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Microvascular dysfunction: An emerging pathway in the pathogenesis of obesity-related insulin resistance

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Abstract The prevalence of type 2 diabetes mellitus (T2DM) and its major risk factor, obesity, has reached epidemic proportions in Western society. How obesity leads to insulin resistance and subsequent T2DM is incompletely understood. It has been established that insulin can redirect blood flow in skeletal muscle from non-nutritive to nutritive capillary networks, without increasing total blood flow. This results in a net increase of the overall number of perfused nutritive capillary networks and thereby increases insulin-mediated glucose uptake by skeletal muscle. This process, referred to as functional (nutritive) capillary recruitment, has been shown to be endothelium-dependent and to require activation of the phosphatidylinositol-kinase (PI3K) pathway in the endothelial cell. Several studies have demonstrated that these processes are impaired in states of microvascular dysfunction. In obesity, changes in several adipokines are likely candidates to influence insulin signaling pathways in endothelial cells, thereby causing microvascular dysfunction. Microvascular dysfunction, in turn, impairs the timely access of glucose and insulin to their target tissues, and may therefore be an additional cause of insulin resistance. Thus, microvascular dysfunction may be a key feature in the development of obesity-related insulin resistance. In the present review, we will discuss the evidence for this

emerging role for the microcirculation as a possible link between obesity and insulin resistance.

Keywords Microcirculation · Type 2 diabetes mellitus · Insulin resistance · Endothelial dysfunction

1 Introduction

The prevalence of obesity is increasing worldwide and has reached epidemic proportions in Western society [1]. Central obesity and a sedentary lifestyle are main causes of insulin resistance and type 2 diabetes mellitus (T2DM) [2, 3]. The increases in their prevalence results in an increased prevalence of T2DM, with an estimated 285 million people with T2DM worldwide and a further seven million people developing T2DM each year [4]. However, how obesity leads to T2DM is incompletely understood, and further insight into this pathophysiology may contribute to more precise risk assessment for the development of T2DM and additionally to new treatment targets for prevention of T2DM.

Microvascular dysfunction may be both cause and consequence of T2DM. On the one hand, it is generally accepted that T2DM causes microvascular dysfunction and microvascular complications such as nephropathy and retinopathy [5]. On the other hand, microvascular dysfunction has been identified as an *antecedent* of T2DM [5]. In addition, we [6–8] and others [9, 10] have demonstrated that microvascular dysfunction is impaired in (central) obesity, and obesity has been suggested as a primary cause of microvascular dysfunction [11]. Therefore, microvascular dysfunction may be an intermediate step linking central obesity to insulin resistance and T2DM [11–14].

In the present review, we will discuss the role of microvascular dysfunction as a key feature in the development of obesity-related insulin resistance and T2DM.

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2 Microcirculation: definition, function, and assessment

The microcirculation can be defined anatomically, i.e. as vessels less than 150 μm in diameter, including all capillaries and venules and small arterioles [15]. However, this definition excludes larger arterioles with a diameter above 150 μm , which may be important to microcirculatory function [15]. An alternative definition is based on arterial/arteriolar vessel physiology, and includes all vessels that respond to increased pressure by a myogenic reduction in lumen diameter. Such a definition also includes the larger arterioles in the microcirculation in addition to capillaries and venules [11, 15, 16].

The microcirculation has three essential functions which serve to regulate whole body and tissue metabolism, and blood pressure [12, 13]: 1) regulation of the exchange of nutrients, oxygen, and hormones; 2) avoidance of large fluctuations in hydrostatic pressure at the capillary level; and 3) determining the overall peripheral resistance, since the quantitatively most substantial drop in hydrostatic pressure occurs at the level of the microcirculation [15]. Microvascular dysfunction is defined as an impairment in one or more of these functions.

Currently, several types of estimates of microvascular (endothelial or smooth muscle cell) function are available [17]. First, assessment of microvascular function in specific microvascular beds is frequently used, such as in 1) skin (by capillaroscopy and laser-Doppler fluxmetry); 2) muscle (by plethysmography and contrast-enhanced ultrasonography); 3) conjunctival bed (by intravital microscopy) and 4) retina (by photography). Responses can be studied in the basal state and after applying a stimulus, such as reactive hyperemia, heating, or local or systemic administration of endothelium-(in)dependent vasoactive agents such as acetylcholine and sodium nitroprusside, with lower responses in general reflecting microvascular dysfunction [18–20]. In addition, in the retinal microvasculature (as evaluated by retinal photography) [21–24] venular dilation and arteriolar narrowing have been associated with, and may be markers of, endothelial dysfunction [25]. Moreover, responses of the retinal microvasculature can be studied after applying a flicker-light stimulus, with lower responses thought to reflect endothelial dysfunction [26]. Second, microvascular function can be assessed with the use of biomarkers. Thus, measurement of plasma levels of endothelium-derived regulatory proteins (e.g. soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), and von Willebrand factor (vWF) [27]) is often used. Increased levels of these markers are thought to reflect endothelial permeability to leucocytes (i.e. sE-selectin, sICAM-1, and sVCAM-1) [27–30], and prothrombotic and procoagulant activity (i.e. vWF) [27, 29, 30]. Note that the microvascular endothelium

is the most important determinant of these plasma markers of endothelial dysfunction [27, 31–33], because of its large surface area and production capacity (i.e. the microvasculature covers 98 % of the total vascular surface area [34]). For these reasons, it is plausible to assume that higher circulating concentrations of these markers reflect microvascular endothelial dysfunction. Another biomarker of microvascular function is urinary albumin excretion or microalbuminuria, which is thought to reflect a generalized increase in endothelial permeability [27], and is frequently used as a marker of general endothelial dysfunction [27, 29, 30, 35–37]. This concept is derived from data showing that microalbuminuria is associated with a greater transcapillary escape rate of albumin, i.e. with greater microvascular permeability [27] and from data showing that microalbuminuria is strongly associated with risk of cardiovascular disease, that this association cannot be explained by conventional risk factors, and that changes in microalbuminuria are paralleled by changes in cardiovascular risk (reviewed elsewhere [38]). In general, assessment of microvascular function in specific vascular beds is technically demanding and time-consuming, and is thus mostly used in relatively small-scale, experimental studies, whereas the use of biomarkers is especially suitable for large-scale observational studies.

3 Microvascular dysfunction: a cause of insulin resistance

Insulin resistance is typically defined as decreased sensitivity and/or responsiveness to the *metabolic* actions of insulin, which results in impaired glucose disposal [11, 13, 39, 40]. In muscle cells, activation of the phosphatidylinositol-kinase (PI3K) pathway leads to translocation of glucose transporter-4 (GLUT-4) to the cell membrane, which activates the downstream pathways of glucose metabolism [11, 13, 39–41]. The translocation of GLUT-4 is believed to be the rate-limiting step for insulin-mediated glucose uptake in muscle [41, 42]. Several studies demonstrated that inappropriate fat accumulation in muscle cells or the release of inflammatory cytokines by fat cells may affect this pathway [41, 43, 44]. This process is referred to as metabolic insulin resistance, and is widely accepted to precede the development of T2DM [45]. Nevertheless, most studies investigating metabolic insulin resistance examined individuals with long-standing T2DM [46]. These studies demonstrated that there are indeed defects in the insulin-signaling pathway in muscle cells in the diabetic state [18, 47]. However, an important and necessary step preceding translocation of GLUT-4 is the perfusion of the microvasculature within the skeletal muscle in order to deliver insulin and glucose to the muscle cells.

Since the 1990s, there has been an increasing interest in this precellular step, and it has become clear that the delivery of insulin and glucose to muscle tissue is regulated by insulin itself via direct effects on microvascular function that require activation of the insulin receptor on endothelial cells [11, 40]. Baron and colleagues [48] first reported that insulin increases total blood flow in skeletal muscle, which is paralleled by an increase in insulin-mediated glucose uptake [48, 49]. Although some studies have confirmed this vascular action of insulin [50, 51], most studies observed only insulin-mediated increases in total limb blood flow after using supra-physiological doses of insulin or after several hours of delay when physiological concentrations were used [52–54]. Therefore, the physiological importance of insulin's ability to increase *total* blood flow remains controversial [11, 18, 53]. However, besides these actions on resistance vessels, insulin can *redirect* blood flow in skeletal muscle from non-nutritive to nutritive capillary networks, *without* increasing total blood flow. This results in a net increase of the overall number of perfused nutritive capillary networks [11], and thereby increases insulin-mediated glucose uptake by skeletal muscle [40, 52]. This process is referred to as functional (nutritive) capillary recruitment. Such capillary recruitment requires physiological concentrations of insulin and has a time course that accords well with the time course for insulin-mediated glucose uptake in skeletal muscle [39, 53, 55]. In addition, this process has been shown to be endothelium-dependent, requiring activation of the PI3K pathway in the endothelial cell [39], including the insulin receptor, insulin receptor substrate 1 (IRS-1), insulin receptor substrate 2 (IRS-2) [56], phosphoinositide-dependent kinase 1 (PDK-1), and protein kinase B (Akt) [39, 41] (Fig. 1). In contrast to muscle cells, this activation does not result in translocation of GLUT-4 to the cell surface, but in the production of NO due to increased endothelial NO synthase (eNOS) activity [39] (Fig. 1). Consequently, insulin-induced stimulation of endothelial cells leads to increased NO production, which stimulates capillary recruitment [39] and transendothelial transport of insulin [57]. In addition, however, insulin also has vasoconstrictor effects through the production of endothelin-1 (ET-1) through stimulation of the intracellular MAP kinase signaling (MAPK) pathway and extracellular signal-regulated kinase-1/2 (ERK-1/2) (Fig. 1) [39]. In physiological conditions, when insulin binds to the insulin or insulin-like growth factor receptor on endothelial cells [58], the net result usually favors NO production [11] and thus vasodilatation and capillary recruitment.

There now is substantial evidence, derived from both animal experiments and observations in humans, that this pathway is an important determinant of insulin-mediated glucose uptake in muscle. Thus, Clark and colleagues [59] were the first to report insulin-mediated capillary recruitment in skeletal

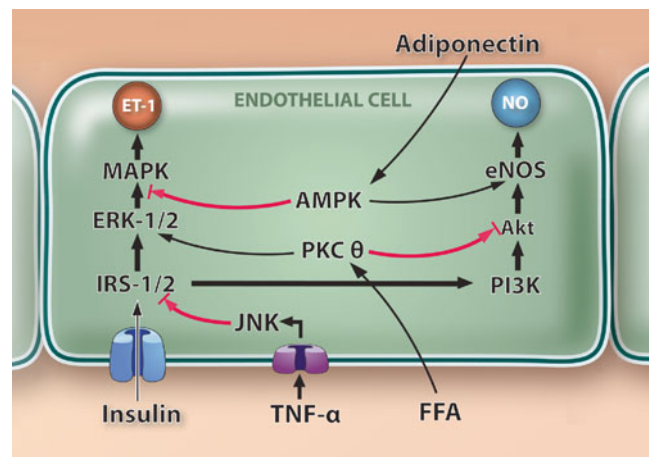


Fig. 1 When insulin binds on the insulin receptor of endothelial cells, the PI3K and MAPK pathways in the endothelial cell are activated. These activations lead to the production of NO and ET-1, resulting in vasodilatation and vasoconstriction respectively. Normally, the net result usually favors NO production, resulting in a redirection of blood flow in skeletal muscle from non-nutritive capillaries to nutritive capillaries, thereby increasing insulin-mediated glucose uptake by skeletal muscle. In obesity, there is an increase in several circulating adipose tissue-derived factors, in particular FFA and TNF- α , whereas the anti-inflammatory adipokine adiponectin is decreased. FFA and TNF- α affect the insulin signaling pathway in endothelial cells by the activation of PKC θ and JNK respectively. In addition, decreased adiponectin levels decrease AMPK phosphorylation. Accordingly, these endocrine factors are likely candidates to influence insulin signaling pathways (i.e. decreased PI3K activation and increased ERK-1/2 activation) in endothelial cells, thereby impairing insulin-mediated vasodilatation and capillary recruitment, and thus skeletal muscle glucose uptake. *black arrows*—stimulation; *red arrows*—inhibition; IRS-1/2—insulin receptor substrate 1/2; PI3K—phosphatidylinositol-kinase-dependent; Akt—protein kinase B; eNOS—endothelial nitric oxide synthase; NO—nitric oxide; ERK-1/2—extracellular signal-regulated kinase-1/2; ET-1—endothelin-1; FFA—free fatty acids; TNF- α —tumor necrosis factor- α ; JNK—intracellular enzyme c-Jun N-terminal kinase; PKC θ —protein kinase C; AMPK—5' adenosine monophosphate-activated protein kinase

muscle of the rat hind limb. In subsequent *in vivo* rat studies, the effect of insulin on capillary perfusion was confirmed [54, 60, 61]. In human muscle, it was established that insulin increased microvascular blood volume [62, 63]. In addition, hyperinsulinemia was shown to enhance skin post-occlusive capillary recruitment and microvascular vasomotion [64, 65]. Moreover, capillary recruitment and acetylcholine-mediated vasodilatation of both skin and resistance arteries were strongly associated with insulin sensitivity [66–70]. In further support of the physiological importance of insulin-mediated capillary recruitment, several studies have demonstrated that insulin-mediated capillary recruitment contributes to glucose disposal in skeletal muscle [6, 52, 55, 56, 63, 71, 72]. In subsequent studies, it was established that approximately 40 % of insulin-mediated glucose uptake by skeletal muscle can be attributed to capillary recruitment [55, 73, 74]. In

pathophysiological conditions, both obese Zucker rats [75] and obese insulin-resistant humans [6–8, 62, 66, 72, 76–80] are characterized by impaired capillary recruitment as well as impaired insulin-mediated glucose uptake by skeletal muscle in the basal state and during hyperinsulinemia.

In accordance, accumulating evidence supports the hypothesis that microvascular dysfunction precedes and even predicts the development T2DM. First, a growing number of prospective studies have shown that microvascular dysfunction is associated with incident impaired fasting glucose (IFG) and incident T2DM. Recently, we analyzed and pooled thirteen [81–93] prospective population-based studies in a meta-analysis [94]. These studies focused on the microvasculature of the skin, eye, and kidney. When all estimates of microvascular dysfunction were combined, we found that, per standard deviation (SD) greater microvascular dysfunction, the incidence of IFG increased by 15 % and the incidence of T2DM increased by 25 %, in a follow-up period ranging from 2.6 to 12 years. In addition, one SD greater endothelial dysfunction, as measured with plasma levels of endothelium-derived regulatory proteins – which are likely to be derived from microvascular endothelium [95] –, was associated with a 50 % higher incidence of T2DM. Second, studies which examined diet-induced insulin resistance in both vasculature and skeletal muscle demonstrated that microvascular endothelial dysfunction develops well before impaired insulin activation of PI3K in skeletal muscle [96, 97]. Third, a recent study demonstrated reduced insulin-mediated glucose uptake by skeletal muscle in tissue-specific knockout mice lacking IRS-2 in endothelial cells [56]. Importantly, glucose uptake by isolated skeletal muscle from these mice was not impaired, indicating that microvascular endothelial dysfunction causes impaired insulin-mediated glucose uptake even while insulin signaling pathways in skeletal muscle cells are intact [56].

In summary, these findings strongly support the hypothesis that microvascular dysfunction is a *cause* of insulin resistance, by affecting insulin-mediated glucose uptake by skeletal muscle through impaired capillary recruitment (Fig. 2) [11–14]. Subsequently, the hyperglycemia and hyperinsulinemia that evolve from metabolic insulin resistance can further impair endothelial dysfunction (and thus capillary recruitment) [98–100] and glucose disposal [18], which results in a vicious circle (Fig. 2).

Importantly, by increasing peripheral vascular resistance, microvascular dysfunction can also contribute to the development of hypertension (Fig. 2), which suggests that microvascular dysfunction constitutes one of the links between insulin resistance and hypertension in the metabolic syndrome (reviewed elsewhere [11–14]).

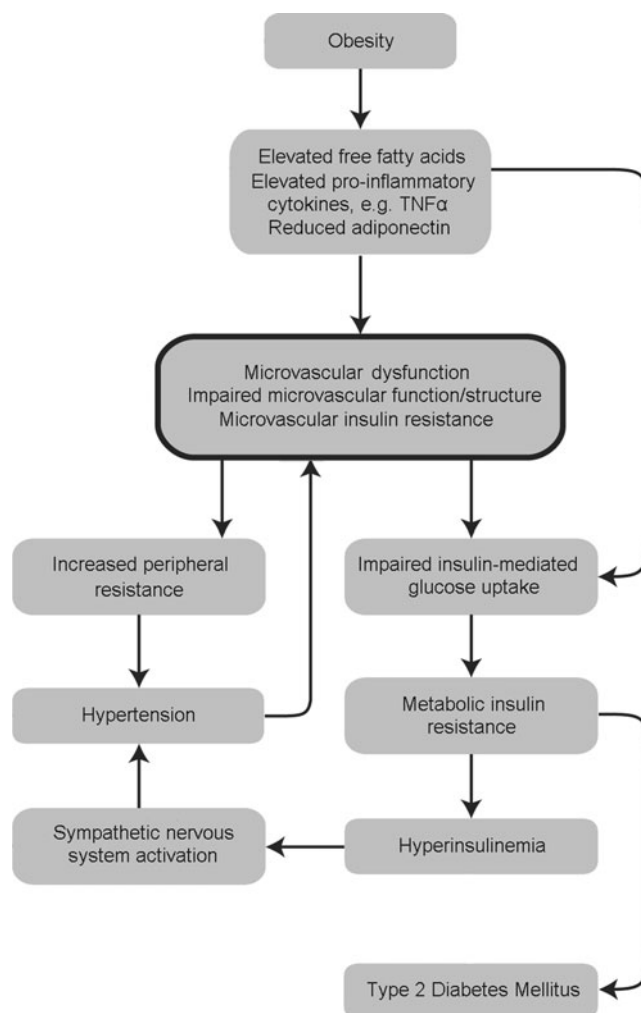


Fig. 2 Hypothesis describing microvascular dysfunction as an intermediate step linking central obesity to insulin resistance (and, downstream, T2DM) and hypertension (adapted from Jonk [18]). TNF- α —tumor necrosis factor- α

4 Obesity: a cause of microvascular dysfunction

4.1 Obesity-related intracellular signaling

We [6–8] and others [9, 10, 62, 101, 102] have demonstrated that microvascular dysfunction is impaired in obesity and that microvascular dysfunction develops progressively along with an increase in adipose tissue [40, 67, 103]. In obesity, insulin-mediated activation of the PI3K pathway in endothelial cells is selectively impaired while the insulin-mediated activation of ERK-1/2 is intact [40, 104], i.e. there is endothelial or microvascular insulin resistance with regard to insulin's vasodilator actions. For example, studies in diet-induced obesity in animals revealed blunted PI3K signaling pathways in endothelial cells through impaired insulin-mediated phosphorylation of Akt [96, 105, 106] and eNOS [56, 96, 105, 107], whereas insulin-mediated ET-1 expression was unchanged [56, 107].

Thus, obesity-related microvascular dysfunction is characterized by cellular defects in endothelial cells that influence the balance between vasodilatation and vasoconstriction. The selective insulin signaling impairment in endothelial cells results in decreased NO production, which reduces insulin-mediated nutritive capillary recruitment [39] and trans-endothelial transport of insulin [57]. Consequently, there is a decreased insulin-mediated glucose uptake in skeletal muscle, i.e. skeletal muscle (i.e. metabolic) insulin resistance (Fig. 2). Therefore, endothelial insulin signaling defects play an important role in the development of obesity-related insulin resistance.

4.2 Obesity-related endocrine signaling

The close association between adipose tissue and microvascular functioning strongly suggests signaling pathways between adipose tissue and the microcirculation [11, 40]. Adipose tissue and in particular visceral adipose tissue cells secrete a variety of bioactive substances called adipokines [11, 40]. In obesity, there is an enhanced production of free fatty acids (FFAs), tumor necrosis factor (TNF)- α , leptin, resistin, and several other inflammatory cytokines [11, 40], whereas the production of adiponectin, an anti-inflammatory adipokine, is reduced [11, 108].

Several studies have demonstrated that systemic FFA infusion inhibits insulin-mediated capillary recruitment [80, 109–111] and forearm blood flow responses [112] with subsequent blunted insulin-mediated glucose uptake by skeletal muscle [109–111, 113–117]. Conversely, lowering FFA levels in obese subjects improved basal and insulin-mediated capillary recruitment in obese insulin-resistant individuals [80]. FFA may induce insulin resistance through activation of protein kinase C (PKC) θ which subsequently inhibits insulin-mediated phosphorylation of Akt and stimulates ERK-1/2 (Fig. 1) [118].

TNF- α inhibits both insulin-mediated capillary recruitment and insulin-mediated glucose uptake by skeletal muscle [103, 119, 120]. TNF- α may induce endothelial insulin resistance through activation of intracellular enzyme c-Jun N-terminal kinase (JNK) [121]. JNK has been shown to regulate both whole-body insulin sensitivity and insulin-mediated cell signaling [40, 122]. Hence, activation of JNK impairs the PI3K pathway and stimulates the phosphorylation of ERK-1/2 in endothelial cells [121, 123], resulting in insulin-mediated vasoconstriction (Fig. 1) [40]. Conversely, a recent study found that specific inhibition of JNK in *db/db* mice can restore the blunted insulin-mediated vasodilatation [124].

Adiponectin has been shown to increase insulin sensitivity and improve vascular function [125]. These beneficial effects are probably attributable to stimulation of 5'

adenosine monophosphate-activated protein kinase (AMPK) phosphorylation by adiponectin, which enhances insulin-mediated vasodilatation through increased eNOS phosphorylation and decreased ERK-1/2 stimulation (Fig. 1) [126, 127]. In addition, adiponectin itself can reduce the production of proinflammatory cytokines, which has favorable effects of insulin-signaling pathways [13, 128, 129]. In obesity, adiponectin levels are decreased, which is probably caused by TNF α and IL-6, as well as by other inflammatory mediators [13, 129, 130].

Another important system that has been suggested to be involved in microvascular functioning is the renin-angiotensin system (RAS) [11]. All the components of the RAS necessary to generate the vasoconstrictor angiotensin II (AngII) are expressed in human adipose tissue [11, 131, 132]. In obese subjects there is enhanced activation of the RAS, which may directly relate to the mass of adipose tissue [11, 133]. Enhanced RAS activity may have detrimental effects on insulin-mediated skeletal muscle glucose uptake by 1) affecting insulin-mediated IRS-1 phosphorylation in endothelial cells [134, 135]; 2) production of reactive oxygen species (ROS), which reduce NO bioavailability [136, 137]; and 3) stimulation of the release of ET-1 [138, 139].

In addition to the above, leptin and other cytokines and chemokines have also been implicated in the pathogenesis of endothelial or (micro)vascular insulin resistance (reviewed elsewhere [13, 129, 140, 141]).

In summary, in obesity, there is an increase in several circulating adipose tissue-derived factors, in particular FFA and TNF- α , whereas the anti-inflammatory adipokine, adiponectin, is decreased. These endocrine factors are likely candidates to influence insulin signaling pathways in endothelial cells, thereby causing both impaired insulin-mediated vasodilatation and impaired skeletal muscle glucose uptake (Fig. 1) [11]. Hence, these endocrine factors provide a potential link between obesity-related microcirculatory dysfunction and obesity-related insulin resistance (Fig. 2) [11]. In addition to these systemic endocrine effects, we have postulated a vasoregulatory role for local deposits of fat next to the microvasculature (i.e. perivascular adipose tissue, PVAT) [11, 40, 142]. Adipokines released by these fat cells may directly inhibit vasodilatory pathways locally, and thus have a local rather than a systemic vasoregulatory effect, which we named “vasocrine” [11, 40, 142]. A recent animal study demonstrated dramatic increases in PVAT in muscle of *db/db* mice. In addition, these mice had a reduction in adiponectin release by PVAT and decreased insulin-mediated vasodilatation [124], indicating that PVAT induces microvascular dysfunction by influencing insulin signaling and thereby insulin’s microvascular effects [12].

5 Conclusion

Considerable evidence exists that microvascular dysfunction is a key feature in the development of obesity-related insulin resistance. Obesity is associated with microvascular dysfunction through alterations in endocrine and vasocrine signals that cause alterations in microvascular endothelial and skeletal muscle intracellular signaling. Microvascular dysfunction, in turn, impairs the timely access of glucose and insulin to their target tissues, and is therefore a contributor to insulin resistance.

Therefore, the microcirculation may present a target for the prevention and treatment of the metabolic syndrome and T2DM [11, 12, 40]. Hence, more studies are required for elucidation of the pathophysiological pathways that contribute to microvascular dysfunction. Unraveling this issue may contribute to more precise assessment of risk of development of the metabolic syndrome and T2DM. In addition, unraveling how microvascular dysfunction is determined and how it leads to the metabolic syndrome and T2DM may lead to new treatment targets as well as to a better understanding of why certain existing treatments (i.e. angiotensin converting enzyme- (ACE-) inhibitors, angiotensin receptor blockers (ARBs), and physical activity) are associated with decreased risk of developing the metabolic syndrome and T2DM.

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