



## REVIEW ARTICLE

## Vascular endothelial dysfunction, a major mediator in diabetic cardiomyopathy

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Diabetes mellitus is currently a major public health problem. A common complication of diabetes is cardiac dysfunction, which is recognized as a microvascular disease that leads to morbidity and mortality in diabetic patients. While ischemic events are commonly observed in diabetic patients, the risk for developing heart failure is also increased, independent of the severity of coronary artery disease and hypertension. This diabetes-associated clinical entity is considered a distinct disease process referred to as “diabetic cardiomyopathy”. However, it is not clear how diabetes promotes cardiac dysfunction. Vascular endothelial dysfunction is thought to be one of the key risk factors. The impact of diabetes on the endothelium involves several alterations, including hyperglycemia, fatty acid oxidation, reduced nitric oxide (NO), oxidative stress, inflammatory activation, and altered barrier function. The current review provides an update on mechanisms that specifically target endothelial dysfunction, which may lead to diabetic cardiomyopathy.

**Keywords:** cardiomyopathy; diabetes; diabetic cardiovascular complications; endothelium; vascular; heart failure; metabolism

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## INTRODUCTION

Cardiovascular disease is a major cause of morbidity and mortality in diabetic patients. It has long been established that diabetes significantly increases the risk of cardiovascular disease, with diabetic men being two times more likely to suffer from congestive heart failure (CHF) than non-diabetic individuals and diabetic women being five times more likely to develop CHF [1, 2]. In fact, 68% of deaths in type 2 diabetic patients are caused by cardiac complications [3–6]. Interestingly, a subset of diabetic patients develops left ventricular dysfunction in the absence of coronary artery disease, hypertension, or vascular disease. This observation, first made by Rubler *et al.* in 1972, is now known as diabetic cardiomyopathy [7]. Currently, diabetic cardiomyopathy is defined as ventricular dysfunction that occurs independently of hypertension or myocardial ischemia in diabetic patients. It is important to note, however, that diabetic cardiomyopathy rarely exists without these features and that the presence of these comorbidities does play a role in enhancing the complications associated with the disease.

While diabetic cardiomyopathy is now recognized as a clinically distinct entity, the mechanisms underlying its pathogenesis are still poorly understood. In this review, we will look at evidence regarding how diabetes-induced endothelial dysfunction mediates cardiac dysfunction. Specifically, we will focus on the hyperglycemia-induced changes that occur in the vascular endothelium during diabetes and how these changes may contribute to the development of diabetic cardiomyopathy. Several potential mechanisms that have been implicated in endothelial dysfunction in diabetic cardiomyopathy will be discussed.

## Clinical manifestations of diabetic cardiomyopathy

Diabetic cardiomyopathy affects both type 1 and type 2 diabetic patients and is characterized by a variety of functional and structural changes in the heart. In early stages of the disease, left ventricular diastolic dysfunction (LVDD) is the most prevalent cardiac complication [8, 9]. Although previous reports using Doppler echocardiography have estimated that LVDD affects ~40%–64% of asymptomatic diabetic patients [10], the emergence of more direct and accurate measurements of diastolic function, such as color M-mode and Doppler tissue imaging, have caused these estimates to rise to as high as 75% [11]. Systolic dysfunction is another characteristic of diabetic cardiomyopathy, although it usually develops during the later stages of the disease after diastolic dysfunction has been established [1, 12]. The development of both diastolic and systolic dysfunction in diabetics can be explained by hyperglycemia and hyperinsulinemia rather than by obesity, high blood pressure, or coronary artery disease [13].

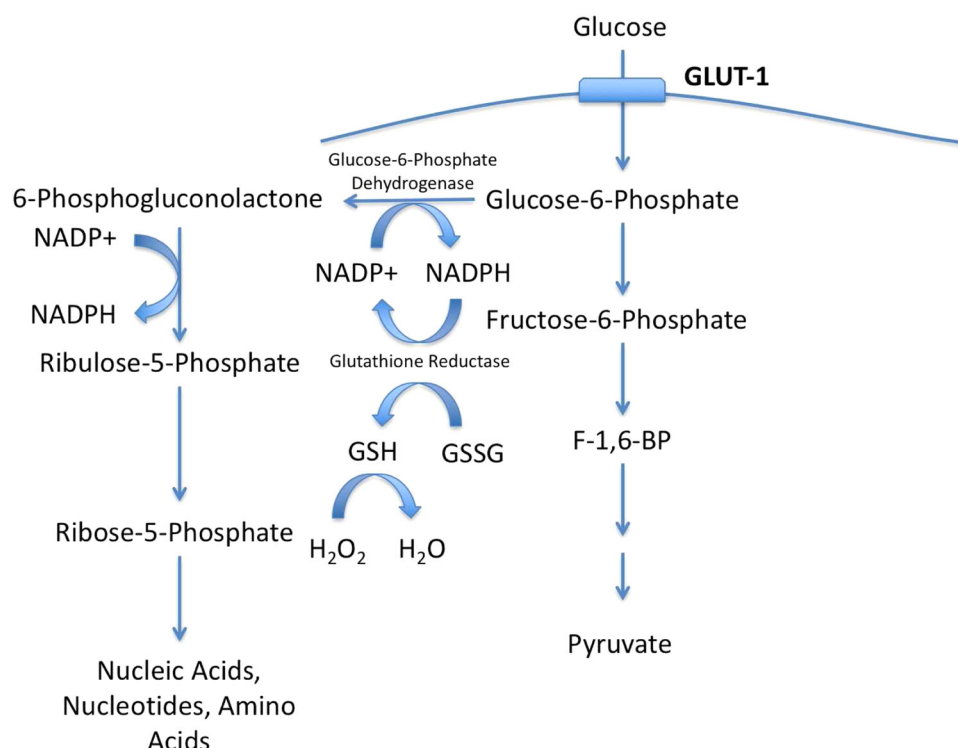
Diabetic cardiomyopathy is also associated with left ventricular hypertrophy [14], independent of coronary artery disease [15]. Hypertrophy only seems to be observed in patients with diabetes, not in patients with impaired fasting glucose or impaired glucose tolerance, suggesting that it is a consequence of long-term diabetic effects [16]. In addition, diabetic cardiomyopathy is characterized by an increase in both perivascular and interstitial fibrosis [17–19].

## Potential mechanisms underlying diabetic cardiomyopathy

The development of diabetic cardiomyopathy is likely to be multifactorial. Several pathways have been implicated, including vascular endothelial dysfunction, glucose toxicity, mitochondrial

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**Fig. 1** Normal endothelial cell metabolism. In healthy endothelial cells, glucose enters the cell through the GLUT-1 receptor, in an insulin independent manner. Energy production occurs mostly by glycolysis, rather than through oxidative phosphorylation. During normal endothelial function, some of the glucose-6-phosphate that is produced during glycolysis gets shunted into the pentose phosphate pathway. The purpose of this pathway is to produce NADPH, an important antioxidant in endothelial cells, as well as pentoses, which can be used to produce nucleic acids, nucleotides, and amino acids. It also allows the conversion of glutathione disulfide (GSSG) back to glutathione (GSH), which helps to prevent oxidative stress by converting H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O

dysfunction, and lipotoxicity [20]. It is difficult to determine how each of these factors individually contributes to cardiac dysfunction in diabetic patients. However, involvement of the vascular endothelium is becoming a front-runner in the pathogenesis of this disease. Hyperglycemia, a hallmark of diabetes, has been identified as one of the key causes of endothelial dysfunction in both type 1 and type 2 diabetes [21, 22]. High glucose levels lead to multiple biochemical modifications in endothelial cells and myocytes. These modifications include enhanced non-enzymatic glycation, sorbitol-myo-inositol-mediated changes, redox potential alterations, and the activation of protein kinase C (PKC) [23]. Hyperglycemia also causes an increase in reactive oxygen species (ROS) in endothelial cells, overwhelming the cell's ability to overcome oxidative stress [24, 25]. In the following section, we will discuss the effects of diabetes on the vascular endothelium and how they may potentially play a role in the development of diabetic cardiomyopathy.

#### Mechanisms of endothelial dysfunction in diabetic cardiomyopathy

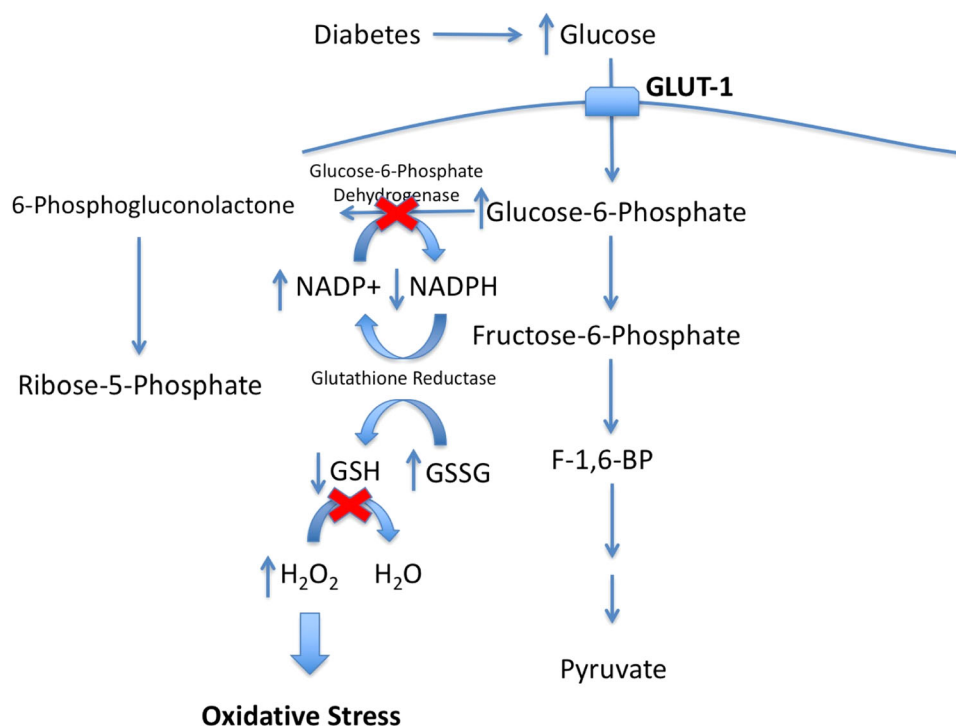
**Normal endothelial cell metabolism.** Under healthy conditions, endothelial cells are quiescent and play a role in maintaining vascular homeostasis. Endothelial cell metabolism is shown in Fig. 1. Glucose enters the cell through the GLUT-1 transporter, whose activity is predominantly regulated by extracellular glucose concentrations [26]. Glucose uptake through this receptor occurs in an insulin independent manner [27]. Consequently, endothelial cells are more susceptible to hyperglycemia-induced damage than other cell types.

The generation of ATP in endothelial cells occurs primarily via the glycolytic pathway rather than through oxidative phosphorylation [28, 29]. During glycolysis, some glucose-6-phosphate (G-6-P) gets shunted into the pentose phosphate pathway, producing

NADPH, an antioxidant found in endothelial cells, as well as pentoses, which can be used to make nucleic acids, nucleotides, and amino acids. This process also allows for the conversion of glutathione disulfide to glutathione, which helps prevent oxidative stress by converting H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O.

Fatty acid oxidation occurs in endothelial cells; however, it is unclear what role this oxidation plays since it seems to contribute very little to energy production in the endothelium [30, 31]. During periods of glucose deprivation, there is an increase in fatty acid oxidation, suggesting a degree of metabolic compensation [32]. Several theories have been proposed for the reason behind fatty acid oxidation in endothelial cells. Fatty acid oxidation is a source of NADPH that helps maintain the redox balance in endothelial cells. The oxidation of lipids may also play a role in regulating endothelial cell permeability [31]. Finally, fatty acid oxidation in endothelial cells may be important in the de novo synthesis of nucleotides by providing the carbon atoms necessary to build aspartate and glutamate [33].

**Diabetes-induced changes in the glycolytic pathway.** Metabolic changes associated with the diabetic myocardium have been extensively studied. Under hypoxic or inflammatory conditions, such as those that occur during diabetic cardiomyopathy, endothelial cells work to revascularize tissue to restore the delivery of oxygen and nutrients to damaged areas. Endothelial cells enhance their glycolytic flux even further during vessel sprouting, doubling their glycolytic rate during periods of proliferation or migration [34]. This enhancement allows the cells to migrate into hypoxic areas and proliferate where oxidative metabolism would be impaired. This alteration, in addition to enhanced glucose transportation due to the increase in GLUT-1 activity that occurs during diabetes, causes an increase in intracellular glucose concentrations in diabetic endothelial cells



**Fig. 2** Hyperglycemia-induced metabolic derangement in endothelial cells. During diabetes, entry of glucose-6-phosphate into the pentose phosphate pathway is inhibited. This causes a reduction in the production of NADPH and a buildup of H<sub>2</sub>O<sub>2</sub>, which both contribute to oxidative stress in diabetic endothelial cells

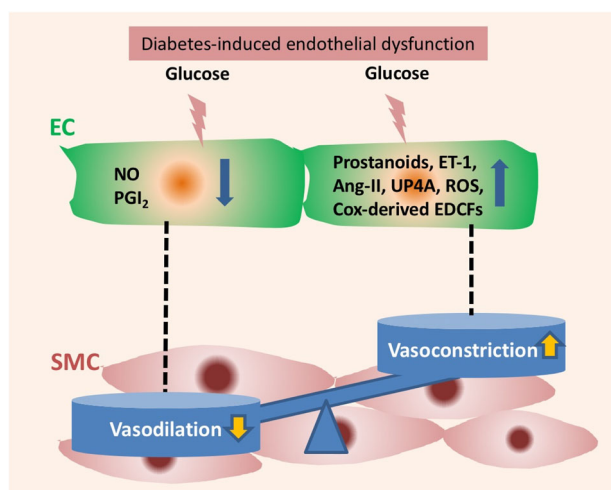
[35]. Endothelial cells address this excess glucose through several mechanisms.

Glucose can be shunted into the side branches of glycolysis, such as the hexosamine biosynthesis pathway, the pentose phosphate pathway, the polyol pathway, and the glycation pathway. The pentose phosphate pathway is a side pathway of glycolysis with two phases: the oxidative phase and the non-oxidative phase. Glucose enters the pentose phosphate pathway as G-6-P. During the oxidative phase, NADPH is produced, whereas during the non-oxidative phase, pentoses are produced. Under hyperglycemic conditions, entry of G-6-P into the pentose phosphate pathway is inhibited, which leads to reduced endothelial cell viability and migration [36]. In addition, inhibition of the pentose phosphate pathway leads to oxidative stress and endothelial dysfunction [37], whereas overexpression of G-6-P dehydrogenase, the first and rate-limiting enzyme of the pentose phosphate pathway, decreases endothelial oxidative stress [38]. The hexosamine biosynthesis pathway converts fructose-6-phosphate (a glycolytic intermediate) to uridine 5'-diphosphate N-acetylglucosamine (UDP-GlcNAc), which under normal conditions, is used for protein glycosylation. However, in diabetic endothelial cells, hyperglycemia-induced glycosylation inhibits endothelial nitric oxide synthase (eNOS) activation [39] and angiogenesis [40]. In the polyol pathway, aldose reductase converts glucose to sorbitol, using up NADPH. Sorbitol then gets converted to fructose and 3-deoxyglucosone, a precursor for the formation of advanced glycation end products (AGEs). Overproduction of AGEs, which occurs during diabetes, causes a variety of detrimental effects in endothelial cells by binding to the receptor for advanced glycation end products (RAGE), such as increasing endothelial cell permeability [41], inhibiting eNOS activity [42], impacting the coagulation system [43, 44], and activating both NADPH oxidase (NOX) and NF-κB [45, 46]. During the glycation pathway, glycolytic intermediates are converted to methylglyoxal, which modifies DNA and proteins. Upregulation of

this pathway has been implicated in diabetic cardiomyopathy, leading to an inhibition of eNOS activity in endothelial cells [47, 48].

**Hyperglycemia and vascular endothelial permeability.** In diabetes, hyperglycemia is caused by reduced insulin levels and/or insulin resistance, leading to excessive levels of glucose circulating in the blood plasma. A blood sugar level with a consistent range between ~5.6 and 7 mmol/L is considered hyperglycemic, whereas a concentration above 11.1 mmol/L is considered diabetic. However, symptoms are usually not noticeable until blood sugars reach even higher levels of ~15–20 mmol/L. Interestingly, short-term hyperglycemia, caused by insulin discontinuation, is associated with an increase in myocardial systolic contractile function in type 2 diabetic patients with or without heart failure [49]. Chronic levels of blood glucose exceeding 7 mmol/L can lead to metabolic derangements in endothelial cells and induce organ damage, as shown in Fig. 2.

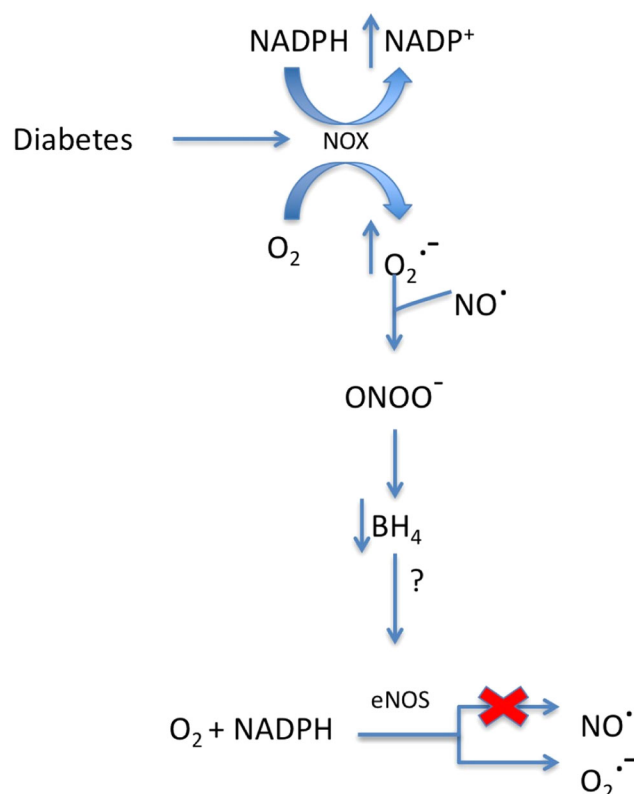
Experimental evidence indicates that hyperglycemic conditions promote increased permeability of the endothelial cell layer in the myocardium while also decreasing capillary density [50]. A potential mechanism for this increased permeability during diabetes is activation of the diacylglycerol (DAG)-PKC signaling pathway [51, 52]. PKC is a family of serine-threonine kinases. The PKC superfamily can be classified into three subfamilies: classical PKC (cPKC; α, β1, β2, and γ), novel PKC (nPKC; δ, ε, η, θ, and μ), and atypical PKC (aPKC; ζ, λ/ι) [53]. The activation of cPKC and nPKC is DAG dependent, whereas the activation of aPKC is not DAG dependent [53]. DAG-dependent subfamilies cPKC and nPKC were previously reported to be associated with vascular permeability in diabetes [54]. Activation of this pathway may contribute to diabetic endothelial dysfunction by increasing endothelial cell permeability and leukocyte adhesion in several tissues, including the heart, retina, and kidney [51]. The mechanism by which PKC activation leads to increased endothelial cell permeability remains



**Fig. 3** Effect of diabetic endothelial dysfunction on vasodilators and vasoconstrictors. Hyperglycemia in diabetes decreases vasodilation through the decreased bioavailability of nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>). It also caused an increase in endothelium-derived contracting factors including prostanoids, endothelin-1 (ET-1), angiotensin-II (Ang-II), dinucleotide uridine adenosine tetraphosphate (UP<sub>4</sub>A), ROS, and cyclooxygenase (COX)-derived prostanoids. EC endothelial cell, SMC smooth muscle cell

unclear. It has recently been shown in human umbilical vein endothelial cells (HUVECs) that hyperglycemia leads to the phosphorylation of myosin light chain (MLC) by PKC, which in turn causes VE-cadherin tyrosine phosphorylation and the disruption of adherens junctions [55]. Proteins associated with endothelial tight junctions include transmembrane, scaffolding, and signaling proteins [56]. In particular, the transmembrane proteins occludin, tricellulin, the claudin family, and junction adhesion molecules, along with the scaffolding zonula occludens proteins (ZO-1, -2, and -3), play major roles in the formation and regulation of tight junctions [57]. Hyperglycemia-mediated activation of PKC is implicated in the development of oxidative stress and is accompanied by an excessive release of free radicals, which promote endothelial barrier dysfunction in many vascular conditions, including diabetes and cerebral ischemia.

**Diabetes-induced inhibition of nitric oxide.** Another hallmark of endothelial dysfunction is a deficiency in the bioavailability of NO, along with a variety of other vasoactive factors produced by endothelial cells. Under healthy conditions, the endothelium releases vasodilators, such as NO, prostacyclin, and bradykinin, as well as vasoconstrictors, such as vasoconstrictor prostanoids, endothelin, and angiotensin-II. The balance between the release of vasoconstrictors and vasodilators by endothelial cells helps maintain coronary vascular structure. During diabetic cardiomyopathy, an imbalance between the release of constricting and relaxing factors occurs, as shown in Fig. 3. Diabetic conditions cause an upregulation of various vasoconstrictors. For example, an increase in endothelin production has been observed in the diabetic heart [58], which may lead to vessel hypertrophy and increased myocardial fibrosis, both characteristics of diabetic cardiomyopathy [59]. Importantly, vasoconstrictor prostanoids, including vasoconstrictor prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), thromboxane A<sub>2</sub> (TXA<sub>2</sub>), and PGF<sub>2</sub>α, are also enhanced in diabetes. These products, in turn, upregulate NADPH oxidase and type 4 and type 5 phosphodiesterases (PDE4 and PDE5), resulting in increased ROS production, degradation of cAMP and cGMP, and vasoconstriction [60, 61]. In addition, in diabetic endothelial cells, there is an impaired response of endothelium-dependent hyperpolarization (EDH) [62]. There are smaller arteries in which endothelium-mediated vasodilation is predominately affected by EDH in



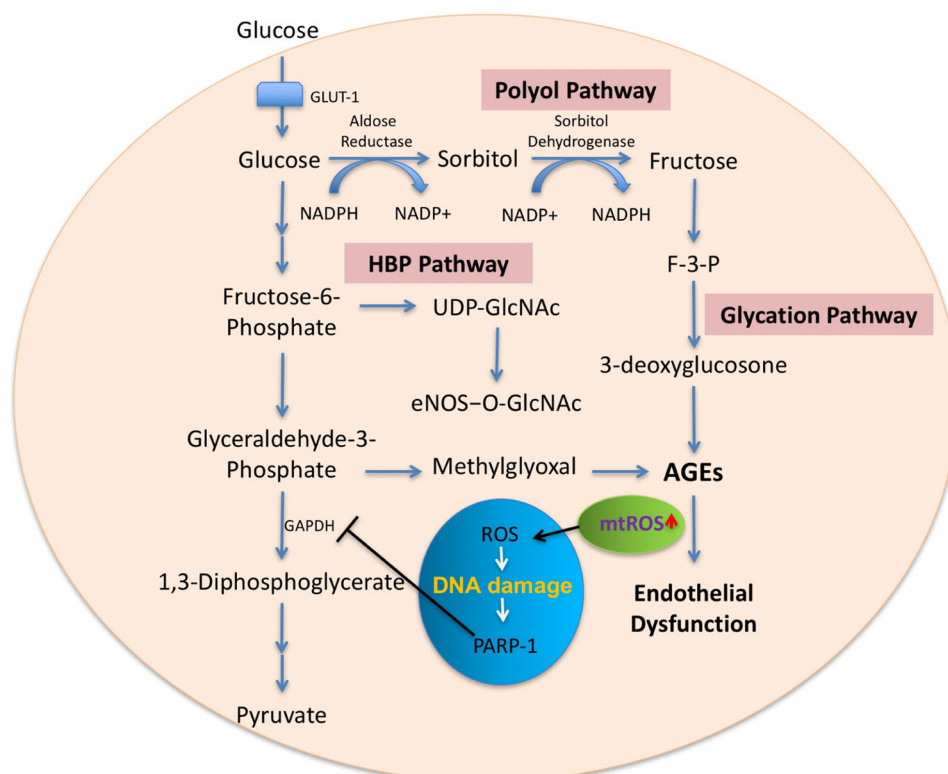
**Fig. 4** Mechanism of eNOS uncoupling. During diabetes, hyperglycemia activates NAD(P)H oxidase (NOX), which is responsible for converting oxygen into the superoxide anion (O<sub>2</sub><sup>-</sup>), using up NADPH during the reaction. Superoxide reacts with NO to form peroxynitrite (ONOO<sup>-</sup>). Peroxynitrite is believed to be the main cause of eNOS uncoupling in endothelial cells. Under normal physiological conditions, NO is synthesized by eNOS from L-arginine and oxygen, using BH<sub>4</sub> as a cofactor. During eNOS uncoupling, however, eNOS produces superoxide instead of NO, leading to oxidative stress in endothelial cells. It is thought that peroxynitrite reacts with BH<sub>4</sub>, and that this loss of BH<sub>4</sub> is the main mechanism by which eNOS becomes uncoupled. However, more recent evidence suggests that other mechanisms may be involved

vascular smooth muscle cells. EDH causes the relaxation of vascular smooth muscle by hyperpolarizing its cell membrane and closing voltage-operated calcium channels, leading to a reduction in intracellular free calcium levels [63].

A key vasoactive factor involved in endothelium-dependent relaxation is NO. In the presence of oxygen, NO is synthesized from L-arginine and NADPH in a reaction catalyzed by nitric oxide synthase (NOS). In diabetic vessels, however, this NO-dependent vasodilatory response is lost [64–66]. This deficiency may be caused by the inactivation of NO due to an increase in free radicals [67] rather than by the reduced activity or expression of eNOS [68, 69]. Treatment of HUVECs and rat heart endothelial cells with D-glucose leads to an increase in the generation of reactive oxygen intermediates (ROIs) [70]. In addition, there is an increase in mitochondrial ROS concentrations in coronary endothelial cells from diabetic rats [71]. Furthermore, endothelium-dependent vasodilation in diabetic rats can be restored by treatment with antioxidants [72], and treatment of aortas from STZ-induced diabetic rats and hamsters with superoxide dismutase (a specific scavenger of superoxide anions) enhances endothelium-dependent relaxation [73, 74]. This evidence suggests a role for antioxidants in enhancing cardiac function by preventing endothelial dysfunction in the presence of diabetes.

Several mechanisms have been proposed to explain how an increase in oxidative stress can lead to decreased NO availability





**Fig. 5** Effects of mitochondrial oxidative stress on endothelial function. During diabetes, there is an increase in mitochondrial oxidative stress, which causes mitochondrial DNA damage. This activates the PARP-1 pathway in the nucleus of endothelial cells, which has been implicated in response to DNA injury. Activation of PARP-1 has been shown to inhibit Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), a key enzyme involved in glycolysis. This inhibition causes the buildup of glycolytic intermediates, which get shunted into the polyol pathway, the hexosamine biosynthesis pathway, or the glycation pathway. These pathways all contribute to endothelial dysfunction. Blue color represents nucleus and green represents the mitochondria

in diabetic endothelial cells, such as reduced tetrahydrobiopterin ( $\text{BH}_4$ ) bioavailability (oxidation of  $\text{BH}_4$ ) and eNOS uncoupling [75]. NOX is an enzyme involved in endothelial cell signaling. It catalyzes the reduction of oxygen to superoxide anions ( $\text{O}_2^-$ ) by using NADPH as an electron donor. During high glucose and insulin-resistant conditions, NOX activity, and thus, superoxide anion production, is increased [76–78]. Superoxide anions react with NO to form peroxynitrite ( $\text{ONOO}^-$ ). Peroxynitrite causes eNOS uncoupling, as described in Fig. 4. Peroxynitrite leads to the oxidation of  $\text{BH}_4$ , a cofactor necessary for NOS activity [79, 80]. However, some evidence suggests that the depletion of  $\text{BH}_4$  is not the main cause of endothelial dysfunction in vivo during periods of oxidative stress [81]. Although it has been shown that  $\text{BH}_4$  supplementation is an effective treatment for suppressing superoxide anion production and improving vasodilation in the endothelium [82–85], many of these studies were done with concentrations of  $\text{BH}_4$  that are 100 times larger than physiological concentrations. Thus, other mechanisms have been proposed. For example, peroxynitrite may cause oxidation of the zinc-thiolate center of eNOS, resulting in eNOS uncoupling [86].

Other mechanisms are involved in NO-induced endothelial dysfunction in diabetes. An endogenous inhibitor of NO synthase, asymmetric dimethylarginine (ADMA), is found at elevated levels in patients with type 2 diabetes [87]. High glucose-induced impairment of dimethylarginine dimethylaminohydrolase (DDAH) activity causes ADMA accumulation and may contribute to a reduction in NO expression and endothelial vasodilator dysfunction in diabetes [88, 89]. The quenching of NO by AGEs in diabetes plays an important role in the development of vasodilatory impairment [90].

*Mito-oxidative stress and mitochondrial dysfunction in diabetes.* Plenty of evidence indicates that myocardial metabolism is altered in diabetes, which likely contributes to diabetic cardiomyopathy. The mitochondria are the center of metabolism and are thus likely to be impacted by impaired metabolism associated with diabetes. Oxidative stress occurs when there is an imbalance between the production of ROS, which include  $\text{O}_2^-$ , NO, the hydroxyl radical, hydrogen peroxide, and peroxynitrite, and the cell's ability to detoxify reactive intermediates. Hyperglycemia mediates the induction of oxidative stress in the pathogenesis of diabetic complications, including diabetic cardiomyopathy [91]. For example, hyperglycemia leads to an increase in mitochondrial ROS in diabetic endothelial cells, which causes oxidative DNA damage. This damage activates the polyADP-ribose polymerase (PARP-1) pathway, as shown in Fig. 5, a pathway involved in DNA injury. Its activation stimulates the transfer of ADP-ribose units from NAD $^+$  to nuclear proteins, resulting in the depletion of NAD $^+$  and ATP from the cell. Inhibition of PARP-1 in endothelial cells may prevent endothelial dysfunction caused by diabetes [92]. Activation of PARP-1 also leads to the inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [93], causing the buildup of glycolytic intermediates. These intermediates get shunted into several different pathways, including the hexosamine biosynthesis pathway, the polyol pathway, and the glycation pathway, all of which are increased in hyperglycemic endothelial cells [93].

Sphingosine-1-phosphate (S1P), which binds to S1P receptors (S1PRs), also regulates an array of biological activities in endothelial cells. It has recently been demonstrated that high levels of glucose enhance ROS generation and markedly reduce NO in endothelial cells and that this effect is completely reversed by either induction

of S1PR1 or reduction in S1PR2 [94]. This finding suggests that S1PR1 and S1PR2 play crucial roles in endothelial dysfunction caused by hyperglycemia-induced ROS production.

In addition, hyperglycemia-induced overproduction of mitochondrial ROS (mtROS) in cultured endothelial cells can be inhibited by AMPK activation [95], which seems to work through the upregulation of MnSOD (a mitochondrial specific antioxidant) [96] and UCP2 [97]. In patients with both coronary artery disease and type 2 diabetes, elevated mtROS levels in the endothelium led to an increase in AMPK activation, which supports a role for AMPK in protection against oxidative stress [98]. The upregulation of AMPK in the endothelium of diabetic mice may also prevent endothelial dysfunction [99]. Thus, AMPK may be a novel therapeutic target in the treatment of diabetes by improving blood glucose homeostasis, lipid profiles, and blood pressure [100]. Taken together, these findings suggest that ROS inhibition may provide beneficial effects in the prevention of diabetic cardiomyopathy.

There is also an imbalance between mitochondrial fission and fusion in diabetic endothelial cells, which may be related to increased oxidative stress. Mitochondrial fission is defined as the division of a mitochondrion into two separate mitochondria, leading to the elimination of damaged and dysfunctional mitochondria. Mitochondrial fusion is the merging of two or more mitochondria into one mitochondrion, which allows for the formation of a dynamic network that can respond to metabolic changes. This balance maintains normal mitochondrial function [101]. An imbalance between mitochondrial fission and fusion may contribute to the development of endothelial dysfunction in diabetes [102]. Exposure of endothelial cells to hyperglycemia leads to an increase in mitochondrial fission, which can be ameliorated by decreasing superoxide anion concentrations [103]. This fission may be caused by an increase in dynamin-related protein 1 (DRP1) expression, an activator of mitochondrial fission, or a decrease in optic atrophy 1 (OPA1), an activator of mitochondrial fusion [103]. Thus, mitochondrial dysfunction in endothelial cells represents a crucial step in the development of endothelial dysfunction.

## CONCLUSION

Although diabetic cardiomyopathy is a complex disease with many possible causes, its underlying mechanisms are still not clear. Metabolic derangements in myocytes have been intensively studied. However, increasing evidence suggests that endothelial dysfunction may be one of the primary mechanisms involved in its pathogenesis. Hyperglycemic conditions in the diabetic heart lead to metabolic derangements and oxidative stress in cardiovascular endothelial cells, which cause multiple effects, such as increased permeability, impaired endothelial relaxation, and a decrease in NO availability. Therefore, endothelial cells represent a potential therapeutic target in the treatment of the disease. More work needs to be done to elucidate the most important pathways involved in the development of diabetic cardiomyopathy. However, studies looking at the prevention of hyperglycemia-induced endothelial dysfunction through BH<sub>4</sub> or antioxidant supplementation show promising results. These findings suggest a significant role for endothelial cells in future diagnosis and treatment of the disease.

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## ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

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