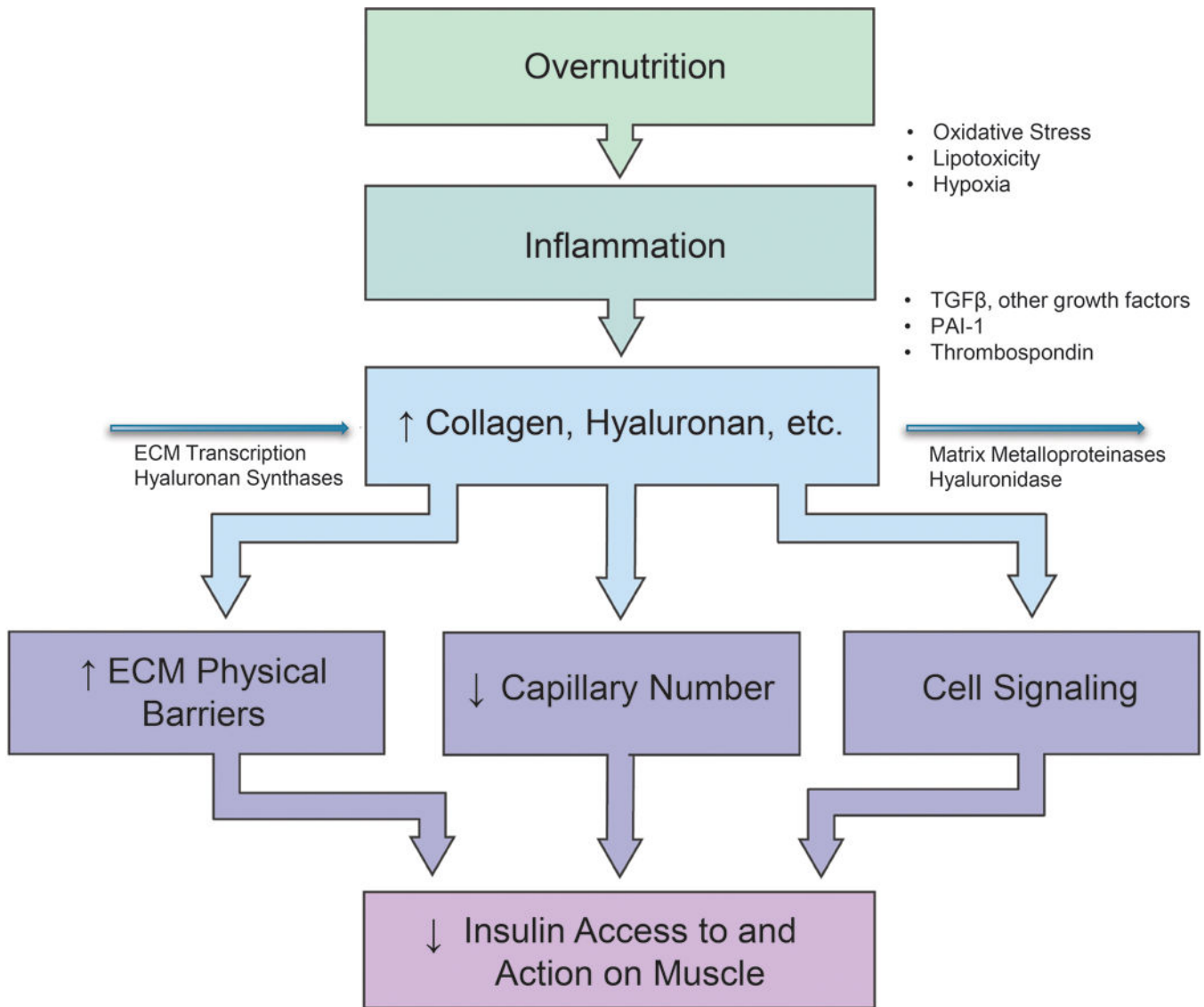
Insulin movement from capillary lumen to interstitium is affected by determinants of fluid phase transport through vesicles or paracellular gaps.



**Figure 3.**

Insulin access is determined by i) vascular reactivity; ii) microcirculatory hemodynamics; and iii) capillary insulin permeability (determined by vesicular and/or paracellular fluid phase transport). Vascular reactivity and microcirculatory hemodynamics are determined by endocrine factors, paracrine factors, cytokines, and microcirculatory architecture. Capillary insulin efflux is determined by the balance between hydrostatic and oncotic pressures.





**Figure 4.** The extracellular matrix (ECM) in the sequelae of prediabetes. Inflammation results in ECM remodeling which creates endothelial dysfunction, capillary regression, spatial barriers, and increased ECM component interaction with cell surface receptors, including the integrin receptor family. These result in a decrease in tissue insulin access and, consequently, insulin action. Modified from Williams et al.<sup>186</sup>

**Table**

## Abbreviations appearing in the text

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<b>AGE</b>	advanced glycation products
<b>ADA</b>	American Diabetes Association
<b>Ang</b>	angiotensin
<b>ARB</b>	angiotensin receptor blocker
<b>ACE</b>	angiotensin-converting enzyme
<b>ARIC</b>	Atherosclerosis Risk in Communities
<b>JNK</b>	c-Jun N-terminal kinase
<b>DPP</b>	Diabetes Prevention Program
<b>DPP-4</b>	dipeptidyl peptidase-4
<b>DI</b>	disposition index
<b>ET-1</b>	endothelin-1
<b>ECM</b>	extracellular matrix
<b>ERK1/2</b>	extracellular signal-regulated kinase 1/2
<b>FPG</b>	fasting plasma glucose
<b>FMD</b>	flow-mediated dilation
<b>FAK</b>	focal adhesion kinase
<b>FS-IVGTT</b>	frequently-sampled intravenous glucose tolerance test
<b>Hb</b>	hemoglobin
<b>HMGB1</b>	high mobility group box 1
<b>HOMA-IR</b>	homeostatic model assessment of insulin resistance
<b>IFG</b>	impaired fasting glucose
<b>IGT</b>	impaired glucose tolerance
<b>IRS1</b>	insulin receptor substrate 1
<b>IGF</b>	insulin-like growth factor
<b>ILK</b>	integrin-linked kinase
<b>IL</b>	interleukin
<b>LDL</b>	low density lipoprotein
<b>MEK</b>	MAPK/ERK kinase
<b>NO</b>	nitric oxide
<b>NOS</b>	nitric oxide synthase
<b>NLRP3</b>	NLR family and PYHIN inflammasome
<b>NLR</b>	nucleotide-binding domain and leucine-rich repeat-containing
<b>MAPK</b>	p38 mitogen activated protein kinase
<b>PI3K</b>	phosphatidylinositol 3 kinase
<b>PDE5</b>	phosphodiesterase 5
<b>PAI-1</b>	plasminogen activator inhibitor-1
<b>PKC</b>	protein kinase C
<b>PYHIN</b>	pyrin and HIN domain
<b>RAGE</b>	receptor for AGE
<b>TNF</b>	tissue necrosis factor

<b>t-PA</b>	tissue-type plasminogen activator
<b>TGF</b>	transforming growth factor
<b>VLDL</b>	very low density lipoprotein
<b>WHO</b>	World Health Organization

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