

## ENDOTHELIAL FACTORS IN METABOLIC SYNDROME

# Endothelial Dysfunction and Metabolic Syndrome

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### ABSTRACT

Atherosclerotic cardiovascular disease (ASCVD) continues to be the leading cause of death worldwide. Metabolic syndrome is associated with an increased risk of ASCVD. With the prevalence of metabolic syndrome continuing to increase, it is important to understand the relationship between these risk factors and development of ASCVD. Endothelial dysfunction (ED), an early, essential step in atherosclerotic plaque formation, is the key link. Here we review diagnostic methods of ED and the mechanisms of each metabolic syndrome component contributing to ED. Finally, the effects of current treatments of metabolic syndrome on ED will also be discussed.

**Keywords:** Atherosclerotic cardiovascular disease, Endothelial dysfunction, Metabolic syndrome.

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### INTRODUCTION

Metabolic syndrome has been recognized as a combination of interrelated metabolic risk factors that directly promote atherosclerotic cardiovascular disease (ASCVD), with the key link between this syndrome and adverse cardiovascular events being endothelial dysfunction (ED). The Adult Treatment Panel (ATP) III criterion is the most commonly used definition: Central obesity (waist circumference  $\geq 102$  cm in men or  $\geq 88$  cm in women), hyperlipidemia (triglyceride level  $\geq 150$  mg/dL or high-density lipoprotein [HDL]-cholesterol  $< 40$  mg/dL in men or  $< 50$  mg/dL in women), hypertension (blood pressure  $\geq 130/85$  mm Hg), and impaired fasting glucose (fasting blood glucose  $> 100$  mg/dL). According to this criterion, approximately 24% of US adults were estimated to have three or more of the above in 2002.<sup>1</sup>

The predominant factors contributing to the metabolic syndrome are thought to be insulin resistance (IR)<sup>2</sup> and

central obesity.<sup>3,4</sup> Indeed, several metabolic pathways have been implicated linking IR as a central driver for the other metabolic risk factors.<sup>5,6</sup> Moreover, central obesity has been associated with accumulation of lipid in muscle and liver tissues, which on its own predisposes to hyperlipidemia and IR.<sup>7</sup> The adipose tissue shows increased production of proinflammatory cytokines and other inflammatory markers, which lead to a state of low-grade inflammation,<sup>8,9</sup> and those with the metabolic syndrome have been found to have elevated levels of proinflammatory cytokines [e.g., interleukin (IL)-6 and tumor necrosis factor alpha (TNF)- $\alpha$  and acute-phase proteins [e.g., C-reactive protein (CRP) and fibrinogen].<sup>10,11</sup> In addition, the metabolic syndrome predisposes to a prothrombotic state, due to elevated levels of procoagulation factors, such as plasminogen activator inhibitor-1, tissue factor, and fibrinogen.<sup>12</sup> These numerous, complex biochemical changes lead to ED and increased risk for subsequent ASCVD.<sup>13</sup>

### ENDOTHELIAL DYSFUNCTION

The healthy endothelial cell layer serves an essential role in maintaining normal vascular homeostasis. In normal physiology, the endothelium produces several paracrine factors that regulate vascular tone, limit expression of proinflammatory molecules, inhibit platelet aggregation, promote fibrinolysis, and limit smooth muscle proliferation.<sup>14-16</sup> Endothelial-derived nitric oxide (NO) is the principal driver of the vasodilatory process. It is generated by the conversion of L-arginine to L-citrulline through the action of endothelial NO synthase (eNOS) and its cofactor tetrahydrobiopterin (BH4).<sup>17</sup> NO molecules vasodilate the local vascular smooth muscles by stimulating guanylyl cyclase and increasing production of cyclic guanosine monophosphate.<sup>17</sup> In addition to its vasodilatory effects, NO also acts as a potent inhibitor of platelet aggregation and adhesion, interferes with leukocyte adhesion, and inhibits proliferation of vascular smooth muscles.<sup>17-19</sup> Disruption of these processes leads to ED, an early, essential step in the development of atherosclerosis.

### ENDOTHELIAL DYSFUNCTION ASSESSMENT

#### Invasive Assessment

Coronary reactivity testing involves intra-arterial infusions of endothelium-dependent vasodilators, such as

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acetylcholine (ACh), bradykinin, or substance P. Acetylcholine activates the endothelial muscarinic receptors that metabolize L-arginine, stimulate NO synthase, and thus generate NO.<sup>20</sup> In response to these infusions, coronary epicardial vessels dilate in the setting of normal or preserved endothelial function. In ED, ACh's direct smooth muscle constrictor effects on epicardial vessels overcome the dilator effects of endothelium-dependent NO release.<sup>21</sup>

### Noninvasive Assessment

Doppler echocardiography, positron emission tomography, and magnetic resonance imaging have been used to noninvasively assess either peripheral or coronary vasculature. Ultrasound-based measurement of brachial artery reactivity or flow-mediated vasodilation (FMD) is the most widely used technique: After a brief period of upper-arm occlusion using a blood pressure cuff, shear stress-mediated brachial arterial dilation is measured using a high-frequency ultrasound transducer.<sup>22</sup> The FMD refers to the vasodilator response due to the shear-mediated NO release of the brachial artery with the stimulus being the ischemia after cuff deflation. Thus, FMD is used as a surrogate measure of endothelial function.

### Associated Biomarkers

Biomarkers have been a subject of research interest for their potential for adding independent prognostic value, in addition to clinical risk factors. Markers of oxidative stress have been studied as a means to assess endothelial injury and dysfunction because reduction in NO bioavailability is often due to increased prooxidant stress.

Glutathione maintains thiol groups of biomolecules in their reduced state and prevents peroxidation of membrane lipids.<sup>23</sup> Similarly, high cysteine levels are indicative of increased oxidative stress. Glutathione is also involved in transportation of NO.<sup>24</sup> Our studies have found associations between increased oxidative stress, measured as lower glutathione and/or higher cysteine levels and FMD, microvascular vasodilator function, arterial stiffness, and arterial thickness.<sup>25-30</sup>

Asymmetric dimethylarginine (ADMA) is a by-product of L-arginine metabolism, i.e., elevated in patients with hypertension, dyslipidemia, and atherosclerosis.<sup>31-34</sup> The ADMA acts as a competitive inhibitor to eNOS, leading to decreased NO production and bioavailability.<sup>31</sup> Plasma ADMA levels correlate with ED<sup>32</sup> and subclinical atherosclerosis.<sup>35</sup>

Following oxidative stress or apoptosis, microparticles are shed from plasma membranes. Those originating from endothelial cells have been thought to impair

endothelium-dependent dilation and the NO pathway. Circulating endothelium-derived microparticles were found to correspond to the severity of coronary artery disease in patients presenting with acute coronary syndromes.<sup>32</sup>

### METABOLIC SYNDROME AND MECHANISMS CONTRIBUTING TO ENDOTHELIAL DYSFUNCTION

Components of the metabolic syndrome—impaired glucose metabolism, obesity, dyslipidemia, and hypertension—are all associated with ED. Endothelial vasodilation is increasingly impaired with the number of components present from the metabolic syndrome.<sup>36</sup> The mechanisms by which these risk factors affect endothelial function are often interrelated and broadly fall into common pathways of endothelial injury, inflammation, reactive oxygen species (ROS) production, and disruption of NO function and bioavailability.

#### Abnormal Glucose Metabolism

Endothelial dysfunction is present even in the early stages of diabetes including impaired glucose tolerance and impaired fasting glucose.<sup>37</sup> Insulin resistance is marked by hyperinsulinemia and hyperglycemia. Under normal conditions, insulin enhances the vasodilatory action of NO and increases its production. Endothelial cells in the insulin-resistant population, however, display paradoxical vasoconstriction when exposed to insulin. The reasons for this are likely multifactorial. In patients with insulin-dependent diabetes mellitus, the serum insulin concentration is inversely correlated with endothelium-dependent vasodilation (EDV).<sup>38</sup> Furthermore, insulin administration itself impairs endothelial function.<sup>39</sup> However, this impairment can be reversed with the administration of antioxidant vitamin C. This suggests that hyperinsulinemia increases oxidative stress in the vasculature.<sup>39</sup> Vascular oxidative stress and overproduction of ROS have a deleterious effect on eNOS activity and synthesis of NO. The ROS in insulin-resistant subjects enhance the oxidation of BH4 to 7,8-dihydrobiopterin (BH2), limiting the amount of active cofactor available for eNOS function. The activity of dihydropyridine reductase, an enzyme that regulates the rate of regeneration of BH4 from BH2, is reduced in IR and compounds on the problem with pteridine metabolism.<sup>40</sup> Finally, ROS directly inactivate NO, thereby decreasing its bioavailability.

Hyperglycemia produces ED by blunting EDV through an assortment of intracellular pathways. Hyperglycemia can lead to the depletion of nicotinamide adenine dinucleotide phosphate, which is essential to the regeneration of antioxidant molecules, such as glutathione,

tocopherol, and ascorbate.<sup>41</sup> Accumulation of advanced glycosylation products formed in chronic hyperglycemia also inactivates NO, creating another avenue for ED.<sup>42</sup> Hyperglycemia also increases the synthesis of diacylglycerol, a key component to the initiation of the protein kinase C (PKC) pathway. Activation of the PKC pathway induces endothelial expression of endothelin-1, a potent vasoconstrictor, as well as decreases the level of eNOS production. The PKC additionally increases production of various growth factors and prothrombotic factors that alter the vascular remodeling process and predispose to thrombosis respectively.<sup>41</sup> Endothelial cells exposed to hyperglycemic conditions also undergo apoptosis that leads to the loss of intimal integrity and detachment of endothelial cells. In certain cases, the endothelium does not detach as an entire cell, forming endothelial microparticles (EMPs) that have procoagulant activity.<sup>43</sup> This process of endothelial denudation normally leads to reparatory mechanisms to restore vascular integrity, such as through the mobilization of endothelial progenitor cells (EPCs). Diabetic patients display a decreased reserve of EPCs secondary to reduced mobilization from the bone marrow, stunted proliferation, and shortened survival.<sup>43</sup>

Interestingly, other studies have shown that the relationship between diabetes and ED may not be unidirectional, but in fact, ED might precede the development of diabetes. A large prospective study of 121,700 women found that the elevated biomarkers of ED, E-selectin, and intercellular adhesion molecule-1 (ICAM-1) predicted incident diabetes.<sup>44</sup> These support the experimental findings in mice with knockout mutations in the eNOS gene that also develop IR.<sup>45</sup> Impaired endothelial permeability limits insulin delivery to the interstitium.<sup>46</sup> Furthermore, insulin delivery to metabolically active muscle tissue is thought to be diminished secondary to impaired endothelial vasodilation, limiting capillary recruitment and compromising microvascular distribution of skeletal muscle blood flow.<sup>47,48</sup>

## Obesity

Obesity has been linked to impaired endothelium-dependent peripheral and coronary vasodilation. The Framingham Heart Study examined this issue in a large community-based sample and found body mass index (BMI) to be inversely correlated with FMD.<sup>49</sup> A similar association was found in the coronary circulation; obese patients with normal or mildly diseased coronary arteries demonstrated significantly attenuated coronary blood flow in comparison with normal-weight subjects with intracoronary ACh.<sup>50</sup>

Excess adipose tissue in obese patients creates a disease state characterized by chronic, low-grade

systemic inflammation. Plasma inflammatory markers, such as CRP, IL-6, TNF- $\alpha$ , fibrinogen, angiotensinogen, and various cell adhesion molecules are uniformly elevated in obese individuals.<sup>15,51,52</sup> Adipocytes function as a metabolically active organ, producing a number of proatherogenic and proinflammatory adipokines. Certain adipokines, such as adiponectin serve a protective role in endothelial function. Adiponectin stimulates NO production and downregulates TNF- $\alpha$ -induced adhesion molecule expression by inhibiting nuclear factor kappa B (NF- $\kappa$ B).<sup>15</sup> Obese individuals have reduced levels of adiponectin. Hypoadiponectinemia is predictably associated with impaired EDV.<sup>53</sup> These stores can be restored with therapeutic lifestyle interventions and weight loss.<sup>54</sup>

Increased production of ROS has also been linked to obesity. Central obesity is tied to oxidative stress through an expanded supply of cytosolic triglycerides in non-adipose tissues, such as muscle, liver, and pancreatic beta cells. Cytosolic triglycerides are the source of the metabolically active long-chain acyl-coenzyme A esters. These esters inhibit the translocation of adenine dinucleotide into the mitochondria, and the resulting intramitochondrial deficiency is a powerful stimulator of mitochondrial oxygen free radical production.<sup>55</sup> Identical to mechanisms at play in IR, oxidative stress decreases NO bioavailability and neutralizes NO function. Endothelin-1 activity is increased in obese patients, and may further exacerbate abnormal vasomotor regulation by disrupting the NO and endothelin-1 balance.<sup>56</sup>

## Dyslipidemia—Low HDL-C and Hypertriglyceridemia

High-density lipoprotein cholesterol works to reverse cholesterol transport and has a protective effect against the development of atherosclerosis. In contrast, decreased HDL-C has been associated with a number of mechanisms that predispose to ED. Hyperlipidemic patients with a low HDL-C have higher levels of vascular cell adhesion molecule-1 (VCAM-1) and ICAM-1. These molecules mediate the adhesion of leukocytes to the endothelium and therefore, contribute to a proinflammatory state.<sup>57</sup> Low HDL-C is also associated with increased low-density lipoprotein cholesterol (LDL-C) oxidation and impaired FMD.<sup>58</sup> The HDL-C has the ability to act as an antioxidant and has been shown to inhibit lipoprotein oxidation.<sup>59</sup> The HDL-C contains antioxidant enzymes and proteins, including platelet-activating factor, acetylhydrolase, and paraoxonase, which are able to counteract LDL-C oxidation.

Oxidized LDL-C induces the activation of NF- $\kappa$ B that ultimately stimulates endothelial cells to express monocyte-specific chemoattractants and cell adhesion

molecules.<sup>60</sup> Oxidized LDL-C also directly decreases NO synthesis through early transcriptional inhibition and destabilization of posttranscriptional messenger ribonucleic acid.<sup>61</sup> The proinflammatory signals are thus intertwined with the oxidative state, demonstrating the importance of HDL-C in protecting the endothelium from these processes.

The contribution of hypertriglyceridemia to ED remains controversial. In a study by Lundman et al,<sup>62</sup> young healthy men with mild-to-moderate hypertriglyceridemia were found to have impaired FMD. Patients in this study also had increased levels of ADMA, an endogenous eNOS inhibitor. Increased ADMA in hypertriglyceridemia consequently reduces the bioavailability of NO, offering one mechanism through which triglycerides can impair endothelial function.<sup>62</sup> Elevated levels of the soluble forms of VCAM-1 and ICAM-1 were also found in subjects with hypertriglyceridemia who otherwise had no history of diabetes, hypertension, or other significant cardiovascular risk factors.<sup>63</sup> Lewis et al<sup>64</sup> found that endothelium-dependent relaxation mediated by ACh was diminished in patients with hypertriglyceridemia and normal LDL-C levels. A study by Chowienczyk et al,<sup>65</sup> in contrast, showed no impairment of the endothelium in patients with severe hypertriglyceridemia in the context of lipoprotein lipase dysfunction and normal LDL-C. This discrepancy may be attributed to lipoprotein lipase deficiency, which is associated with a selective increase in chylomicrons and large very low-density lipoproteins. By virtue of their size, these lipoproteins are unable to invade the vessel wall and initiate the process of atherogenesis.<sup>63</sup> Lewis et al<sup>64</sup> hypothesized that in their study, the difference may have been due to the higher BMI of the test subjects compared with the controls, indicating that there may have been a confounding effect of IR that was not accounted for in their study.

## Hypertension

Patients with essential hypertension have blunted responses to ACh-mediated endothelial vasodilation in both the peripheral and coronary vascular beds.<sup>66,67</sup> When simultaneously exposed to ACh and NG-monomethyl-L-arginine (L-NMMA), an inhibitor of the endothelial synthesis of NO, hypertensive patients do not demonstrate a significant reduction in vasodilation compared with control patients.<sup>68</sup> This phenomenon suggests impairment in NO bioactivity in hypertension. In hypertension, this impairment is largely attributable to NO breakdown and inactivation by ROS, as opposed to decreased endothelial NO synthesis. Intra-arterial administration of high-dose antioxidant vitamin C to patients with hypertension and impaired forearm blood flow can acutely reverse this defect, giving more credence to this theory.<sup>69</sup> Vitamin C

scavenges radical oxygen species allowing for NO activity, which then un masks the effect of L-NMMA.<sup>69</sup> In parallel to increased oxidative stress, dysfunctional endothelium in hypertensive patients is associated with increased endothelial-derived constricting factors (EDCFs). These factors include endothelin-1, angiotensin II, and cyclooxygenase-derived products, such as thromboxane A2 and prostaglandin H2. The EDCFs cause vasoconstriction, further contributing to ED.<sup>70</sup>

## Metabolic Syndrome

Metabolic syndrome encompasses the aforementioned conditions of hypertension, IR, dyslipidemia, and obesity. Many of these conditions coexist with one another, leading to a complex array of inflammation, NO inactivation and depletion, oxidative stress, and prothrombotic states. The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study evaluated 1,016 subjects aged 70 years and measured EDV after intra-arterial ACh infusion. This study found that patients with metabolic syndrome (per National Cholesterol Education Program/ATP III criteria) had EDV that was progressively more impaired with the increasing number of criteria met for metabolic syndrome. When the criteria were analyzed separately and in multiple regression analysis, abdominal obesity was most closely related to EDV.<sup>36</sup> Of course, abdominal obesity is commonly a comorbid condition to the other criteria, and so it is important to recognize that multiple mechanisms are working together additively, if not synergistically in metabolic syndrome to result in ED.

## TREATMENT OF METABOLIC SYNDROME AND ITS EFFECTS ON ENDOTHELIAL DYSFUNCTION

### Lifestyle Modifications

Lifestyle modifications, comprising of diet, exercise, and tobacco cessation, are the first-line intervention for metabolic syndrome. Weight reduction and maintenance of a lower weight should be the first priority.<sup>71</sup> The American Diabetes Association recommends the Mediterranean diet, promoting high consumption of whole-grain foods, fruits, and vegetables for their beneficial effects on glycemic control and cardiovascular risk factors.<sup>72</sup> In the PREDIMED trial, 3,541 patients without diabetes at high cardiovascular risk were assigned to one of two Mediterranean diets, supplemented with either free virgin olive oil or nuts. Those assigned to the Mediterranean diets had lower risk of diabetes compared with the low-fat control diet.<sup>73</sup>

A randomized trial studied the effects of a Mediterranean-style diet on ED in patients with metabolic syndrome with a follow-up of 2 years.<sup>74</sup> The ED was measured by platelet aggregation response to adenosine

after applying a blood pressure cuff and administering L-arginine. Those assigned to the Mediterranean diet had an improved endothelial function score, but it remained unchanged in the control group. Marin et al<sup>75</sup> also conducted a smaller trial in 20 elderly subjects and assessed the effects of dietary fat on the release of EMPs and EPCs. The Mediterranean diet led to a lower EMP and higher EPC levels. Furthermore, they also found lower urinary isoprostane concentrations after consumption of a Mediterranean diet, signifying improvement in oxidative stress.

The American Heart Association (AHA) and American College of Sports Medicine recommend at least 30 minutes of moderate-intensity physical activity for at least 5 days of the week, or 20 minutes of vigorous aerobic exercise 3 days a week, or a combination of the two,<sup>76</sup> and for those with metabolic syndrome, regular exercise is crucial.<sup>71</sup> In healthy males, regular aerobic exercise for 3 months resulted in a 30% increase in ACh-mediated vasodilation.<sup>77</sup> These beneficial effects of physical activity on endothelial function were also found in those with chronic heart failure<sup>78</sup> and in those with cardiovascular risk factors.<sup>79</sup> Exercise has been shown to improve endothelial function, independently of its reduction in cardiovascular risk factors<sup>79</sup>—although exercise training significantly improved FMD, plasma lipids, blood pressure, blood glucose, waist-to-hip ratio, or BMI were unchanged after 8 weeks in patients underlying vascular dysfunction. Furthermore, aerobic interval training was found to be more effective in improving ED, compared with continuous moderate exercise (9% vs 5%;  $p < 0.001$ ) in 32 metabolic syndrome patients, suggesting that exercise intensity is an important factor for improving cardiorespiratory fitness and reversing cardiovascular risk factors.<sup>80</sup>

Smoking is a well-known and most important modifiable risk factor for ASCVD and has been associated with ED in asymptomatic young adults without any other risk factors.<sup>81</sup> The proposed mechanisms are thought to be secondary to smoking increasing adherence of platelets and macrophages to the vessel wall, developing a procoagulant and inflammatory environment.<sup>82</sup> Smoking cessation, for only 2 weeks, has been found to reduce platelet aggregations, and thus, decrease oxidative stress.<sup>83</sup> A study of 1,504 current smokers found that after 1 year of cessation, FMD improved despite weight gain, while in those who did not quit, FMD did not change.<sup>84</sup>

### Insulin Sensitizers

Metformin is a biguanide that reduces IR without directly affecting insulin secretion and causes weight loss.<sup>85</sup> Metformin reduces the incidence of metabolic syndrome by 17% compared with placebo.<sup>86</sup> Patients with metabolic

syndrome who received metformin showed significant improvement in EDV compared with placebo.<sup>87</sup> Subjects with type II diabetes managed with diet but without metabolic syndrome had improvement in ACh-stimulated vasodilation in addition to IR.<sup>88</sup> Potential mechanisms for metformin's effect include the improvement in IR, antioxidant effects, and its effects on lipids and free fatty acids.<sup>89</sup>

Troglitazone, an activator of peroxisome proliferator activator receptor  $\alpha$ , improved IR and controlled diabetes. Patients treated with troglitazone for 12 weeks had improved FMD, which strongly correlated with improved fasting insulin levels.<sup>90</sup> Rosiglitazone also improved ED after 12 weeks in patients with type II diabetes, an effect attributed to increase in NO.<sup>91</sup>

### Statins

The recent 2013 American College of Cardiology (ACC)/AHA guidelines recommend statin therapy for primary prevention in patients with diabetes between the ages of 40 and 75 years, with calculated 10-year ASCVD risk of 7.5% or higher, with LDL cholesterol levels of 190 mg/dL or higher, and those with cardiovascular disease.<sup>92</sup> The evidence for statins' widespread use for primary ASCVD prevention, and specifically its benefits on ED, dates back to the 1990s. Six months of lovastatin dose 40 mg twice-daily therapy significantly improved endothelium-mediated responses in 23 patients with atherosclerosis.<sup>93</sup>

The effects of statins on ED were then found to occur earlier than 6 months in subsequent studies. Six weeks of 40 mg of daily pravastatin increased FMD in patients with acute coronary syndrome, when compared with placebo, in the Reduction of Cholesterol in Ischemia and Function of the Endothelium (RECIFE) trial.<sup>94</sup> Another study found a significant increase in FMD in hypercholesterolemic, postmenopausal women receiving atorvastatin as early as 2 weeks.<sup>95</sup>

Interestingly, the RECIFE trial did not find any correlations between the changes in FMD and decreases in total and LDL cholesterol,<sup>94</sup> suggesting another mechanism of ED improvement other than lipid lowering. Indeed, statins have been shown to increase the bioavailability of NO: Patients with both normal and high levels of cholesterol had significant improvement in EDV after L-NMMA was administered and blocked statin therapy.<sup>96,97</sup> Statins may also reduce leukocyte adhesion and thus improve endothelial function by reducing circulating levels of adhesion molecules P-selectin and ICAM-1.<sup>98</sup>

### CONCLUSION

The vascular endothelium serves as a modulator of vascular disease, and ED is a critical early step in the development of atherosclerosis. The components of

metabolic syndrome render patients more susceptible to ASCVD as their endothelial function is disrupted by inflammation, oxidative stress, and biomechanical stress. This is a promising study population to identify potential biomarkers or other modes of endothelial function assessment to ultimately better risk-stratify patients who are likely to develop ASCVD and sustain major cardiovascular events. Prospective trials are needed to assess the prognostic value of risk assessment in these patients. Furthermore, novel, primary preventive interventions could be developed targeting this early stage of atherosclerosis to decrease the ASCVD burden that continues its devastation worldwide.

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