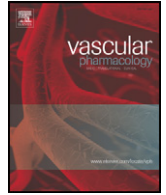




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Review

## Diabetic microangiopathy: Pathogenetic insights and novel therapeutic approaches



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ABSTRACT

Diabetic microangiopathy, including retinopathy, is characterized by abnormal growth and leakage of small blood vessels, resulting in local edema and functional impairment of the depending tissues. Mechanisms leading to the impairment of microcirculation in diabetes are multiple and still largely unclear. However, a dysregulated vascular regeneration appears to play a key role. In addition, oxidative and hyperosmolar stress, as well as the activation of inflammatory pathways triggered by advanced glycation end-products and toll-like receptors, have been recognized as key underlying events. Here, we review recent knowledge on cellular and molecular pathways of microvascular disease in diabetes. We also highlight how new insights into pathogenic mechanisms of vascular damage in diabetes may indicate new targets for prevention and treatment.

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**Abbreviations:** AGEs, advanced glycation end products; PKC, protein kinase C; DAG, diacylglycerol; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; ECs, endothelial cells; VSMCs, vascular smooth muscle cells; vWF, von Willebrand Factor; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinases; PARP, poly-ADP-ribose polymerase; NF- $\kappa$ B, nuclear factor- $\kappa$ B; TLR, toll-like receptors; EPCs, endothelial progenitor; MCP, monocyte chemoattractant protein; SDF, stromal cell derived factor; Ang, angiotensin; DPP-4, dipeptidyl peptidase-4; SMPs, smooth muscle progenitor cells; BMP6, Bone morphogenetic protein 6; TNF, tumor necrosis factor; FOXO1, Forkhead box O1; CREB, cAMP-responsive element binding; PDGF, platelet-derived growth factor; TGF- $\beta$ , Transforming growth factor  $\beta$  signaling; HMGB1, high mobility group box 1; IL, interleukin; VCAM, vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; LPS, lipopolysaccharide; AQP1, Aquaporin-1; COX, Cyclooxygenase; NFAT, nuclear factor of activated T; TonEBP, Tonicity-responsive binding-protein; FGF, fibroblast growth factor.

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## 1. Introduction

Macro- and microvascular complications are important causes of morbidity and mortality in patients with type 1 (T1DM) and type 2 diabetes (T2DM). The pathogenesis of vascular dysfunctions in diabetes is incompletely understood [1,2]. Hyperglycemia, a key feature of diabetes, is known to exacerbate macrovascular disease, but is also considered the main mechanism setting the stage for the onset of microvascular diseases, now the leading cause of blindness (retinopathy), end-stage renal failure (kidney disease), diabetic cardiomyopathy and peripheral neuropathy. One mechanism, possibly an important final common pathway, through which hyperglycemia causes or aggravates micro- and macrovascular damage, is oxidative stress caused by high glucose. It induces endothelial damage by triggering the polyol pathway, the formation of advanced glycation end products (AGEs), and the activation of the protein kinase C (PKC)-diacylglycerol (DAG) and hexosamine pathways (reviewed in [1,2]). The link between reactive oxygen species (ROS), vascular endothelial growth factor (VEGF) signaling and retinal neovascularization in a condition mimicking hyperglycemia, is the focus of the research work by Park and co-authors published in this issue of *Vascular Pharmacology* [3]. Here, the authors found that betaine (trimethylglycine), a natural specific type of *zwitterion*, known to improve visual acuity, has antioxidant properties and inhibits pathological neovascularization of human retinal microvascular endothelial cells exposed to high glucose levels, by attenuating ROS production, and subsequently suppressing both the VEGF receptor (VEGFR)-2 signaling pathway and VEGF production [3]. Betaine was, here, reported to act on the hyperosmolar component of hyperglycemia [4], a biophysical mechanism known to contribute to the development of microvascular disease in diabetes [5–7]. Thus, betaine would act on one pathway leading from hyperglycemia to microangiopathy.

Other pathways, besides hyperosmolarity, are, however, recognized to contribute to diabetic microangiopathy. These include a dysregulated vessel regeneration and impaired function of vascular cells (*i.e.*, endothelial cells (ECs), vascular smooth muscle cells (VSMCs), stromal cells, pericytes, inflammatory cells, circulating and tissue-resident vascular stem/progenitor cells), all involved in the maintenance of vascular homeostasis and permeability [8,9]. The present review summarizes recent advances in the research of cellular and molecular pathways of microvascular disease in diabetes, provides insights into pathogenic mechanisms of vascular damage in diabetes, and indicates potential new targets for prevention and treatment strategies. This review should thus help putting the original work by Park et al. [3] in the broader context of the available pertinent literature.

## 2. Pathologic features of diabetic microvascular damage

Diabetic microvascular disease is pathologically characterized by abnormal growth and permeability of microcirculatory vessels [10]. Arterioles, capillaries and venules are the smallest functional unit of the microvascular bed. Unlike macrovessels, the specific role of which is to convey blood to the microcirculation in all organs and tissues, microvessels have specific roles in oxygen and micronutrient delivery. Permeability to small molecules, regulation in the physical dimensions and functional properties of the basement membrane are main microcirculatory features, which vary in different types of microvascular beds, such as the glomeruli, the retina, the myocardium, the skin and the muscle [10]. Apoptotic death of podocytes and pericytes are specific changes in diabetic microvessels, occurring in the kidney and in the retina, respectively [11]. In particular, in the diabetic retina pericytes undergo accelerated apoptosis, thereby contributing to an increase in vascular permeability and retinal edema [12]. Furthermore, the loss of pericytes activates a disordered capillarization, consisting of acellular capillaries and micro-aneurysms, which are responsible for impaired perfusion and consequent tissue hypoxia, as well as dysregulated

neovascularization [12]. These aspects will be now described in greater detail.

## 3. Cellular and molecular pathways in diabetic microvascular disease

Endothelial cell damage and malfunction are common in diabetes, and contribute to the progressive loss of microvascular repair mechanisms [13]. Over the past decade, there has been increasing evidence that the integrity of the vascular wall is maintained by diversified cell populations, dedicated to endothelial repair and angiogenesis [8,9,14,15]. Diabetic patients often show deterioration of those cell types, especially in the presence of other cardiovascular complications [13,16]. Since hyperglycemia negatively affects the growth reserve of progenitor cells and the cellular capacity of vessel wall repair, vascular complications of diabetes may reflect a “stem cell vasculopathy”, in which the defective stem cell compartment is unable to regenerate dying ECs or VSMCs, or in which the dysfunctional stem cell compartment itself contributes to the development of the disease (Fig. 1).

### 3.1. Endothelial cells and vascular smooth muscle cells

Several studies have shown that exposure to high levels of glucose leads to a series of biochemical, structural and functional changes in mature vascular ECs and VSMCs, which can be summarized as follows [2,17]: 1) biochemical changes: accumulation of advanced glycation end-products (AGEs); increased production of the procoagulant protein von Willebrand Factor (VWF); increased apoptosis, induced by increased oxidative stress; increase in intracellular  $Ca^{2+}$ ; mitochondrial dysfunction; changes in intracellular metabolism of fatty acid; activation of the mitogen-activated protein kinase (MAPK) signaling pathways; and reduced phosphorylation/activation of protein kinase B (also known as Akt); 2) structural changes: increased production of extracellular matrix proteins, collagen and fibronectin, and of related enzymes (*i.e.*, matrix metalloproteinases, MMPs); 3) functional changes: reduction in cell proliferation and migration; impairment of endothelium-dependent vasodilatation, linked to decreased production of vasodilators and increased production of vasoconstrictors; induction of ischemia and neo-angiogenesis [17]. In human retinal ECs, both the poly-ADP-ribose polymerase (PARP) and the nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling play central roles, as described below. Diabetic injury activates PARP, which in turn induces NF- $\kappa$ B activation preferentially through the toll-like receptor (TLR) signaling pathway, and causes cell apoptosis [18]. Similar to the NF- $\kappa$ B pathway, but with opposite biological effects, the evolutionarily conserved Notch-1 pathway, centered around Notch-1, a member of the Notch family of receptors involved both in stem/progenitor cell fate and orientation, and in the life cycle of adult cells, plays a key role in EC function regulation and VSMC proliferation, differentiation, and apoptosis [19]. The existence of a fine interaction between these signaling pathways has been suggested, and growing evidence indicates a complex cooperation between Notch-1, TLR-4 and NF- $\kappa$ B [19]. Recent studies have found that apoptosis is increased in diabetic mice and in human retinal ECs treated *in vitro* with high glucose, through the activation of PARP and cleaved caspase-3, as well as through the reduced expression of Notch-1 and p-Akt. Notch-1 signaling participates in the interaction of PARP and the p50 NF- $\kappa$ B component, and inhibits PARP- and p50-mediated apoptosis. Thus, Notch-1 signaling protects human retinal ECs from PARP- and NF- $\kappa$ B-induced apoptosis occurring under high glucose [20]. In addition, human retinal ECs and VSMCs show aberrant expression of the Notch-1 ligand jagged 1, and abnormal angiogenesis [21].

The Wnt signaling pathway also plays a fundamental role in multiple physiological and pathological processes in ECs, including angiogenesis and inflammation [22]. The loss or gain of function of Wnt pathway components causes abnormal vascular development and angiogenesis. Mutations in Wnt co-receptors, such as Frizzled, or in other upstream

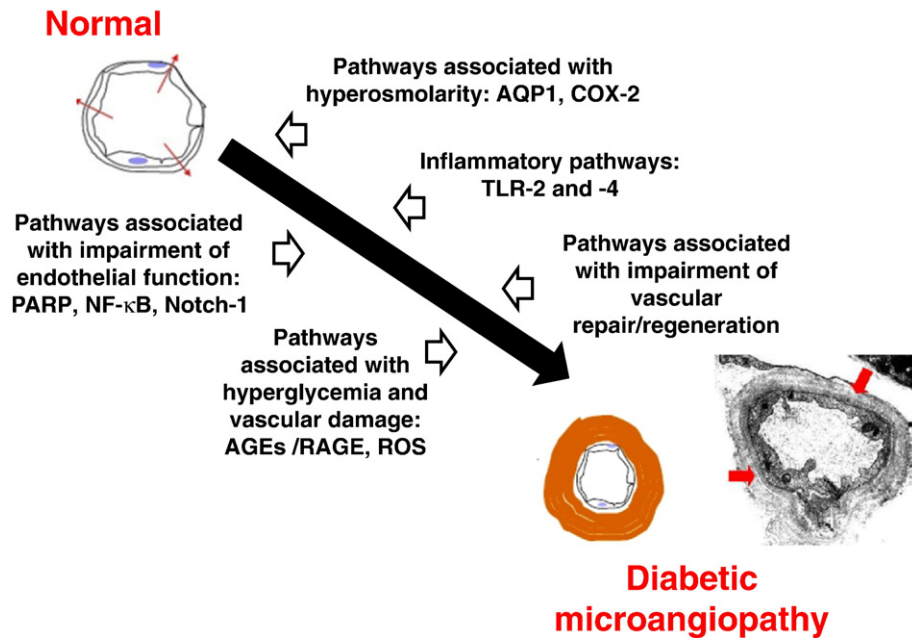


Fig. 1. Pathways associated with diabetic microangiopathy.

molecules result in severely defective retinal vascularization [22]. Furthermore, kallistatin, an endogenous Wnt antagonist, exerts anti-angiogenic and anti-neuroinflammatory effects by inhibiting canonical Wnt signaling in murine models of diabetic retinopathy [23].

### 3.2. Endothelial progenitor cells

Diabetic patients develop vascular dysfunction together with impaired circulating endothelial progenitor cells (EPCs) (see [24,25]) for reviews on characterization and current methods of isolation of EPCs) especially in the presence of comorbidities. While the percentage of EPCs (defined as CD34<sup>+</sup>/CD133<sup>+</sup>/VEGFR2<sup>+</sup> cells) in a healthy adult subject is only 0.01% or less of peripheral blood mononuclear cells, in diabetic patients the number of EPCs is decreased, and later restored by intensive glycemic control [13,26]. EPCs have a therapeutic impact on cardiovascular tissue repair and regeneration by playing various roles in the formation of new blood vessels, including mobilization, migration, adhesion, differentiation of vascular cells, and the production of adequate circulating levels of growth factors and chemokines, necessary for tissue repair and regeneration [27–30]. In fact, experimental and clinical studies suggest that EPCs can home to sites of a pre-existing vessel, and from there form neovessels under the influence of VEGF, chemokines and integrins, including monocyte chemoattractant protein (MCP)-1, stromal cell derived factor-1 (SDF-1), angiopoietin (Ang-1) and the  $\alpha 4\beta 1$  integrin [31–35]. Dysregulated levels of these molecules may contribute to a decrease in the number and function of EPCs in diabetes [31–34,36]. These defects are associated with a more rapid progression of microvascular disease [37]. Increasing EPC number and function might be a goal of stem cell-based cellular therapies for diabetic vascular disease. The therapeutic use of EPCs with restored numbers and functions has been proposed and put in practice in diabetes. For example, the chemokine SDF-1 is involved in the traffic of EPCs from the bone marrow to the peripheral blood, and directs EPC homing to damaged tissues [38]. Interestingly, SDF-1 is a physiologic substrate of the enzyme dipeptidyl peptidase-4 (DPP-4, also known as CD26). Oral DPP-4 inhibitors are widely used for the treatment of T2DM, as they increase the bioavailability of incretin hormones, which regulate insulin release in response to a meal [39,40]. It has recently been shown that a 4-week treatment with the DPP-4 inhibitor sitagliptin in T2DM patients increases the number of circulating EPCs, with concomitant

upregulation of SDF-1 levels [39]. Furthermore, mice that are deficient for DPP-4 (CD26 knock-out mice) are protected from ischemic myocardial injury, with a concomitant upregulation of tissue and plasma level of SDF-1 [41].

### 3.3. Circulating smooth muscle cell progenitors

Besides EPCs, peripheral blood also contains smooth muscle progenitor cells (SMPCs), also known as myofibroblast progenitor cells. Like EPCs, SMPCs can contribute to the repair and regeneration of atherosclerotic vessels [42]. SMPCs can be cultured from human peripheral blood mononuclear cells *in vitro* and express smooth muscle cell  $\alpha$ -actin. More detailed cell subset analysis revealed that these cells may be derived from a population of CD14<sup>+</sup>/CD105<sup>+</sup> monocyte-like cells. The abundance of CD14<sup>+</sup>/CD105<sup>+</sup>-derived SMPCs in the peripheral blood of patients with diabetes, expressing reduced expression of the anti-fibrotic bone morphogenetic protein 6 (BMP6) [43], are increased compared to healthy subjects [42]. Thus, diabetic patients may also have a disturbed balance in vascular progenitor cells involved not only in the maintenance of endothelial integrity (*i.e.*, EPCs), but also in atherogenesis (*i.e.*, SMPCs) and this is, at least in part, responsible for the increased susceptibility of the diabetic vessel wall to atherosclerosis and restenosis.

### 3.4. Pericytes

Vascular pericytes have recently come into the focus of investigators as key regulators of vascular maturation, stabilization and repair, therefore constituting potential targets for therapy [44]. Pericyte abundance, function and recruitment are remarkably altered in diabetes, contributing to microvascular damage in the retina, the kidney and the heart [45]. In fact, an early histopathologic feature of diabetic microangiopathy is the selective degeneration of pericytes in capillary vessels [46]. In the retina of diabetic patients, pericytes regress due to accelerated apoptosis, thus contributing to increased vascular permeability and retinal edema [47]. The loss of pericytes activates a disordered capillarization, represented by acellular capillaries and microaneurysms, which are responsible for areas of underperfusion of the depending tissues [48]. Mechanisms underlying pericyte apoptosis include the formation of AGEs and the tumor necrosis factor (TNF)- $\alpha$ -dependent activation of

the Forkhead box O1 (FOXO1) transcription factor [49–51]. Pericytes from healthy subjects contribute to reparative angiogenesis through paracrine mechanisms and the physical interaction with resident ECs, involving angiopoietin 1, platelet-derived growth factor (PDGF)-BB and cAMP-responsive element binding (CREB)/miR-132 [52]. In addition, pericytes promote the recruitment of proangiogenic CD14<sup>+</sup>/CD16<sup>+</sup> cells through a chemokine-mediated mechanism involving the fractalkine receptor CX3CR1 [52].

Transforming growth factor  $\beta$  signaling (TGF- $\beta$ ) is another pathway involved in the pathogenesis of diabetic retinopathy, because it regulates the differentiation of retinal pericytes during vascular development of the retina. In transgenic Tgfb2 <sup>$\Delta$ eye</sup> mice, deletions in elements of the TGF- $\beta$  pathway have been shown to determine the impairment of pericyte differentiation, which resulted in structural and functional changes in the retina that mimic those of diabetic retinopathy [53].

### 3.5. Prominent role of the AGEs/RAGEs system

Increased production of AGEs via non-enzymatic glycation and glycoxidation processes is one of the most important mechanisms involved in the pathophysiology of micro- and macrovascular complications of diabetes [54–57] (Figure 1). The intracellular accumulation of AGEs and their interaction with associated receptors, RAGEs, alter the cytoplasmic and transcription nuclear factors, including proteins involved in the regulation of gene transcription [57]. AGEs also lead to the formation of stable cross-links with collagen, which causes chemical and biophysical changes of collagen structure and consequent functional changes, such as a thickening of the basement membrane and increased resistance to proteolytic digestion [58]. In addition, AGEs/RAGEs interaction leads to cellular signaling, including NF- $\kappa$ B activation, increased expression of cytokines and adhesion molecules, induction of oxidative stress, and increased formation of cytosolic ROS [59]. A number of RAGE ligands have been identified in diabetic patients, including members of the S100 calgranulin family and high mobility group box 1 (HMGB1). The interaction of these ligands with RAGEs can trigger the interaction with TLRs and innate immune system signaling molecules, particularly TLR-4 [59]. Furthermore, a new class of molecules, the soluble RAGEs (sRAGEs), has recently added its contribution to this complex scenario. The total pool of sRAGEs in the plasma is involved in a wide range of micro- and macrovascular damage, even in non-diabetic subjects [60]. Several variants of sRAGEs exist, including endogenous soluble RAGEs (esRAGEs) and circulating truncated variants of the RAGE isoform, which are able to neutralize the AGE-mediated damage by competing with cell-surface RAGEs for ligand binding. Therefore, esRAGEs are an expression of the antioxidant status, with a key role in protection against early vascular damage [61]. Among all the biochemical mechanisms involved in diabetic vascular damage, the AGE pathway appears to be the most important in the pathogenesis and progression of microvascular complications. AGEs have multiple intra- and extracellular targets. As a result, they can be seen as a “bridge” between the intracellular and extracellular damage. Moreover, whatever the level of hyperglycemia, AGE-related intracellular glycation of mitochondrial respiratory chain proteins has been found to produce more abundant ROS [62], which further promotes AGE formation. Excessive AGE formation leads to a thickening of the microvessel, hypertension, endothelial dysfunction, loss of pericytes, decreased platelet survival and increased platelet aggregation. All these abnormalities may promote a procoagulant state, resulting in ischemia and induction of growth factors, with angiogenesis and neovascularization [63].

### 3.6. The TLR-2 and -4 inflammatory signaling pathways

Inflammation is one of the key events that characterizes the early steps of diabetic microangiopathy (Fig. 1). Accordingly, the activation of NF- $\kappa$ B caused by high glucose determines an enhanced expression

of various inflammatory molecules and evokes inflammatory responses in monocyte/macrophages, the microglia, and retinal ECs. In addition, TLR-2 and -4 signaling pathways appear to be the main triggers of these inflammatory responses. Recent *in vitro* studies have shown that high concentrations of glucose significantly increase TLR-2 and TLR-4 mRNA and protein expression in human microvascular ECs, as well as the activation of NF- $\kappa$ B p65, the expression of inflammatory markers such as interleukin (IL)-8, IL-1 $\beta$ , TNF- $\alpha$ , MCP-1, and vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 [64,65]. All these events were reversed by TLR-4 or TLR-2 inhibition, or dual inhibition of these pathways by TLR4/2 inhibitory peptide. In addition, antioxidant treatments reduce TLR-2 and TLR-4 expression and downstream inflammatory events. Collectively, these data suggest that hyperglycemia induces TLR-2 and TLR-4 activation and downstream inflammatory signaling, possibly through ROS [65].

The crucial role of TLR-4 was also confirmed by data showing that deletions or mutations in the TLR-4 gene may either protect against or exacerbate fatty acid-induced insulin resistance and obesity induced by diet [66–68]. A +896 A > G single nucleotide polymorphism (SNP) causes the substitution of Asp299 with Gly, modifying the normal structure of extracellular region of TLR-4. This +896 A > G TLR-4 polymorphism may be associated with decreased ligand recognition or protein interaction, and altered responsiveness to lipopolysaccharide (LPS). In particular, T2DM patients carrying the AG or the GG genotypes feature an increased risk of developing retinopathy compared with patients carrying the AA genotype [69–71].

### 3.7. Biophysical pathways: the role of hyperosmolar stress

The hyperosmolar component of hyperglycemia is an important biophysical mediator of diabetic vascular disease [72] (Fig. 1). In overt T1DM and T2DM, hyperglycemia causes an increase in plasma osmolarity, resulting in the osmotic efflux of water, which reduces the intracellular volume (cell shrinking) and promotes adaptive responses, including the activation of hyperosmolarity-responsive genes, involved in glucotoxicity and vascular injury [73]. Aquaporin-1 (AQP1) [74] is an example of proteins induced by hyperosmolarity. Aquaporins are a family of 10 different water-specific, membrane-channel proteins expressed in diverse tissues [74]. Aquaporins have been shown to play an important role in controlling the interplay between vascular permeability and angiogenesis [74]. AQP1 is a membrane water channel playing an important role in increasing the water permeability of cell membranes and promoting the water transport across cells driven by osmotic pressure. AQP1 is specifically and strongly expressed in many microvascular ECs outside the brain, in adult and embryonic fibroblasts, in the retina and in adipose tissue-derived mesenchymal stem cells [74], and has a promoting role in angiogenesis and vascular development [75]. AQP1 is also expressed in atherosclerotic lesions following balloon injury, especially in neointimal VSMCs [76].

Cyclooxygenase (COX)-2 has prominent pro-angiogenic effects [77]. COX-2, which is induced by cytokines, mitogens and endotoxin, regulates the expression and activity of MMP-9 [78]. Although the role of COX-2 in atherothrombosis remains controversial [79], endothelial COX-2 and MMPs have been shown to co-localize in unstable plaques and the retina, where they play an essential role in angiogenesis and related complications, such as plaque destabilization [80], as well as proliferative diabetic retinopathy [6], both characterized by excessive angiogenesis [8,81]. MMP-9 is strongly induced by cytokines in macro- and microvascular endothelial cells, while MMP-2 is refractory to these stimuli or less markedly induced [82]. In human monocyte/macrophages [83], and ECs [83], high glucose was shown to induce the expression of MMP-2 and -9, and of COX-2. The transcription factor, Tonicity-responsive binding-protein TonEBP/NFAT5, also called nuclear factor of activated T cells (NFAT)-5, is the molecular link between hyperosmotic stress and pro-inflammatory/proangiogenic molecules, such as COX-2 and MMPs. TonEBP/NFAT5 is a master transcription

factor of osmosensing genes responsive to hyperosmolar stress, including pro-inflammatory cytokines such as TNF- $\alpha$  in macrophages [84]. In renal medullary epithelial cells, hyperosmolar stress increases COX-2 promoter activity in a luciferase reporter assay [85,86]. In addition, hypertonic saline has been reported to induce COX-2 in rat macrophages [87] and human ECs [88]. The participation of NFAT transcription factors in COX-2 activation was also already shown in several additional cell types [89–94]. In fact, previous research had shown that the angiogenic signaling involved in the induction of COX-2 by VEGF in human ECs requires the activation of NFAT proteins [91]. Furthermore, TonEBP/NFAT5 is necessary for the hyperosmolar induction of COX-2 in renal epithelial cells [89]. An upregulation of the NFAT/MMP-2 pathway was also demonstrated in the development of metastatic osteosarcoma, where the secretion of active MMP-2 is under the transcriptional regulation of NFAT [90]. We have demonstrated that induction of COX-2 in microvascular endothelial cells and in the retina of diabetic *Ins2Akita* mice is largely hyperosmolarity-related, and that TonEBP/NFAT5 is here involved [6]. We have also shown that, in diabetic *Ins2Akita* mice, retinal COX-2 and AQP1 are upregulated in conjunction with increased angiogenesis [6]. Finally, interruption of the AQP1 axis by siRNA attenuated *in vivo* angiogenesis in response to high glucose [6]. These results showed that the hyperosmolar stress is a biophysical mechanism through which excessive angiogenesis can occur in diabetes.

### 3.8. Betaine and hyperglycemia-induced vascular damage

Betaine is an organic osmolyte, which serves as a source of methyl groups and plays a role in the control of the osmotic pressure inside the intestinal epithelial cells and renal cells. Betaine usually derives from the diet or choline oxidation. Betaine methylates homocysteine to methionine, also producing *N,N*-dimethylglycine. Betaine is important in development, from pre-implantation embryo to infancy. Betaine failure may contribute to metabolic syndromes, including lipid disorders and diabetes, and may have a role in atherosclerosis and other diseases [95]. Betaine supplementation improves animal health, but the effect of its long-term supplementation in humans is not known, although reports that it improves athletic performance will stimulate further studies. Subgroups of subjects that may benefit from betaine supplementation could be identified in the laboratory, particularly those who lose betaine excessively through the urine. Plasma betaine levels are highly variable, being typically 20–60  $\mu\text{M/L}$  in women and 25–75  $\mu\text{M/L}$  in men. Plasma dimethylglycine is typically <10  $\mu\text{M/L}$ . Urine betaine excretion is minimal, even after a large dose of betaine. It is constant, highly variable among individuals, and usually <35 mmol/mol creatinine.

In this issue of *Vascular Pharmacology*, Park et al. [3] report that betaine inhibits retinal neovascularization associated with oxidative stress and the *in vitro* growth of human retinal microvascular ECs. This study demonstrates that betaine attenuates ROS production, and subsequently suppresses VEGFR-2 signaling pathway and VEGF production. Dietary betaine supplementation has been shown to increase fibroblast growth factor (FGF)21 levels, to improve glucose homeostasis, and to reduce the hepatic lipid accumulation in mice [96]. Betaine plasma levels in humans are reduced in insulin-resistant conditions, and correlate closely with insulin sensitivity. However, betaine administration failed to improve glucose homeostasis and fat content in the liver of FGF21 knockout mice, suggesting that FGF21 is required for the beneficial effects of betaine. Taken together, betaine supplementation warrants further investigation as a nutritional supplement for patients with T2DM for its effects on diabetic microvasculopathy.

## 4. Conclusions

A significant number of promising therapeutic targets have been shown in preclinical models of diabetic microangiopathy, as summarized and discussed above. In humans, at present, the most effective

strategy to prevent microvascular complications of diabetes still remains, however, the intensive treatment of hyperglycemia or the improvement of glycometabolic control. While glycemic control has still uncertain results in controlling macrovascular complications, its role in curbing the risk of retinopathy is clear [1,2]. While we eagerly wait for better insights into the pathogenesis of diabetic microangiopathy to explore new therapeutic options, our best goal at present still is to continue to ensure a tighter glycemic control in diabetic patients. This step should be taken as early as possible in the natural history of the disease, as it is currently the only viable option for preventing and slowing down the progression of diabetic microangiopathy [97–100].

## Conflicts of interest declaration

The authors declare no conflicts of interest as to the content of this review paper.

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