

## Reversibility of endothelial dysfunction in diabetes: role of polyphenols

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### Abstract

The endothelium, a thin single sheet of endothelial cells, is a metabolically active layer that coats the inner surface of blood vessels and acts as an interface between the circulating blood and the vessel wall. The endothelium through the secretion of vasodilators and vasoconstrictors serves as a critical mediator of vascular homeostasis. During the development of the vascular system, it regulates cellular adhesion and vessel wall inflammation in addition to maintaining vasculogenesis and angiogenesis. A shift in the functions of the endothelium towards vasoconstriction, proinflammatory and prothrombotic states characterise improper functioning of these cells, leading to endothelial dysfunction (ED), implicated in the pathogenesis of many diseases including diabetes. Major mechanisms of ED include the down-regulation of endothelial nitric oxide synthase levels, differential expression of vascular endothelial growth factor, endoplasmic reticulum stress, inflammatory pathways and oxidative stress. ED tends to be the initial event in macrovascular complications such as coronary artery disease, peripheral arterial disease, stroke and microvascular complications such as nephropathy, neuropathy and retinopathy. Numerous strategies have been developed to protect endothelial cells against various stimuli, of which the role of polyphenolic compounds in modulating the differentially regulated pathways and thus maintaining vascular homeostasis has been proven to be beneficial. This review addresses the factors stimulating ED in diabetes and the molecular mechanisms of natural polyphenol antioxidants in maintaining vascular homeostasis.

**Key words:** Polyphenols: Endothelial dysfunction: Diabetes: Cell signalling

### Endothelial cells and their vascular functions

Endothelial cells lining the lumen of the vascular bed serve as a metabolic interface between blood and tissues, which is indispensable for the maintenance of vascular homeostasis<sup>(1)</sup>. These cells are the predominant players in regulating a variety of blood vessel functions including blood fluidity and passage of nutrients, hormones and macromolecules to the surrounding tissues<sup>(2)</sup>. The major functions of endothelial cells including maintenance of vascular tone, cell adhesiveness, platelet aggregation, leucocyte trafficking, coagulation cascade, inflammation, permeability, regulation of thrombosis and fibrinolysis<sup>(1,3)</sup> will be discussed in the following section (Fig. 1).

### Vascular tone

The degree of constriction experienced by a blood vessel relative to its maximally dilated state is referred to as the vascular tone, which is modulated by the release of endothelium-derived relaxing and constricting factors<sup>(4)</sup>.

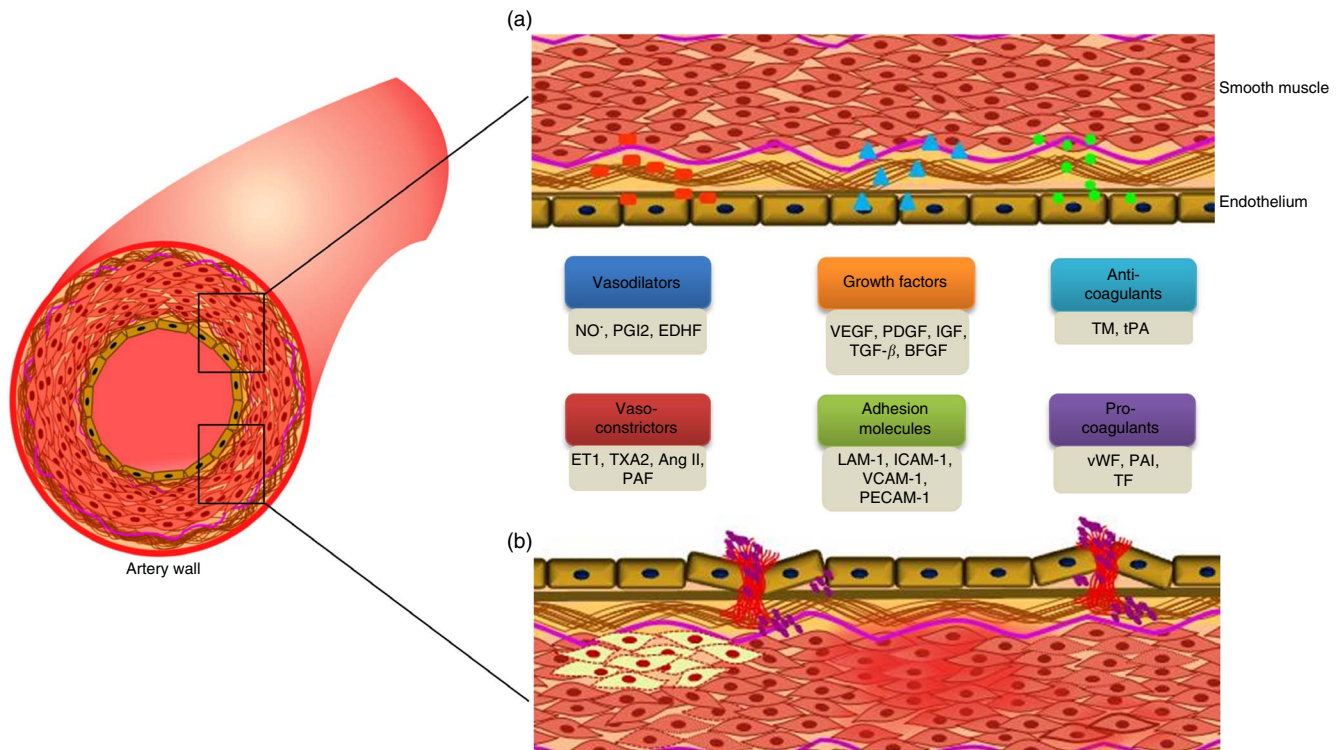
The vasoprotective effects of a healthy endothelium are vasodilation, suppression of smooth muscle cell growth and inhibition of inflammatory responses, in which the vasodilators act against the effects of endothelium-derived vasoconstrictors. Vascular tone is maintained by homeostasis between the vasodilators such as nitric oxide (NO<sup>\*</sup>), prostacyclin (PGI<sub>2</sub>) and endothelium-derived hyperpolarising factor and vasoconstrictors, including endothelin-1 (ET-1), angiotensin II (Ang II), thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and platelet-activating factor released by the endothelial cells<sup>(5)</sup>. Damage to the endothelium creates an imbalance between vasodilation and vasoconstriction, resulting in the initiation of a cascade of events such as increased endothelial permeability, platelet aggregation, leucocyte adhesion and generation of cytokines<sup>(6)</sup>.

### Coagulation and fibrinolysis

Endothelial cells have major roles in regulating haemostatic balance, preventing the activation of thrombin and inhibiting platelet adhesion, thereby mediating anticoagulant activity<sup>(7)</sup>.

**Abbreviations:** ADMA, asymmetric dimethylarginine; AGE, advanced glycation end products; AMPK, AMP-activated protein kinase; BH<sub>4</sub>, (6R)-5,6,7,8-tetrahydro-L-biopterin; cGMP, cyclic GMP; EGCG, epigallocatechin gallate; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ET-1, endothelin-1; GLP-1, glucagon-like peptide-1; ICAM, intracellular adhesion molecule; IRE1, inositol-requiring enzyme 1; IRS-1, insulin receptor substrate 1; Nrf2, nuclear factor-E2-related factor 2; MCP-1, monocyte chemoattractant protein-1; NO<sup>\*</sup>, nitric oxide; NOX, NADPH oxidase; PKC, protein kinase C; ROS, reactive oxygen species; SGLT2, sodium–glucose co-transporter 2; STZ, streptozotocin; T1D, type 1 diabetes; T2D, type 2 diabetes; UPR, unfolded protein responses; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; XO, xanthine oxidase.

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**Fig. 1.** Factors secreted by endothelium, and its functions. (a). A healthy endothelium mediates endothelium-dependent vasodilation and suppresses thrombosis and inflammation. It displays the vasodilatory function with high levels of vasodilators such as nitric oxide (NO<sup>•</sup>), prostacyclin (PGI<sub>2</sub>) and endothelium-derived hyperpolarising factors (EDHF). It plays a vital role in cell proliferation and differentiation function with the secretion of growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), transforming growth factor-β (TGF-β), and basic fibroblast growth factor (bFGF). It also has an anticoagulative phenotype with the release of thrombomodulin (TM) and tissue plasminogen activator (tPA). (b). A dysfunctional endothelium mediates vasoconstriction with the secretion of endothelin-1 (ET1), thromboxane A<sub>2</sub> (TXA<sub>2</sub>), angiotensin II (Ang II) and platelet-activating factor (PAF). Endothelial cell inflammation has been associated with the expression of cell adhesion molecules such as leucocyte adhesion molecule-1 (LAM-1), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and platelet cell adhesion molecule-1 (PECAM-1). In addition, it secretes pro-coagulants such as von Willebrand factor (vWF), plasminogen activator inhibitor type-1 (PAI-1) and tissue factor (TF). A colour figure is available in the online version of the paper.

These cells secrete an integral membrane protein, thrombomodulin, which causes a reduction in blood coagulation along with the assistance of platelet tissue factor, plasminogen activator inhibitor-1 (PAI-1) and urokinase-type plasminogen activator<sup>(8)</sup>. The release of endothelial products by thrombin has a critical role in coagulation, inflammation and vascular homeostasis, which is largely arbitrated by the thrombin receptor, also known as protease-activated receptor-1 (PAR-1). The activation of PAR-1 mediates intracellular signalling pathways by (a) enhancement in the production of NO<sup>•</sup> and PGI<sub>2</sub>, which induce vasodilation and regulation of platelet activation; (b) stimulating tissue plasminogen activator (tPA), which binds to fibrin and mediates effective fibrinolysis; and (c) activation of Weibel–Palade bodies that release von Willebrand factor (vWF), angiotensin-2 and P-selectin, which are involved in the modulation of inflammatory response<sup>(9)</sup>.

### Cell growth and differentiation

The synchronised regulation of vasculogenesis or *de novo* differentiation of bone marrow-derived endothelial progenitor cells (EPC), that is, angioblasts into endothelial cells followed by angiogenesis, is essential for the development of the vascular system. Angiogenesis is the major mechanism of vascularisation

involving endothelial cell sprouting, vessel branching and intussusceptions of the vasculature from the pre-existing blood vessels<sup>(10)</sup>. Vascular endothelial growth factor (VEGF) secreted by the endothelial cells is known to be the main growth factor specific for the vascular endothelium, and hence expression of VEGF receptors has been considered a critical regulator of endothelial cell development<sup>(11)</sup>. Expression of growth factors such as fibroblast growth factor, heparin-binding epidermal growth factor, platelet-derived growth factor (PDGF) and transforming growth factor β in addition to tyrosine kinase receptors tie-2 (tek) and angiopoietins (Ang-1 and Ang-2) assists in the vascular development and promotes angiogenesis, a major event in embryonic development, regeneration and wound healing<sup>(12)</sup>.

### Adhesion and permeability

Endothelial cells produce specific adhesion molecules, namely E-selectin, intracellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM), for the regulation of cell adhesion and permeability<sup>(13)</sup>. The intact endothelium expresses low levels of these adhesion molecules, and upon activation, over-expression of these molecules has been reported, which play a role in maintaining endothelial barrier integrity

and also mediate paracellular and transcellular migration<sup>(14)</sup>. In addition, leucocyte passage from blood to underlying tissues involves the interaction between leucocyte carbohydrate ligand and endothelial E- and P-selectins. Further, firm adhesion mediated by the interactions between leucocyte integrins and endothelial adhesion molecules and their up-regulation increases endothelial permeability<sup>(15)</sup>. Moreover, binding and accumulation of circulating cells such as platelets, leucocytes and erythrocytes to the endothelial cells restricts blood flow<sup>(16)</sup>.

### Endothelial dysfunction

The inability of the endothelium to maintain vascular homeostasis is referred to as endothelial dysfunction, a systemic pathological state that alters endothelial cell phenotype, characterised by a shift in the normal functions of the endothelium towards reduced vasodilation, proinflammatory and prothrombotic state. Endothelial dysfunction is triggered by a number of factors including turbulent blood flow, shear stress, hypoxia, ageing, hyperglycaemia, hypercholesterolaemia and hypertension<sup>(17,18)</sup>. The pathophysiology of endothelial dysfunction in diabetes, an initial event in the development of microvascular and macrovascular complications, is emphasised. The microvascular complications in diabetes encompass long-term complications affecting small blood vessels including diabetic retinopathy, nephropathy and neuropathy. The macrovascular complications include the diseases of large blood vessels throughout the body including coronary and peripheral arteries leading to cardiovascular and cerebrovascular diseases and stroke.

### Factors promoting endothelial dysfunction in diabetes

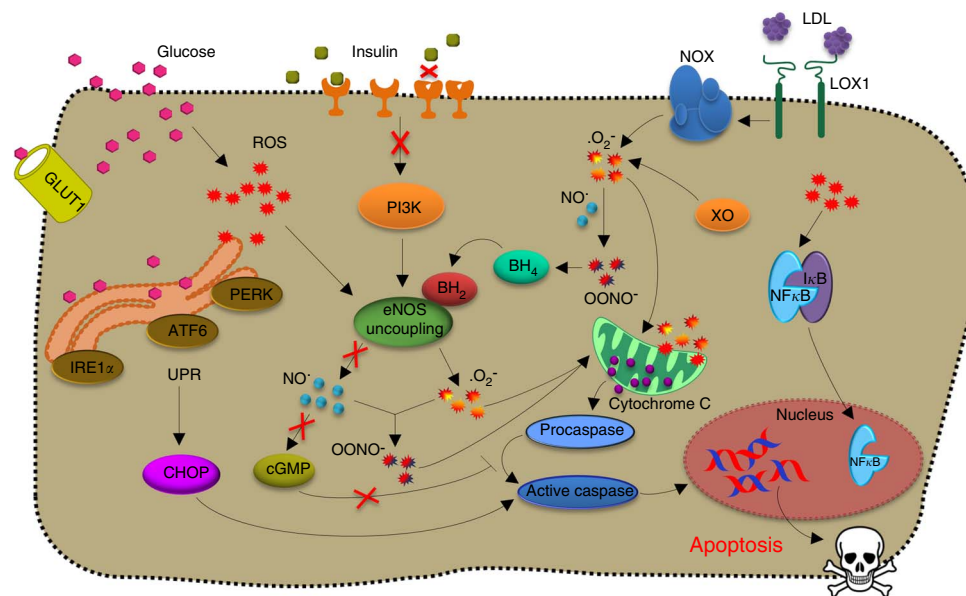
Vascular homeostasis regulates glucose metabolism in the endothelial cells by balancing insulin levels and stimulating

glucose transport<sup>(19)</sup>. High glucose disrupts the vascular homeostasis and promotes both microvasculature and macrovasculature modifications by induction of phenotypic switch and altering intracellular signalling pathways. In the recent years, significant achievements have been made in understanding the mechanism of endothelial cell dysfunction triggered under diabetic conditions and the differences in etiopathogenesis between type 1 diabetes (T1D) and type 2 diabetes (T2D). In T1D, uncontrolled hyperglycaemia with low levels of endogenous insulin mediates endothelial dysfunction<sup>(20)</sup>, whereas the pathogenesis of endothelial dysfunction in T2D involves effects of fatty acids and insulin resistance<sup>(21)</sup>. The metabolic milieu in diabetes, i.e. hyperglycaemia, insulin resistance, hyperinsulinemia and obesity, induces a wide range of events. The major causal factors for endothelial dysfunction including oxidative stress, endoplasmic reticulum (ER) stress and inflammation<sup>(22,23)</sup> are over-viewed below (Fig. 2).

### Hyperglycaemia

Increased blood glucose level, the hallmark of diabetes, is a major risk factor for endothelial dysfunction-mediated vascular complications. In hyperglycaemia, endothelial dysfunction is triggered by the inhibition of the glycolytic enzyme, D-glyceraldehyde 3-phosphate dehydrogenase. This causes accumulation of glycolytic intermediates leading to the activation of major metabolic pathways such as the protein kinase C (PKC) pathway, the hexosamine pathway and the sorbitol pathway<sup>(24)</sup>, in addition to the generation of methylglyoxal and advanced glycation end products (AGE).

Accumulated evidence shows altered phenotype of endothelial cell including apoptosis<sup>(25)</sup> through the activation of



**Fig. 2.** Fate of endothelial cells in diabetes. Hyperglycaemia, insulin resistance and obesity interrupt normal endothelial cell functions and promote the generation of reactive oxygen species (ROS). Induction of oxidative and endoplasmic reticulum (ER) stresses up-regulate inflammatory and apoptotic pathways leading to apoptosis. IER1 $\alpha$ , inositol-requiring enzyme 1 $\alpha$ ; ATF6, activating transcription factor 6; PERK, protein kinase RNA-like endoplasmic reticulum kinase; UPR, unfolded protein responses; CHOP, C/ERB homologous protein; cGMP, cyclic GMP; eNOS, endothelial nitric oxide synthase; XO, xanthine oxidase; NOX, NADPH oxidase; LOX1, LDL receptor 1; BH<sub>4</sub>, (6R)-5,6,7,8-tetrahydro-L-biopterin; NO<sup>•</sup>, nitric oxide;  $\cdot$ O<sub>2</sub><sup>-</sup>, superoxide; ONOO<sup>-</sup>, peroxynitrite. A colour figure is available in the online version of the paper.

caspases, increased reactive oxygen species (ROS) and inflammatory markers<sup>(26)</sup> because of increased glucose concentration in the blood, altered metabolic activity with increased levels of lipid peroxidation products<sup>(27)</sup> and enhanced intracellular calcium levels<sup>(28)</sup>. In addition, high glucose also triggers the production of vasoconstrictors such as prostanoids and thromboxanes, resulting in vascular contractility. Further alteration in insulin signalling mediates an increase in the levels of vasoactive peptides such as Ang II and ET-1, contributing to endothelial dysfunction<sup>(29)</sup>.

### Insulin resistance

Under physiological conditions, insulin delivery is the rate-limiting step in insulin-stimulated glucose uptake in the skeletal muscle. This is mediated by the involvement of endothelial cells, which regulate the capillary recruitment and glucose uptake<sup>(30)</sup>. Insulin resistance is associated with the impairment of GLUT-4 translocation in fat tissues and muscles with reduced NO<sup>•</sup> production, this weakens the insulin signalling and glucose uptake, mediating vasodilation<sup>(31)</sup>. Attenuation of Akt phosphorylation in response to insulin<sup>(32)</sup> has been reported in the microvascular endothelial cells in T2D models resulting from impairment of the phosphoinositide 3-kinase-protein kinase B (PI3K-Akt) pathway.

### Hyperinsulinemia

Metabolic insulin resistance is usually accompanied by increased levels of circulating insulin or hyperinsulinemia which impairs endothelial function<sup>(33)</sup>. Imbalance in PI3K- and mitogen-activated protein kinase (MAPK)-dependent functions of insulin and down-regulation of endothelial nitric oxide synthase (eNOS) has been evidenced in hyperinsulinemia<sup>(34)</sup>. Elevated insulin levels stimulate VCAM-1 and E-selectin through the MAPK-dependent pathway and ET-1 secretion, causing pronounced abnormal vascular function<sup>(35)</sup>. ET-1 induces NAD(P)H oxidase (NOX) expression with increased generation of superoxide anion ( $\cdot\text{O}_2^-$ ), as reported in rat aortic endothelium<sup>(36)</sup>. Hyperinsulinemia also contributes to cellular hypertrophy with increased synthesis and accumulation of elements of the extracellular matrix, thereby altering the structure of the vessel wall<sup>(37)</sup>.

### Obesity and dyslipidaemia

Metabolic disorders including obesity, hypertension and lipid abnormalities linking atherosclerosis cluster with insulin resistance. Dyslipidaemia and obesity are the major risk factors for cardiovascular events in diabetes<sup>(38)</sup>. It has been demonstrated that obesity induces endothelial dysfunction before the development of insulin resistance<sup>(39)</sup>. In T2D, the reduced endothelium-dependent vasodilation is mediated by the attenuation of NO<sup>•</sup> production, decreased sensitivity to NO<sup>•</sup> decreased Akt phosphorylation and enhanced vasoconstrictor tone<sup>(40–42)</sup>. The abnormal lipids affect endothelial cells directly, by the activation of lectin-like oxidised LDL receptor, stimulation of NOX-dependent  $\cdot\text{O}_2^-$  formation and release of cytokines, culminating in apoptosis<sup>(43)</sup>.

### Oxidative stress

Increased oxidative stress has been reported to be a common feature of endothelial dysfunction often associated with NOX, xanthine oxidase (XO), aldehyde oxidase and glucose oxidase, the enzymes involved in the generation of ROS.

#### Mitochondrial reactive oxygen species production.

Mitochondria are known to be one of the major intracellular sources of ROS production, formed as a by-product of oxidative phosphorylation along the respiratory chain<sup>(44)</sup>. Increasing evidence suggests that morphological and functional changes in mitochondria of diabetic subjects are linked to vascular complications<sup>(45)</sup>. Davidson *et al.* reported hyperglycaemia to be associated with impaired mitochondrial activity in vascular tissues leading to ROS-mediated endothelial dysfunction<sup>(46)</sup>. Excess ROS lead to mitochondrial fragmentation in coronary endothelial cells of T1D mice<sup>(47)</sup>. In addition, increased ROS alters mitochondrial calcium homeostasis and hence mediates various vascular complications<sup>(48,49)</sup>. Hence, ROS generation and calcium overload exacerbate mitochondrial metabolism that represents a principal step in the development of endothelial dysfunction.

**NADPH oxidase.** NOX, is a transmembrane enzyme and a major source of  $\cdot\text{O}_2^-$  and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) in the vascular cells<sup>(50)</sup>. Physiologically, NOX-derived ROS has been implicated in the regulation of vascular tone either directly by modulating vasodilation or indirectly by quenching  $\cdot\text{O}_2^-$  to form peroxynitrite ( $\text{ONOO}^-$ )<sup>(51)</sup>, thus decreasing NO<sup>•</sup> bioavailability. It also exhibits a pathophysiological role in provoking endothelial dysfunction, inflammation, hypertrophy, apoptosis, migration, fibrosis, angiogenesis and important processes underlying cardiovascular and renal remodelling in hypertension and diabetes. Hyperglycaemia has also been reported to increase lung vascular permeability with increased generation of vascular  $\cdot\text{O}_2^-$  through NOX<sup>(52)</sup>.

**Xanthine oxidase.** XO uses molecular  $\text{O}_2$  as an electron acceptor producing  $\cdot\text{O}_2^-$  and  $\text{H}_2\text{O}_2$ . It acts as an important biological source of ROS, and its involvement in various pathological processes, including diabetes, has been well documented<sup>(50,53)</sup>. The circulating and tissue XO levels contribute to increased  $\cdot\text{O}_2^-$  production and have been reported in both diabetic animals as well as diabetic patients<sup>(54,55)</sup>. Increased expression of XO results in ATP:ADP ratio imbalance and elevated levels of ROS in streptozotocin (STZ)-induced diabetic mice, demonstrating the role of the enzyme in the pathogenesis of endothelial dysfunction<sup>(53)</sup>. It has also been observed that treatment of diabetic animals with allopurinol, a potent inhibitor of XO, improved endothelium-dependent vasodilation and reduced diabetic complications<sup>(55)</sup>.

It is clear that the increase in NOX and XO contributes to ROS production in mitochondria, which in turn mediates eNOS uncoupling thereby triggering endothelial dysfunction. Inhibition of NOX and XO abolishes the increase in mitochondrial ROS, thus preventing eNOS uncoupling.



**Endothelial nitric oxide synthase uncoupling.** NO<sup>•</sup> is a potent endogenous vasodilator produced by the enzyme nitric oxide synthase (NOS). eNOS produces NO<sup>•</sup> which is involved in various physiological events and is a critical regulator of endothelial cell migration, survival and angiogenesis<sup>(56)</sup>. Studies relate reduced NO<sup>•</sup> production in diabetes to the pathogenesis of diabetic endothelial dysfunction<sup>(57,58)</sup>. Functional eNOS oxidises its substrate L-arginine to L-citrulline and NO<sup>•</sup> by dimerisation of the enzyme with the presence of an essential cofactor, (6R)-5,6,7,8-tetrahydro-L-biopterin (BH<sub>4</sub>), which is one of the potent naturally occurring reducing agents<sup>(59,60)</sup>. NO<sup>•</sup> then activates the enzyme guanylate cyclase, resulting in the formation of cyclic GMP (cGMP), a critical second messenger responsible for initiation and maintenance of vasorelaxation<sup>(61)</sup>. Diminished levels of BH<sub>4</sub> due to its oxidation into BH<sub>2</sub> promotes  $\cdot\text{O}_2^-$  production by eNOS, referred to as 'eNOS uncoupling'<sup>(56)</sup>. Inactivation of NO<sup>•</sup> by  $\cdot\text{O}_2^-$  and other ROS tends to be the major mechanism in impaired vasoregulation<sup>(62)</sup>. eNOS uncoupling-mediated endothelial dysfunction has been documented in diabetes<sup>(63)</sup>. Meanwhile, few reports evidenced that eNOS uncoupling linked to reduced BH<sub>4</sub> availability resulted in the production of  $\cdot\text{O}_2^-$  radicals rather than NO<sup>•</sup><sup>(64,65)</sup>. Further, the prevention of PKC-mediated activation of NOX caused a reduction in eNOS uncoupling and  $\cdot\text{O}_2^-$  production via the inhibition of vascular ONOO<sup>-</sup> formation and decreased oxidation of BH<sub>4</sub>, in STZ-induced diabetic rats<sup>(66)</sup>. The supplementation of BH<sub>4</sub> has been reported to improve endothelium-dependent vasodilation by increasing NO<sup>•</sup> activity in T2D patients<sup>(59,60)</sup>.

Excess  $\cdot\text{O}_2^-$  inhibits the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which mediates the accumulation of asymmetric dimethylarginine (ADMA), an endogenously produced inhibitor of NOS<sup>(67)</sup>. This elevated ADMA inhibits NO<sup>•</sup> synthesis by eNOS or uncouples the enzyme, which in turn enhances oxidative stress<sup>(56,68)</sup>. Reports highlight elevated plasma ADMA levels to be the risk factors for cardiovascular events and kidney disease in both T1D and T2D patients<sup>(69,70)</sup>. Increased oxidative stress in the vasculature is not restricted to the endothelium but also affects the smooth muscle cell layer. Increased  $\cdot\text{O}_2^-$  production by the endothelial cells has important consequences in eNOS uncoupling and its downstream signalling, resulting in decreased expression of soluble guanylyl cyclase and the cGMP-dependent protein kinase I, which controls vessel tone and smooth muscle cell proliferation<sup>(68,71)</sup>.

### Endoplasmic reticulum stress

ER stress known as unfolded protein responses (UPR) has a crucial role in the pathogenesis of diabetes, contributing to pancreatic  $\beta$ -cell loss and insulin resistance. Vascular endothelial cells of hyperglycaemic subjects are characterised by altered rough endoplasmic reticulum (rER) and protein folding, which leads to ER stress<sup>(38,72)</sup>. As reported by Sheikh-Ali *et al.*<sup>(72)</sup>, hyperglycaemia-induced ER stress in endothelial cells may be initiated through different stress signalling molecules of the glucose metabolism such as pyruvate, glycerol and dihydroxyacetone. The accumulation of unfolded or misfolded

proteins in the ER initially restores the normal function of ER by halting translation, degrading misfolded proteins and activating signalling pathways to increase molecular chaperones involved in protein folding. Sustained UPR has been implicated in the initiation of several cell death pathways mediated by the activation of caspase-12<sup>(73)</sup>, apoptosis signal-regulating kinase 1, the c-Jun N-terminal kinase (JNK) pathway<sup>(74)</sup>, the protein kinase RNA-like-like endoplasmic reticulum kinase (PERK) pathway and C/ERB homologous protein (CHOP) pathway<sup>(75)</sup>. Provoked ER stress increases the activity of JNK and catalytic I $\kappa$ B kinase subunits and induces inflammation associated with impairment of insulin receptor substrate 1 (IRS-1) signalling, thus linking diabetes and vascular endothelial dysfunction<sup>(12,76)</sup>.

### Effect of hyperglycaemia on the blood vessels

Hyperglycaemia activates the hexosamine pathway leading to the formation of O-linked N-acetylglucosamine (O-GlcNAc) in the blood vessels. In the vasculature, it competes with NOS phosphorylation and impairs NO<sup>•</sup>-mediated arteriolar dilations. The more O-GlcNAc, the less NO<sup>•</sup> produced in the vasculature leading to vasoconstriction, thus increasing the risk of high blood pressure in diabetic subjects<sup>(77)</sup>. In addition, an elevated level of O-GlcNAc impairs angiogenesis in endothelial cells, by inhibiting Akt signalling, cell migration and capillary-like structure formation<sup>(78)</sup>. In T2D subjects, the lymphatic barrier dysfunction reduces lymph flow, thereby trapping lipids and cholesterol in the tissue, inhibiting lymphatic transport of cholesterol bound to HDL from the tissues to the liver, aggravating atherosclerosis and dyslipidaemia<sup>(79)</sup>.

Insulin is a known vasodilator that increases blood flow mainly by stimulating NO<sup>•</sup> synthesis<sup>(80)</sup>. Baron *et al.*<sup>(81)</sup> demonstrated that insulin controls its own access and that of other substrates such as glucose, lipids and several signalling molecules to peripheral tissues, by increasing blood flow, but this effect is compromised in states of insulin resistance. It has been shown that insulin infusion increases myocardial blood flow in both T1D and T2D<sup>(82,83)</sup>.

The role of insulin in regulating glucose availability is well established in peripheral tissues, such as skeletal muscle and adipose tissues. Insulin normally stimulates capillary recruitment of skeletal muscle and subcutaneous adipose tissue, thus increasing blood flow mainly after a meal or physical exercise. This function is impaired in insulin resistance and T2D, reflecting early onset of vascular dysfunction. Failure of insulin to increase muscular and adipose tissue blood flow consequently decreases glucose disposal. Numerous studies have shown that in states of decreased insulin sensitivity the postprandial blood flow in adipose tissue is reduced<sup>(84)</sup>. Reports highlight insulin enhancement of NOS-dependent vascular actions, increased total muscular blood flow and recruitment of muscle capillaries<sup>(85)</sup>.

### Diabetes-mediated structural alterations in endothelial cells

Under normal physiological conditions, endothelial cells show stable interaction with smooth muscle surface and scanty organelles with flat nucleus, separated from the underlying connective tissues by a thin, extracellular matrix of tissues

named as 'basal lamina', which functions in heterotypic tissue interactions. In addition, normal endothelial cells are actively engaged in transcytosis, a process by which the transcellular transfer of molecules across the endothelium takes place<sup>(86)</sup>.

The ultrastructure of the endothelial cell and the extracellular matrix tends to be altered under hyperglycaemia. A significant enrichment of biosynthetic organelles such as golgi complex in the aortic and capillary endothelial cells, abundance of rER in aortic arch of retinal venules and femoral artery have been reported<sup>(87)</sup>. Further predominant thickening of endothelial cell basal lamina accounting to impairment in the circulation of metabolic products between tissues<sup>(88)</sup> has been reported (Fig. 3). Hyperglycaemia also mediates endothelial dysfunction by causing endothelial barrier injury leading to hyperpermeability and plasma leakage<sup>(89)</sup>.

### Antidiabetic drugs improving endothelial function

Currently available conventional therapies for diabetes are supported by clinical and preclinical evidences that are aimed at improving endothelial function. Some of the antidiabetic agents such as metformin, glucagon-like peptide-1 (GLP-1) receptor agonists, inhibitors of phosphodiesterase-5 (PDE5), dipeptidyl peptidase-4 (DPP4) and sodium-glucose co-transporter 2 (SGLT2) act in different modes to preclude diabetic complications especially by preventing endothelial dysfunction, which is a key event in the pathogenesis.

#### Metformin

Metformin, an antidiabetic drug, significantly improved endothelium-dependent vasodilator response. Reduction in the

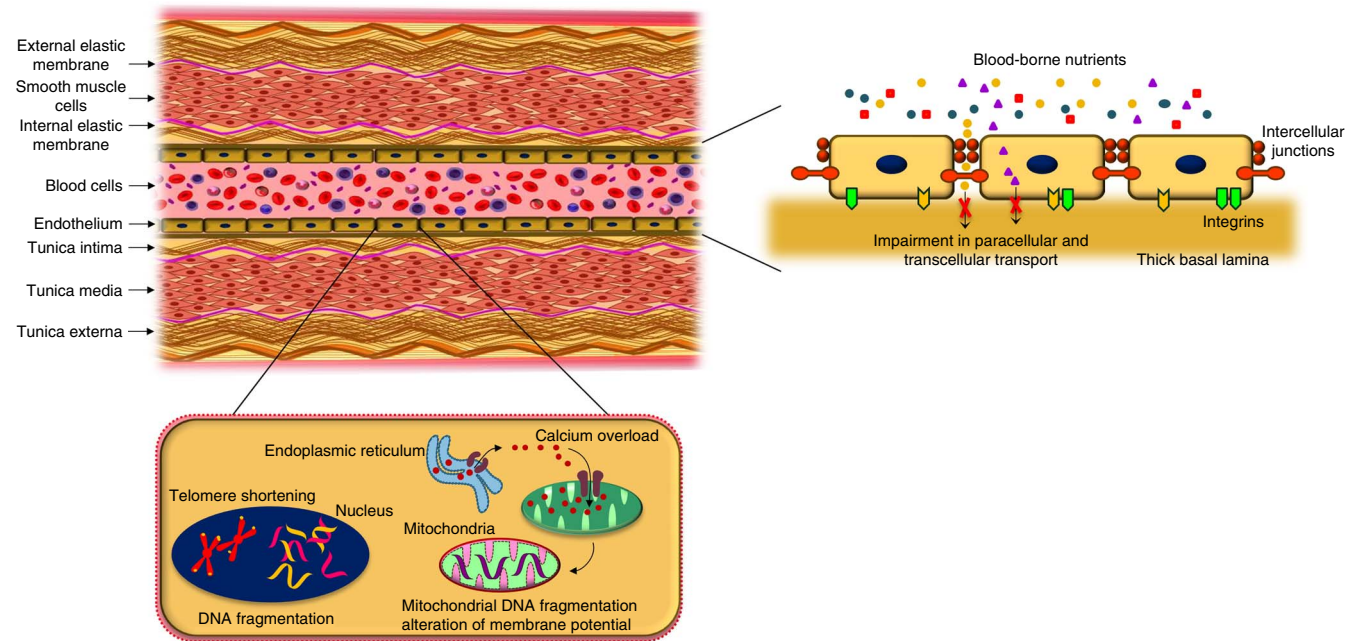
levels of endothelial markers such as vWF, VCAM-1, tPA, PAI-1 and ICAM-1<sup>(90)</sup> have been reported to be the mechanism underlying the protective effects of metformin. In addition, long-term treatment with metformin improved endothelial function and decreased inflammatory response<sup>(91)</sup>.

#### Glucagon-like peptide-1 receptor agonists

GLP-1 receptor agonists mimic GLP-1 and increase incretin effect in patients with T2D. They stimulate the release of insulin and improve the endothelial function through direct vascular action<sup>(92)</sup>. Recent report showed that exenatide, a GLP-1 receptor agonist, reverted glucose- and lipid-induced endothelial dysfunction. Exenatide activated eNOS and NO<sup>•</sup> production in endothelial cells, in addition to induced vasorelaxation and reduced endothelial dysfunction in arterioles<sup>(93)</sup>. In obesity and pre-diabetic patients, exenatide treatment showed a significant change in inflammation and oxidative stress status. Effects of exenatide have also improved postprandial vascular endothelial dysfunction in T2D patients<sup>(94)</sup>.

#### Phosphodiesterase-5 inhibitors

PDE5 is a cytosolic enzyme that primarily functions to degrade cGMP and induce vasoconstriction. PDE5 inhibitors up-regulate eNOS transcription and increase NO<sup>•</sup> release, causing long-term vasodilator effects. They are also reported for improving endothelium-dependent vasorelaxation in diabetic rats and for their potential to reduce glomerular hypertension in diabetic nephropathy<sup>(95)</sup>. Sildenafil, a PDE5 inhibitor, attenuates diabetic nephropathy in Otsuka Long-Evans Tokushima Fatty rats<sup>(96)</sup>.



**Fig. 3.** Structural modifications in endothelial cells under hyperglycaemia. Hyperglycaemia induced thickening of endothelial basal lamina impairs the transport of metabolic products and nutrients between blood and tissues. Further, hyperglycaemia induces telomere shortening, DNA fragmentation, intracellular calcium enhancement leading to mitochondrial calcium overload causing mitochondrial DNA fragmentation and alteration in membrane potential. A colour figure is available in the online version of the paper.

### Dipeptidyl peptidase-4 inhibitor

Endothelial cells are the main endogenous sources of DPP4, an enzyme involved in inactivating active GLP-1 (7–36) to GLP-1 (9–36). The inhibitors of DPP-4 have been used in the treatment of T2D<sup>(92)</sup>. DPP4 interacts with pro-inflammatory pathways and impairs endothelial function through incretin-dependent and incretin-independent mechanisms. It has been reported that the DPP-4 inhibitor des-fluoro-sitagliptin enhanced acetylcholine-induced endothelium-dependent vasodilatation in mouse aortic rings<sup>(97)</sup>. Vildagliptin, another DPP-4 inhibitor, improved endothelial function, as indicated by measurement of forearm blood flow during acetylcholine infusion in T2D patients<sup>(98)</sup>.

### Sodium–glucose co-transporter inhibitors

A newly developed therapeutic strategy for the treatment of diabetes is the use of the SGLT2 inhibitors, which offer a novel insulin-independent approach for the control of hyperglycaemia and are currently in phase 2 and 3 clinical trials. These inhibitors target the SGLT2, the main GLUT in the kidney that is also responsible for the reabsorption of >90% of the glucose in the kidney<sup>(99)</sup>. SGLT2 inhibition reduces the reabsorption of glucose and therefore enhances urinary glucose excretion preventing glucotoxicity and consequently decreasing both fasting and postprandial hyperglycaemia. SGLT2 inhibitor therapy has been reported to reverse glucose-induced vascular dysfunction by reducing glucotoxicity, oxidative stress, inflammation and restoring insulin signalling, thereby reversing endothelial dysfunction<sup>(100)</sup>. Empagliflozin has been reported to cause reduction in cellular glucotoxicity, prevention of oxidative stress, AGE signalling and inflammation via NOX inhibition, decreased AGE precursor and methylglyoxal thereby maintaining the normal endothelial function in STZ-animal T1D model<sup>(100)</sup>. In addition, SGLT-2 inhibitors improved the vascular architecture in connective tissue participating in arterial stiffening and regulated NO•-dependent relaxation in mouse pulmonary arteries<sup>(101)</sup>.

### Alternative treatment strategies

Adverse side effects of currently available therapies for diabetes and their associated complications added to the insufficiency in meeting the increasing therapeutic demand warrants the development of alternative approaches for the treatment and prevention of fatal vascular complications. A promising strategy encompasses the use of antioxidants, such as vitamins, that reduce oxidative stress. In addition, exercise training, organic nitrate and nitrite, tetrahydrobiopterin, L-arginine, taurine and magnesium supplementation are the other therapeutic possibilities for endothelial dysfunction in diabetes.

### Exogenous nitrate treatment

Administration of exogenous NO• in the form of organic nitrates rather than improving vascular function leads to an increased formation of ONOO<sup>-</sup> mediating adverse effects. In particular,

nitroglycerin induces clinical tolerance, oxidative stress and endothelial dysfunction similar to other nitrates such as isosorbide-5-mononitrate (ISMN) and isosorbide dinitrate. These are more likely to induce endothelial dysfunction and increase oxidative stress via activation of the vascular NOX. In contrast, pentaerythritol tetranitrate (PETN) reduces oxidative stress in vascular tissue through its interaction with the powerful antioxidant enzyme heme oxygenase-1 (HO-1). Regulation of cardiac oxidative stress and lipid peroxidation in STZ-induced diabetic rats treated with PETN treatment proved a clear correlation between NOX activity, endothelial dysfunction and nitrate resistance<sup>(102)</sup>. In STZ-induced rats, PTEN treatment prevented the activation of NOX, inhibited XO-mediated  $\cdot\text{O}_2^-$  production, eNOS uncoupling and thereby reduced oxidative stress, which was not the case with ISMN. In addition, PETN modified the metabolism of glucose in diabetic rats via the AMP-activated protein kinase (AMPK) pathway<sup>(103)</sup>.

### Exercise

Moderate exercise tends to be the stimulator of NO• release, and it influences endothelial function, which in turn reduces the cardiovascular profile in diabetic patients<sup>(104)</sup>. It has been reported that exercise training improved the vascular endothelial function in patients with long-term T1D<sup>(105)</sup>. Currently, the beneficial effects of exercise training in T2D patients have been well established. Participation in regular physical activity maintained glucose metabolism, improved energy metabolism and insulin sensitivity and delayed T2D by attenuating the lipid profile, blood pressure and cardiovascular events<sup>(106,107)</sup>. Lee *et al.*<sup>(108)</sup> demonstrated that exercise recovered eNOS phosphorylation, increased antioxidant enzymes such as superoxide dismutase (SOD)-1 and SOD-2, increased NO• bioavailability and also decreased pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6, in the T2D mouse model.

### Antioxidant therapy

Antioxidants have been accounted to improve endothelial dysfunction in diabetes by re-coupling eNOS and mitochondrial function, by increasing the activity of  $\cdot\text{O}_2^-$  scavenging enzymes or by decreasing vascular NOX activity.

**Vitamin C.** In endothelial cells, L-ascorbic acid blocks the oxidative stress-induced increase in endothelial permeability by maintaining NO• levels<sup>(109)</sup>. In adults with T1D, the combination of euglycaemia and ascorbic acid therapy has been observed to normalise endothelial function<sup>(110)</sup> in addition to the reduction in acute hyperglycaemia-induced impairment of endothelial function<sup>(111)</sup>. L-Ascorbic acid reversed the endothelial dysfunction in patients with coronary artery disease. The study of Heitzer *et al.* highlighted the mechanism of oxidative stress-driven progression of atherosclerotic disease and the beneficial effect of vitamin C in scavenging the increased  $\cdot\text{O}_2^-$ -derived free radicals. The positive response to vitamin C has been documented against the higher risk of



cardiovascular events where oxidative stress has a major role<sup>(59)</sup>. Vitamin C has been also reported to improve the endothelium-dependent, NO<sup>•</sup>-mediated vasodilation in diabetic patients, in relation to an imbalance between increased oxidative stress and depleted antioxidant defence. Further, endothelium-dependent vasodilation has been improved by a supraphysiological concentration of vitamin C<sup>(59)</sup>. A recent study showed that ascorbic acid supplementation ameliorated skeletal muscle oxidative stress during hyperinsulinemia and improved insulin-mediated glucose disposal in T2D<sup>(112)</sup>.

**Folic acid.** Folic acid, a tetrahydrofolic acid precursor, has been shown to improve endothelial dysfunction in patients with T1D<sup>(113)</sup> and T2D<sup>(114)</sup> by potentially reversing the uncoupling of eNOS. The reduced folate levels have been associated with reduced flow-mediated vasodilation in T1D patients, which demonstrates the direct effect of folic acid on endothelial function<sup>(115)</sup>. On the contrary, latter studies demonstrated that folic acid fails to improve endothelial dysfunction in diabetic nephropathy patients<sup>(116)</sup>. Furthermore, increased cancer incidence and mortality with folate and vitamin B<sub>12</sub> supplementation have limited their use<sup>(117)</sup>.

**Vitamin D.** Low vitamin D levels have been associated with an increased risk of cardiovascular disease and endothelial dysfunction in adults with diabetes<sup>(118)</sup>. Evidence demonstrates that a single large dose of vitamin D markedly improved flow-mediated vasodilation in adult T2D<sup>(119)</sup>. Vitamin D is a natural ER stress reliever, and the absence of vitamin D receptor in macrophages triggers ER stress in T2D patients<sup>(120)</sup>.

**Vitamin E.** Vitamin E has a potential to prevent hyperglycaemia-induced endothelial dysfunction, as demonstrated in *in vivo* models. Deficiency of vitamin E is deleterious for endothelial function in diabetes. Hyperglycaemia-induced reduction in basal NO<sup>•</sup> production has been significantly prevented by vitamin E supplementation<sup>(121)</sup>. A study with T1D patients showed that vitamin E improved the endothelial vasodilator function<sup>(122)</sup> as well as enhanced vascular reactivity of micro- and macrocirculation<sup>(123)</sup>.

### Polyphenols in the amelioration of endothelial dysfunction

Numerous therapeutic strategies have been developed for restoring endothelial dysfunction under diabetic conditions. In addition to antioxidant therapy, many studies also confirmed the protective effects of polyphenol antioxidants against diabetic vascular complications<sup>(124,125)</sup>. Many clinical trials have revealed the beneficial activity of these polyphenol antioxidants against endothelial dysfunction; for example, the effects of green tea and cocoa polyphenols against endothelial dysfunction in patients with diabetes, red-wine polyphenols against microvascular dysfunction and citrus fruit consumption on vascular protection<sup>(126,127)</sup>. The major drawbacks in the effects of polyphenol antioxidants are (i) their presence in large amounts in natural food, (ii) differences in their

bioavailability<sup>(128)</sup> and (iii) the chemical nature of the active component, which might not be polyphenol but its derivative<sup>(129)</sup>.

Other limitations of the currently available interventional trials are the relatively short duration of the therapy, inadequacy of the doses of antioxidants used and the timing of the initiation of antioxidant therapy<sup>(130)</sup>. However, a detailed scientific investigation of polyphenol antioxidants confirmed that this tends to be the safe and effective approach for diabetes and its vascular complications. In addition, results from several experimental studies suggested that polyphenol antioxidants improved endothelial function by multiple mechanisms. Extensive literature demonstrates the beneficial effect of polyphenol antioxidants on endothelial dysfunction and revealed NO<sup>•</sup> signalling pathway as a major target in diabetes management.

The molecular mechanisms underlying the antioxidant properties of polyphenols and some of the important signalling pathways mediating vascular endothelial cell protection under hyperglycaemia including (i) NO<sup>•</sup> signalling pathway, (ii) VEGF-mediated angiogenic pathway, (iii) ER stress pathway, (iv) inflammatory and (v) nuclear factor-E2-related factor 2 (Nrf2)-mediated antioxidant pathway (Fig. 4) are discussed.

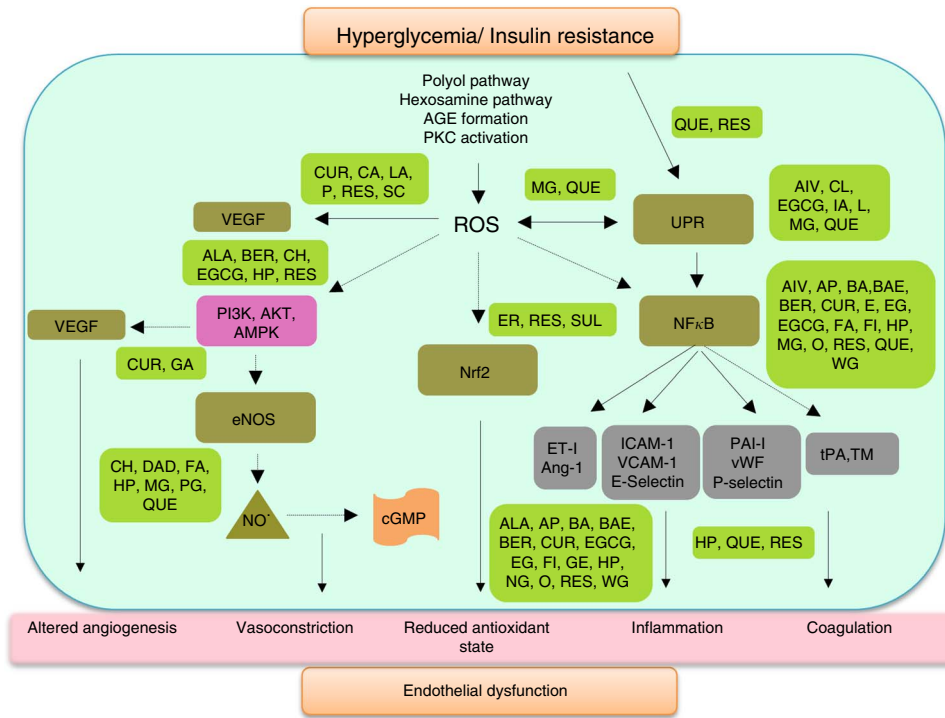
### Nitric oxide signalling

NO<sup>•</sup> production in endothelial cells is mainly regulated by the constitutive expression of eNOS in the presence of its substrate L-arginine and cofactors such as NADPH, FAD, FMN and BH<sub>4</sub><sup>(131)</sup>. eNOS is regulated at multiple sites by phosphorylation of serine (Ser), threonine (Thr) and tyrosine (Tyr) residues by Akt kinase, cyclic AMP (cAMP)-dependent PKA and AMPK in endothelial cells<sup>(132,133)</sup>. Further, the activation of IRS-1 by insulin subsequently phosphorylates eNOS through PI3K/Akt signalling pathways<sup>(134)</sup>. eNOS is activated in response to fluid shear stress and numerous agonists via cellular events such as increased intracellular calcium, interaction with substrate and cofactors, protein phosphorylation and cellular factors such as VEGF, IGF and so on. Dysregulation of these processes attenuates eNOS expression and reduces NO<sup>•</sup> levels, a characteristic of numerous pathophysiological disorders, including diabetes. Under conditions of insulin resistance, IRS-1 mutation reduces the insulin-stimulated eNOS phosphorylation in endothelial cells<sup>(135)</sup> and impairs NO<sup>•</sup> bioavailability<sup>(136)</sup> through PI3k/Akt signalling. In addition, NOX localised in the endothelium serves as a major source of  $\cdot\text{O}_2^-$ , and its interaction with eNOS leads to uncoupling, which is implicated in diabetic complications<sup>(137)</sup>. OONO<sup>-</sup> formed by the reaction of NO<sup>•</sup> with  $\cdot\text{O}_2^-$  reduces the availability of NO<sup>•</sup>, causing induction of VCAM-1, ICAM-1 and E-selectin in endothelial cells<sup>(138)</sup>. The activated endothelium expresses chemotactic factors such as monocyte chemoattractant protein-1 (MCP-1) and other proinflammatory cytokines such as macrophage colony-stimulating factor and TNF- $\beta$ . Expression of these factors contributes to the development of inflammation within the arterial wall and promotes atherogenesis.

Modulation of NO<sup>•</sup> and the role of antioxidants in restoring endothelial dysfunction is gaining momentum. Polyphenol antioxidants are being investigated for enhancement of the







**Fig. 4.** Various signalling targets of polyphenol antioxidants in endothelial cell protection. AIV, astragaloside; ALA,  $\alpha$  linolenic acid; AP, apigenin, BA, baicalin; BAE, baicalein; BER, berberine; CA, chlorogenic acid; CH, catechin hydrate; CL, cycloastragenol; CUR, curcumin; DAD, daidzein; EG, emodin-6-O- $\beta$ -D-glucoside; E, emodin; EGCG, epigallocatechin gallate; ER, eriodictyol; FA, ferulic acid; FI, fisetin; GA, gallic acid; GE, genistein; HP, hesperetin; IA, ilexgenin A; L, luteolin; LA,  $\alpha$ -lipoic acid; MG, mangiferin; NG, naringin; O, orientin; P, paeonol; PG, propyl gallate; QUE, quercetin; RES, resveratrol; SC, scutellarein; SUL, sulfuraphane; WG, wogonin; AMPK, AMP-activated protein kinase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; cGMP, cyclic GMP; Nrf2, nuclear factor-E2-related factor 2; UPR, unfolded protein responses; ET-1, endothelin-1; Ang-1, angiotensin 1; ICAM-1, intracellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; PAI-1, plasminogen activator inhibitor-1; vWF, von Willebrand factor; tPA, tissue plasminogen activator; TM, thrombomodulin. A colour figure is available in the online version of the paper.

release of NO<sup>\*</sup> from the endothelium to assess the possibility of reversing endothelial dysfunction caused by decreased production of NO<sup>\*</sup> in diabetes. Polyphenols are considered to be the most effective antioxidants. One of the most studied polyphenols, resveratrol, is present in red grapes and other fruits. It has been reported to restore the IRS-1/Akt/eNOS signalling pathway in endothelial cells under palmitate-induced insulin resistance<sup>(139)</sup>. Further, resveratrol is effective in restoring the vascular functions mediated through eNOS in diabetic rats<sup>(140)</sup>.

Machha *et al.*<sup>(141)</sup> reported modulation of endothelium-derived NO<sup>\*</sup> bioavailability in aortic tissues of diabetic rats by quercetin, a flavonol. Recently, the effect of quercetin against high-glucose-induced toxicity mediated by sirtuin 1-dependent eNOS and intracellular cGMP expression in bone marrow-derived EPC<sup>(142)</sup> has been reported. The vasoprotective role of quercetin has also been evidenced by phosphorylating eNOS at Ser1179 through cAMP/PKA signalling and enhancing the production of NO<sup>\*</sup><sup>(143)</sup>.

Gallic acid, a trihydroxy benzoic acid, restored eNOS activity in glomerular endothelial cells of diabetic rats through oxidative stress reduction in diabetic nephropathy<sup>(144,145)</sup>. Daidzein, a phyto-oestrogen, reversed changes in vascular reactivity through NO<sup>\*</sup> and PG-related pathways, and attenuated oxidative stress in aortic tissue of diabetic rats<sup>(146)</sup>. Other polyphenolic compounds such as epigallocatechin gallate (EGCG), hesperetin,  $\alpha$ -linolenic

acid, ferulic acid and catechin hydrate have also been reported to reverse endothelial dysfunction through the activation of the PI3K/Akt/eNOS pathway, as demonstrated in both *in vitro* and *in vivo* diabetic models<sup>(147-151)</sup>.

ADMA, the endogenous NOS inhibitor, is disposed to be one of the causative factors in endothelial dysfunction. Tang *et al.*<sup>(152)</sup> demonstrated that EGCG, a polyphenolic catechin, attenuated endothelial dysfunction in diabetic models by decreasing ADMA level via increasing DDAH activity. In addition, silibinin, a flavonolignan, markedly improved endothelial function in T2D mice by reducing circulating and vascular ADMA levels<sup>(153)</sup>.

The protective role of berberine, an alkaloid, has been reported to increase the expression of AMPK and eNOS, whereas it down-regulates NOX, thereby ameliorating endothelial dysfunction in palmitate-exposed endothelial cells<sup>(154)</sup>. Wang *et al.*<sup>(155)</sup> showed that berberine attenuated high-glucose-induced endothelial dysfunction through the activation of AMPK/eNOS signalling, mediating NO<sup>\*</sup> and cGMP production, and endothelium-dependent vasodilatation.

#### Vascular endothelial growth factor-mediated angiogenesis

VEGF, a potent pro-angiogenic growth factor, and its receptors in endothelial cells promote angiogenesis and cell proliferation<sup>(156)</sup>. Under diabetic conditions, elevated VEGF level acts as a



pathological angiogenic stimulus leading to ocular neovascularisation (retinopathy) and nephropathy, whereas low levels of VEGF activity lead to cardiomyopathy and peripheral neuropathy because of insufficient angiogenesis<sup>(157)</sup>. The relationship between VEGF and vascular complications is an intricate process, particularly in patients with diabetes. This situation provides an insight into the complicated interplay between VEGF, VEGF receptors and the resulting systemic effects on microvascular and macrovascular diseases<sup>(157)</sup>.

In diabetic retinopathy, VEGF receptors are up-regulated in retinal endothelial cells. Increased VEGF favours the proteolysis of endothelial basement membrane, which is the limiting step in angiogenesis, leading to retinopathy. In addition, a direct correlation between VCAM-1 and VEGF in the vitreous fluid of retinopathy patients has been reported<sup>(158)</sup>. VEGF and its receptor expression levels are critical in maintaining normal glomerular podocytes and renal tubular function. VEGF expressed in glomerular podocytes activates VEGF receptor 2 on glomerular capillary endothelial cells, regulating endothelial fenestrations and permeability<sup>(159)</sup>. Hyperglycaemia together with ROS elevates VEGF level and increases glomerular permeability, hyperfiltration and hypertrophy contributing to diabetic nephropathy<sup>(160)</sup>.

Diabetic macrovascular complications such as cardiomyopathy are characterised by impaired angiogenesis. Cardiomyopathy, an inability of the heart to circulate blood throughout the body, has been reported with reduced myocardial VEGF and its receptor expressions. In addition, it results in decreased capillary density accompanied by decreased myocardial perfusion and progressive left ventricular dysfunction<sup>(161)</sup>. VEGF has an important role in nerve regeneration, and its regulation reflects the functional state of peripheral nerves. It protects the dorsal root ganglia against diabetes-induced neurotoxicity. The reduced levels of VEGF under hyperglycaemia lead to late-onset motor neuron degeneration<sup>(162)</sup>. Under hyperglycaemic conditions, impaired wound healing mediated by down-regulation of VEGF receptors results in impaired angiogenesis, which in turn is the cause for EPC dysfunction, low platelet count, reduced granulation and defective lymphatic vasculature<sup>(163)</sup>.

Reversal from endothelial dysfunction, evidenced by a few polyphenol antioxidants, is effected by down-regulation of angiogenesis mediated by VEGF. Curcumin, a well-known antioxidant, inhibits VEGF expression in STZ-induced diabetic retina and kidney, thereby mediating vascular protection<sup>(164–166)</sup>. Chlorogenic acid found abundantly in coffee significantly decreased VEGF levels and thereby reduced retinal vascular hyperpermeability and leakage in the diabetic retinopathy<sup>(167)</sup> rat model.

VEGF mediates the activation of PKC, and its translocation to endothelial cell membranes triggers the angiogenesis process<sup>(168)</sup>. The antiangiogenic activity of curcumin was demonstrated in human retinal endothelial cells reported to be caused by a reduction in glucose-induced mRNA levels of VEGF expression, and it also inhibits PKC $\beta$ II translocation induced by VEGF<sup>(169)</sup>. The protective role of paeonol has been studied against high-glucose-induced vascular endothelial cell injury in a co-culture model system with vascular smooth muscle cells. Restored vascular homeostasis was evidenced by down-regulation of VEGF and PDGF-B, rat sarcoma (Ras),

phosphorylated rapidly accelerated fibrosarcoma (p-Raf) and phosphorylated extracellular signal-regulated kinases (p-ERK) protein expressions in the vascular smooth muscle cells<sup>(170)</sup>. Scutellarein, a flavone found in *Scutellaria lateriflora*, suppresses VEGF and cell proliferation<sup>(171)</sup> in high-glucose-treated human retinal endothelial cells. Resveratrol down-regulated VEGF/fetal liver kinase-1 (Flk-1) (VEGF receptor-2) expression and thereby modulated hyperpermeability and junction disruption in glomerular endothelial cells. It also ameliorates high-glucose-induced hyperpermeability mediated by overexpressed caveolin-1 in aortic endothelial cells<sup>(172)</sup>.

R-(+)- $\alpha$ -lipoic acid inhibits VEGF-stimulated proliferation in microvascular and macrovascular endothelial cell isolated from the diabetic patients independent of their vascular origin. It reduces both apoptosis and proliferation of retinal endothelial cells through the activation of Akt and retinoblastoma protein transcription factor (E2F-1)<sup>(173)</sup>. Hyperglycaemia and increased AGE in diabetes cause inactivation of the VEGF receptors such as Flk-1 and hence altered VEGF expression, affecting endothelial growth and migration<sup>(174)</sup>. VEGF is vital in promoting collateral vessel formation after ischaemic events and plays a key role in wound healing<sup>(156)</sup>. Defective wound healing in diabetic patients is often associated with impaired VEGF expression resulting in failure to form the vasculature<sup>(175)</sup>. VEGF also mediates the survival of immature vessels through Flk-1 via the PI3K/Akt pathway<sup>(176)</sup>. In addition to cell growth, VEGF inhibits apoptosis through the Akt pathway<sup>(177)</sup>. Nakamura *et al.*<sup>(178)</sup> demonstrated the antiapoptotic potential of VEGF in EPC isolated from cord blood through the activation of Akt signalling. The abnormal cross talk between VEGF-A and NO $\cdot$  pathways is fuelled by the hyperglycaemia-induced oxidative stress<sup>(179)</sup>.

Yang *et al.* reported that the elevated glucose down-regulated VEGF through attenuation of p42/44 MAPK phosphorylation with the activation of mitochondrial apoptosis pathway mediated by ROS and Ca leading to endothelial dysfunction. Restoration of VEGF explains its role in apoptosis inhibition with a decrease in the B-cell lymphoma 2-associated X protein:B-cell lymphoma 2 (Bax:Bcl-2) ratio, thereby inhibiting caspase-3 activation<sup>(180)</sup>.

With cultured human glomerular endothelial cells under high-glucose and vascular endothelial growth factor receptor 1 (VEGFR1) inhibition, induced apoptosis and oxidative stress are mediated by the suppression of PI3K-Akt phosphorylation<sup>(181)</sup>. T2D mice with VEGFR1 inhibition showed apoptosis of the glomerular cells with albuminuria, mesangial matrix expansion and inflammatory cell infiltration, with the inactivation of eNOS-NOx signalling. The uncoupling of the VEGF-NO $\cdot$  axis in the glomeruli resulting from eNOS inactivation has been reported in glomerular endothelial dysfunction<sup>(182)</sup>. Tian *et al.*<sup>(145)</sup> confirmed the improvement of glomerular pathological changes in diabetic rats through oxidative stress reduction and VEGF-NO $\cdot$  axis recovery and prevention of glomerular endothelial dysfunction by propyl gallate.

### Endoplasmic reticulum stress pathway

ER is the organelle in which proteins fold and attain their native conformation and post-translational modifications such as



*N*-linked glycosylation, disulfide bond formation, lipidation, hydroxylation and oligomerisation<sup>(183)</sup>. Any perturbation in the protein folding machinery leads to the accumulation of unfolded/misfolded proteins and activates UPR. Elevation in levels of glucose, oxidised phospholipids and homocysteines disturbs the endothelial cells and trigger ER stress<sup>(184,185)</sup>. As a counter mechanism to maintain ER homeostasis, UPR up-regulates ER chaperons and foldases, and activates endoplasmic reticulum-associated degradation and also down-regulates the expression of secretory proteins, thereby reducing the accumulation of unfolded proteins<sup>(186)</sup>. ER stress sensors such as PERK, inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) play a central role in the initiation and regulation of UPR<sup>(187)</sup>. However, when the stress is beyond the holding capacity of UPR, it up-regulates IRE1, which activates JNK signalling and caspase-12<sup>(188)</sup>; in addition to CHOP activation via phosphorylation<sup>(189)</sup>, these events culminate in the suppression of the antiapoptotic protein Bcl-2, rendering the cells to undergo apoptosis<sup>(190)</sup>.

The ER stress responses has been implicated in the endothelial dysfunction observed in T1D, T2D and obesity<sup>(191,192)</sup>. Experimental conditions mimicking hyperglycaemia with supra-physiological dextrose concentrations and over-expression of exogenous GLUT-1 increased the ER stress in human umbilical vein endothelial cells (HUVEC)<sup>(72)</sup>. It has also been evaluated that ER stress activates IRE1 via JNK phosphorylation. It perturbs insulin signalling in endothelial cells by inhibiting IRS-1 and reducing NO production<sup>(38)</sup>. PERK mediated hyperglycaemia-induced endothelial inflammation and retinal vascular leakage in T1D mouse model<sup>(191)</sup>, and endothelial cell apoptosis under high-glucose conditions have been reported<sup>(193)</sup>.

Experimental evidence spotlights ER stress to be a major causative factor for neurodegenerative diseases in diabetes<sup>(72,194)</sup>. For instance, ER stress is a potential mediator of inflammation in diabetic retinopathy<sup>(195)</sup>, which has been well documented with the experiment of Chen *et al.*<sup>(191)</sup>. Moreover, diabetic retinopathy-associated distorted retinal angiogenesis has been linked to ER stress<sup>(196,197)</sup>. Hence, inhibition of ER stress and its specific mediators has been acknowledged to be a potential target for vascular complications under hyperglycaemia. Antioxidants such as  $\alpha$ -tocopherol and ascorbic acid did not ameliorate glucose-induced ER stress condition<sup>(198)</sup>. Research studies focusing on endothelial ER stress under hyperglycaemic conditions are limited, and only few polyphenolic compounds are reported to overcome the UPR. Recent research with human endothelial cells by Song *et al.* indicated provoking of ER stress with increased ROS production in hyperglycaemia. Mangiferin, a polyphenolic compound, effectively inhibited ER stress-associated oxidative stress by reducing ROS production and by attenuating IRE1 phosphorylation<sup>(199)</sup>.

Inflammasome is a multiprotein intracellular complex and an innate immune system receptor that regulates the activation of caspase-1 and induces inflammation under stress conditions<sup>(200)</sup>. The important components of the inflammasome complex include the nucleotide-binding domain and leucine-rich repeat-containing proteins (also known as NOD (nucleotide oligomerisation domain)-like receptors (NLR))<sup>(201)</sup>. Upon sensing stress stimuli, the relevant NLR activates

caspase-1, which subsequently functions to cleave the proinflammatory IL-1 cytokines into their active forms: IL-1 $\beta$  and IL-18. It has been reported that ER-activated inflammasome formation evokes endothelial inflammation and apoptosis, playing a critical role in the onset of endothelial dysfunction<sup>(202)</sup>. Mangiferin, a xanthonoid, has been reported to reduce the expression of thioredoxin-interacting protein (TXNIP) and its NOD-like receptor protein 3 (NLRP3) inflammasome. It also inhibited ER stress-associated IRE1 $\alpha$  phosphorylation and regulated endothelial homeostasis in an AMPK-dependent manner<sup>(199)</sup>.

Polyphenolic compound combinations such as quercetin, luteolin and EGCG alleviate palmitate-induced ER stress in endothelial cells. They were found to maintain the endothelial homeostasis by inhibiting ER stress-associated TXNIP and NLRP3 inflammasome activation, and they restored mitochondrial function and protected cells against inflammation and apoptosis<sup>(203)</sup>. Few other compounds such as ilexgenin A<sup>(204)</sup>, astragaloside IV and cycloastragenol<sup>(205)</sup> have been reported to improve endothelial function under palmitate-induced ER stress in an AMPK-dependent manner and by inhibiting TXNIP/NLRP3 inflammasome pathway.

Experimental ER stress has been induced with pharmacological agents such as thapsigargin, tunicamycin, A23187 and brefeldin A<sup>(206)</sup>. The contemplated possible involvement of ER stress in endothelial dysfunction has been studied by our group in tunicamycin-induced HUVEC. Tunicamycin blocks *N*-glycosylation of proteins and causes an extensive protein misfolding, thus activating UPR in the endothelial cells<sup>(207)</sup>. The protective effect of quercetin against the activation of ER stress was attributed to the up-regulation of markers such as 78 kDa glucose-regulated protein (GRP78), a molecular chaperone and CHOP in unresolved diabetic and experimental ER stress conditions<sup>(208)</sup>. Quercetin pre-treatment decreased tunicamycin-induced ER stress marker expression in HUVEC<sup>(209)</sup>. Poly ADP ribose polymerase (PARP), the end point of a number of cell signalling pathways, is known to control many physiological and pathological outcomes and tends to mediate immunity and inflammation<sup>(210)</sup>. In addition, it has been demonstrated that quercetin re-establishes ER homeostasis by reducing caspase-3 and caspase-dependent PARP cleavage and normalising the antioxidant enzymes SOD1 and catalase (CAT) under ER stress<sup>(209)</sup>. Further, thapsigargin-induced ER calcium depletion due to inhibition of the calcium-ATPase has been restored with resveratrol in the endothelium<sup>(211)</sup>, and it also prevented retinal vascular degeneration induced by tunicamycin<sup>(212)</sup>.

### Inflammatory pathway

In addition to the mediators of vasomotor functions, vascular endothelial cells release inflammatory mediators that are fore-runners in the initiation, amplification and resolution of the inflammatory response. Endothelial cells are more prone to various stresses including glucose toxicity, which stimulates the secretion of proinflammatory cytokines and adhesion factors<sup>(213)</sup>. Endothelial dysfunction focusing vascular inflammation has been implicated in the induction of vasoconstrictors, adhesion



molecules, Ang II and ET-1<sup>(214)</sup>. ROS tends to be the competent stimulator of ET-1, which in turn activates NOX and XO, worsening the cellular oxidative stress contributing to vascular remodelling and endothelial dysfunction<sup>(215,216)</sup>.

In addition, hyperglycaemia activates NF- $\kappa$ B, the master regulator and major proinflammatory transcription factor that controls multiple proinflammatory and proatherosclerotic targets in endothelial cells, and thus activates inflammation<sup>(217)</sup>. The regulation of NF- $\kappa$ B is primarily associated with the I $\kappa$ B family of transcription factor inhibitor proteins, and its phosphorylation implies the most important step in its activation<sup>(218)</sup>. At the downstream, NF- $\kappa$ B activates the transcription of proinflammatory genes including TNF- $\alpha$ , IL-1, IL-8, E-selectin, VCAM-1 and ICAM-1 in vascular endothelial cells, as reviewed by Xiao *et al.*<sup>(219)</sup>, and it also promotes the expression of MCP-1 and adhesion of leucocyte and monocytes to the endothelial cells, which is followed by their infiltration and differentiation into macrophages under hyperglycaemic conditions<sup>(220)</sup>.

It has been well documented that under conditions of insulin resistance and T2D, inflammation has been reflected with an increase in TNF- $\alpha$ , IL-6, PAI-1, ET-1 and high-sensitive C-reactive protein relating it to endothelial dysfunction<sup>(221)</sup>. In addition, it has been explicated that NEFA-induced ROS activates NF- $\kappa$ B, inhibitor of kappaB kinase (IKK) $\alpha$  and impairs insulin-stimulated activation of eNOS and NO $\cdot$  production in endothelial cells<sup>(222)</sup>.

Many polyphenolic compounds have been reported to ameliorate endothelial dysfunction by inhibiting inflammatory mediators. A recent study with palmitate-induced insulin resistance model reveals that resveratrol suppressed IKK $\beta$ /NF- $\kappa$ B phosphorylation, TNF- $\alpha$  and IL-6 production and restored the IRS-1/Akt/eNOS signalling pathway in endothelial cells<sup>(139)</sup>. Resveratrol has been reported to block TNF- $\alpha$ -induced activation of NF- $\kappa$ B in coronary arterial endothelial cells and to inhibit inflammatory mediators<sup>(223)</sup>, exerting its effect through action on (IKK) cascade, thereby attributing to its antioxidant properties.

Ferulic acid combined with astragaloside IV is known to protect vascular endothelial dysfunction in STZ-induced diabetic rats by promoting the release of NO $\cdot$  and eNOS, and inhibiting the hyper-stimulation of MCP-1, TNF- $\alpha$  and NF- $\kappa$ B P65 in aorta<sup>(147)</sup>. The administration of the flavonoids such as EGCG, quercetin and delphinidin increased the bioactivity of NO $\cdot$  and prevented endothelial cell apoptosis by modulating inflammatory pathways<sup>(224)</sup>. Curcumin inhibits proinflammatory cytokines, TNF- $\alpha$ , ICAM-1, NOX2 and cyclo-oxygenase-2 expressions and reduces leucocyte-endothelium interaction in diabetes-induced vascular inflammatory models<sup>(225–227)</sup>. The report of Mahmoud *et al.* confirmed that quercetin confers protection to diabetes-induced vasoconstriction, leading to low-grade inflammation with concomitant reduction in serum levels of both TNF- $\alpha$ , CRP and inhibition of NF- $\kappa$ B in aorta<sup>(228)</sup>.

In addition, increased adhesion of monocytes to bovine aortic endothelial cells (BAEC) and expression of VCAM-1 in response to TNF- $\alpha$  treatment was reversed by pre-exposure with hesperetin<sup>(148)</sup>. In a clinical study, hesperetin treatment increased flow-mediated dilation with the reduction in

circulating inflammatory biomarkers such as CRP, serum amyloid A protein and soluble E-selectin<sup>(148)</sup>. Yamagata *et al.* reported increased expression of ICAM-1 and VCAM1 in the endothelial cells under exposure to high glucose and TNF- $\alpha$ ; further, the treatment with apigenin significantly inhibited the expression of adhesion molecules and also IKK $\alpha$  and IKKi, thereby mediating the protection against atherosclerotic vascular diseases<sup>(229)</sup>.

The molecular link between the activation of PKC and up-regulation of VCAM-1 expression under hyperglycaemic conditions involving NF- $\kappa$ B activation has been studied<sup>(230)</sup>. EGCG has been reported to inhibit the vascular inflammation in hyperglycaemia by suppression of the PKC/NF- $\kappa$ B signalling pathway, and it also prevented monocyte adhesion in HUVEC cells<sup>(231)</sup>.

MAPK signalling was reported to regulate the high-glucose-induced inflammatory cytokines<sup>(232)</sup>. A study with emodin (3-methyl-1, 6, 8-trihydroxyanthraquinone), an anthraquinone in HUVEC, reported the inhibition of glucose-induced phosphorylation of ERK 1/2 and p38 MAPK, thereby protecting the cells against inflammation<sup>(233)</sup>. Brazilin markedly inhibited the high-glucose-induced MAPK/ERK signal transduction pathway, thereby inhibiting the phosphorylation of extracellular signal-regulated kinase and transcription factor NF- $\kappa$ B in HUVEC cells<sup>(234)</sup>. The report of Kim *et al.*<sup>(232)</sup> demonstrated that hesperidin, naringenin and resveratrol reduced high-glucose-induced ICAM-1 expression via the p38 MAPK signalling pathway, contributing to the inhibition of monocyte adhesion to endothelial cells. In STZ-induced diabetic rats and high-glucose-induced HUVEC cells,  $\alpha$ -linolenic acid treatment decreased the expression of P-selectin, ICAM-1 and neutrophil adhesion via Akt phosphorylation<sup>(150)</sup>.

In high-glucose-induced vascular inflammation in HUVEC and mouse models, increased vascular permeability, monocyte adhesion, expressions of CAM, formation of ROS and activation of NF- $\kappa$ B, with induced expressions of MCP-1 and IL-8, were ameliorated not only by emodin-6-O- $\beta$ -D-glucoside<sup>(235)</sup> but also by other polyphenols such as fisetin, orientin and naringin model, thereby reducing diabetic complications and atherosclerosis<sup>(236–238)</sup>. A recent study with three structurally related polyphenols such as baicalin, baicalein and wogonin inhibited endothelial cell barrier disruption, suggesting its protection against vascular inflammatory diseases<sup>(239)</sup>. Chronic resveratrol treatment in T2D rats showed a vasoprotective effect through the inhibition of inflammation mediators such as IL-1 $\beta$  and IL-6, decreasing the circulating vWF levels, recovered vascular permeability in both carotid artery and thoracic aorta. In addition, resveratrol showed an inhibitory effect against NF- $\kappa$ B p65, proinflammatory mediators including TNF- $\alpha$ , ICAM-1 and MCP-1 in endothelial cell lines<sup>(240)</sup>.

The protective effect of genistein was proven in the human aortic endothelial cells under exposure to high glucose by its inhibitory action on monocytes, and suppression of MCP-1 and IL-8<sup>(241)</sup>. The combination of quercetin, EGCG and curcumin attenuated high-glucose-induced membrane fluidity and transmembrane potential of HUVEC cells by reducing the AGE products formed and limiting the release of pro-inflammatory

**Table 1.** The molecular mechanism of polyphenol antioxidants in modulating endothelial dysfunction under diabetes

Sl. no.	Compounds	Type/systematic name/functional group	Mechanism of action	Experimental model	References
1	$\alpha$ -Linolenic acid	Carboxylic acid/(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	Increased phosphorylation of Akt at Ser <sup>437</sup> and Thr <sup>308</sup> Reduced TNF- $\alpha$ -stimulated phosphorylation of NF- $\kappa$ B's inhibitory subunit, I $\kappa$ B $\alpha$ Enhanced eNOS activity, inhibited iNOS, NADPH oxidase expression and ONOO <sup>-</sup> production Decreased the expression of P-selectin, ICAM-1 and neutrophil adhesion	<i>In vitro</i> <i>In vitro, In vivo</i>	(173) (150)
2	Apigenin	Flavone/5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one/5-OH, 7-OH, 4'-OH and a C(2)=C(3) double bond and C(4)=O functionalities	Inhibited the expression of adhesion molecules, ICAM-1 and VCAM-1	<i>In vitro</i>	(229)
3	Astragaloside IV and cycloastragenol	–	Enhanced AMPK phosphorylation Inhibited TXNIP/NLRP3 inflammasome activation and attenuated ER stress-induced IRE1 $\alpha$ phosphorylation	<i>In vitro</i>	(205)
4	Baicalin	Flavone/(2S,3S,4S,5R,6S)-6-(5,6-dihydroxy-4-oxo-2-phenylchromen-7-yl)oxy-3,4,5-trihydroxyoxane-2-carboxylic acid/5,6,7-OH, ortho-dihydroxyl group in ring A	Decreased vascular permeability, NF- $\kappa$ B, cell adhesion molecules and prevented monocyte adhesion	<i>In vitro, In vivo</i>	(239)
5	Baicalein	Flavone/5,6,7-trihydroxy-2-phenylchromen-4-one/5,6,7-OH	Decreased vascular permeability, NF- $\kappa$ B, cell adhesion molecules and prevented monocyte adhesion	<i>In vitro, In vivo</i>	(239)
6	Berberine	Isoquinoline alkaloid/5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium	Activated AMPK, enhanced phosphorylation of eNOS at Ser <sup>1177</sup> and promoted the association of eNOS with HSP90, thereby increasing NO <sup>•</sup> production Reduced NF- $\kappa$ B activation, expression of adhesion molecules and suppressed THP1 monocytes adhesion	<i>In vitro, Ex vivo</i>	(154,155)
7	Brazilin	Red pigment/(6aS,11bR)-7,11b-dihydro-6H-indeno[2,1-c]chromene-3,6a,9,10-tetrol	Increased the phosphorylation of eNOS and ERK	<i>In vitro</i>	(234)
8	Catechin hydrate	Flavanols/(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol;hydrate/3,5,7,3',4'-OH	Decreased the expression of cell adhesion molecules and NF- $\kappa$ B Activated PI3K and eNOS, increased the generation of NO <sup>•</sup>	<i>In vivo</i>	(151)
9	Chlorogenic acid	Phenolic acid-hydroxycinnamic acids/(1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid/3,4-OH,orthodiphenolic functionalities	Decreased VEGF levels in diabetic retinopathy	<i>In vivo</i>	(167)
10	Curcumin	Curcuminoids/(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione/8,8'-OH, 7,7'-OCH <sub>3</sub> O-methoxyphenol group and methylenic hydrogen	Inhibited the PKC $\beta$ II translocation induced by VEGF in human retinal endothelial cells Increased GSH levels Inhibited the levels of IL-1 $\beta$ , VEGF and NF- $\kappa$ B Inhibited expression of VEGF in diabetic retina and kidney Increased VEGF and transforming growth factor $\beta$ 1, hypoxia-inducible growth factor-1 $\alpha$ , stromal cell-derived growth factor-1 $\alpha$ , and HO-1 expression in diabetic wound healing Reduced leucocyte-endothelium interaction, inhibited ICAM-1 and NOX2 Increased GSH, SOD and CAT levels Reduced the level of TNF- $\alpha$ Suppressed COX-2 and NF- $\kappa$ B levels, inhibited PKC- $\beta$ II and improved the ratio of prostanoid products PGI <sub>2</sub> :TXA <sub>2</sub>	<i>In vitro</i> <i>In vivo</i> <i>In vivo</i> <i>In vivo</i> <i>In vivo</i> <i>In vivo</i> <i>In vivo</i> <i>In vivo</i>	(169) (165) (164,166) (260) (225) (226) (227)
11	Daidzein	Isoflavone/7-hydroxy-3-(4-hydroxyphenyl)chromen-4-one/7,4'-OH	Activated NO <sup>•</sup> Reversed the increased MDA content and activity of SOD	<i>Ex vivo</i>	(146)
12	Emodin-6-O- $\beta$ -D-glucoside	Anthraquinones	Decreased vascular permeability, expressions of adhesion molecules, monocyte adhesion and NF- $\kappa$ B	<i>In vitro, In vivo</i>	(235)

Role of polyphenols in endothelial dysfunction

Table 1. Continued

Sl. no.	Compounds	Type/systematic name/functional group	Mechanism of action	Experimental model	References
13	Emodin	Anthraquinones/1,3,8-trihydroxy-6-methylantracene-9,10-dione/1,6,8-OH, 3-CH <sub>3</sub>	Inhibited the phosphorylation of ERK 1/2 and p38 MAPK	<i>In vitro</i>	(233)
14	Epigallocatechin-3-gallate	Flavanols/[(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate/phenolic hydroxyl groups on rings B and D,3-OG (G = galloyl)	Increased the phosphorylation of eNOS, IRS-1 and Akt Increased the level of nitrite/nitrate and the activity of DDAH Decreased the levels of LDH, ADMA, TNF- $\alpha$ and MDA Decreased NF- $\kappa$ B and phospho-I $\kappa$ B $\alpha$ expression Inhibited PKC, VCAM-1 expression and the adhesion of THP-1 monocytes Reduced the nuclear translocation of NF- $\kappa$ B p65 Decreased the levels of MCP-1, serum VCAM-1, ICAM-1 levels and prevented monocyte adhesion	<i>In vivo</i> <i>In vitro</i> , <i>In vivo</i> <i>In vitro</i> <i>In vitro</i> , <i>In vivo</i>	(149) (152) (231) (261)
15	Eriodictyol	Flavanone/(2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-2,3-dihydrochromen-4-one/5,7,3',4'-OH	Enhanced ERK/Nrf2/ARE-dependent HO-1 expression	<i>In vitro</i>	(256)
16	Fisetin	Flavone/2-(3,4-dihydroxyphenyl)-3,7-dihydroxychromen-4-one/3, 3', 4'-OH, double bond between C-2 and C-3 conjugated with 4-oxo group	Decreased the vascular permeability, expressions of adhesion molecules, monocyte adhesion and activation of NF- $\kappa$ B Decreased the number of THP-1 monocytes adhesion	<i>In vitro</i> , <i>In vivo</i>	(237)
17	Genistein	Isoflavones/5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one/5,7,4'-OH and a C(2) = C(3) double bond and C(4) = O functionalities	Inhibited monocyte adhesion, and suppressed MCP-1, IL-8 and serum ICAM-1 and VCAM-1 levels Exhibited the antiadhesion effect by suppressing monocyte-endothelial cell interaction mediated through the cAMP/PKA pathway	<i>In vitro</i> , <i>In vivo</i>	(241)
18	Hesperetin	Flavanones/(2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydrochromen-4-one/5,7,3'-OH, 4'-OCH <sub>3</sub>	Stimulated phosphorylation of Src, Akt, AMP kinase, eNOS to produce NO <sup>*</sup> , reduced the concentrations of circulating inflammatory biomarkers such as C-reactive protein, serum amyloid A protein, soluble E-selectin Reduced the TNF- $\alpha$ -stimulated expression of VCAM-1 and adhesion of monocytes	<i>In vitro</i> , Clinical	(148)
19	Ilexgenin A	–	Increased eNOS phosphorylation, NO <sup>*</sup> production and $\Delta\psi_m$ Enhanced AMPK phosphorylation, inhibited PERK and IRE1 $\alpha$ phosphorylation Reduced TXNIP induction, IL-6 and VCAM-1 production	<i>In vitro</i> , <i>In vivo</i> , <i>Ex vivo</i>	(204)
20	Mangiferin	Xanthonoid/1,3,6,7-tetrahydroxy-2-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]xanthen-9-one/6,7-dihydroxylated structure (catechol moiety)	Increased AMPK phosphorylation (AMPK $\alpha$ T172), restored the loss in NO <sup>*</sup> production, $\Delta\psi_m$ and inhibited ET-1 secretion Prevented IRE1 $\alpha$ phosphorylation, inhibited TXNIP expression and suppressed NLRP3 inflammasome activation	<i>In vitro</i>	(199)
21	Naringin	Flavanone/(2S)-7-[(2S,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-2-yl]oxy-5-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one/5,4'-OH, 7-O-rutinoses	Suppressed the adhesion of THP-1 monocytes and NF- $\kappa$ B expression	<i>In vitro</i>	(236)
22	Orientin	Flavone/2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one/O-phenolic hydroxyl in the B ring	Decreased the vascular permeability, expression of cell adhesion molecules, monocyte adhesion and activation of NF- $\kappa$ B	<i>In vitro</i> , <i>In vivo</i>	(238)
23	Paeonol	Phenol/1-(2-hydroxy-4-methoxyphenyl)ethanone	Decreased VEGF and PDGF-B Reduced LDH release	<i>In vitro</i>	(170)
24	Propyl gallate	Ester (condensation of gallic acid and propanol)/propyl 3,4,5-trihydroxybenzoate/orthodiphenolic functionalities	Restored eNOS activity and recovered VEGF–NO <sup>*</sup> axis Restored SOD, CAT, GSH-Px levels and decreased MDA levels	<i>In vivo</i>	(144,145)

Table 1. Continued

Sl. no.	Compounds	Type/systematic name/functional group	Mechanism of action	Experimental model	References
25	Quercetin	Flavonols/2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one/meta-dihydroxyl group in ring A (on 5 and 7 position) and an ortho-dihydroxyl group in ring B (on 3', 4' position)	Increased eNOS phosphorylation, NO <sup>•</sup> production and cGMP levels with enhanced SIRT1 expression	<i>In vitro</i>	(142)
			Increased the phosphorylation of eNOS at Ser1179 and also the expressions of PKA, Akt and ERK1/2	<i>In vitro, Ex vivo</i>	(143)
			Increased the levels of antioxidant enzymes, SOD and CAT	<i>In vitro</i>	(209)
			Reduced the expressions of GRP78 and CHOP Reduced the serum level of both TNF- $\alpha$ and CRP, inhibited NF- $\kappa$ B expression	<i>In vivo</i>	(228)
26	Resveratrol	Stilbenes/5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol/3,5,4'-OH and acidic para-4'-OH group. Stilbenic double bond	Increased IRS-1/Akt/eNOS signalling and also regulated AMPK and SIRT1	<i>In vitro, In vivo</i>	(139)
			Decreased the secretion of endothelin-1, plasminogen activator inhibitor-1 and suppressed IKK $\beta$ /NF- $\kappa$ B phosphorylation		
			Increased eNOS protein expression	<i>In vivo</i>	(140)
			Down-regulated VEGF and Flk-1 in diabetic nephropathy	<i>In vivo</i>	(262)
			Increased transcription level of Ang-2 mRNA		
			Down-regulated the expressions of VEGF, VEGF receptor-2 expression, caveolin 1 and suppressed hyperpermeability	<i>In vitro</i>	(172)
			Enhanced cytosolic Ca increase	<i>Ex vivo</i>	(211)
			Inhibited monocyte adhesion and expressions of iNOS, IL-6 and adhesion molecules	<i>In vitro</i>	(223)
			Inhibited inflammatory mediators such as IL-1 $\beta$ , IL-6, NF- $\kappa$ B p65, TNF- $\alpha$ , ICAM-1, MCP-1 and also decreased the circulating vWF levels	<i>In vivo</i>	(240)
			Increased the transcriptional activity of Nrf2 and up-regulated Nrf2/ARE-driven genes such as NAD(P)H: quinone oxidoreductase 1, $\gamma$ -glutamylcysteine synthetase and HO-1	<i>In vitro, In vivo</i>	(254)
27	Scutellarein	Flavone/5,6,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one	Inhibited cell proliferation via down-regulating VEGF expression in diabetic retinopathy	<i>In vitro</i>	(171)
28	Silibinin	Flavonolignans/(2R,3R)-3,5,7-trihydroxy-2-[(2R,3R)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]-2,3-dihydrochromen-4-one/OH at C5 and C3 in addition to the carbonyl group at C4 and two carboxylate groups	Reduced circulating and vascular ADMA levels	<i>In vivo</i>	(153)
29	Sulforaphane	Isothiocyanate/1-isothiocyanato-4-methylsulfanylbutane	Promoted nuclear localisation of Nrf2 and increased the expression of GSH synthesis enzymes, GCLC and GCLM	<i>In vivo</i>	(255)
			Up-regulated HO-1 expression through the ERK/Nrf2/ARE-dependent pathway, prevented activation of hexosamine and PKC pathways and formation of methylglyoxal	<i>In vitro</i>	(258)
			Increased the expression of Nrf2 and its downstream HO-1 and NQO-1 Reduced I $\kappa$ B kinase phosphorylation, NF- $\kappa$ B, iNOS and COX-2 expression, and the levels of TNF- $\alpha$ and IL-6	<i>In vitro, In vivo</i>	(259)
30	Wogonin	Flavone/5,7-dihydroxy-8-methoxy-2-phenylchromen-4-one/C-2,3 double bond and the positions of the phenolic polyhydroxyl groups on A-ring, intramolecular hydrogen bond between 5-OH and 4-CO	Decreased vascular permeability, NF- $\kappa$ B, cell adhesion molecules and monocyte adhesion	<i>In vitro, In vivo</i>	(239)

eNOS, endothelial nitric oxide synthase; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; AMPK, AMP-activated protein kinase; TXNIP, thioredoxin-interacting protein; NLRP3, NOD-like receptor protein 3; ER, endoplasmic reticulum; IRE1, inositol-requiring enzyme 1; HSP90, heat shock protein 90; NO<sup>•</sup>, nitric oxide; VEGF, vascular endothelial growth factor; PKC, protein kinase C; NOX2, NADPH oxidase isoform 2; SOD, superoxide dismutase; CAT, catalase; COX-2, cyclo-oxygenase 2; IRS-1, insulin receptor substrate 1; DDAH, dimethylarginine dimethylaminohydrolase; ADMA, asymmetric dimethylarginine; MCP-1, monocyte chemoattractant protein-1; Nrf2, nuclear factor-E2-related factor 2; ARE, antioxidant response elements; ET-1, endothelin-1; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PDGF-B, platelet-derived growth factor B; cGMP, cyclic GMP; CHOP, C/ERB homologous protein; SIRT1, sirtuin 1; Ang 2, angiopoietin 2; HO-1, heme oxygenase-1; cAMP, cyclic AMP; vWF, von Willebrand factor; AMPK, AMP-activated protein kinase; MDA, malondialdehyde; THP-1, engineered human reporter monocytes; iNOS, inducible nitric oxide synthase; GCLC, glutamate-cysteine ligase, catalytic subunit; GCLM, glutamate-cysteine ligase, modifier subunit; GSH-Px, glutathione peroxidase.

factors such as MCP-1, thereby preventing chronic inflammation<sup>(242)</sup>.

### Nuclear factor-E2-related factor 2-mediated antioxidant pathway

Nrf2 is a transcription factor that is perhaps the most prominent cellular defence mechanism against oxidative stress. Under basal conditions, Nrf2 is primarily localised in the cytoskeleton as a complex with Kelch-like erythroid cell-derived protein with cap (n) collar homology [ECH]-associated protein 1 (Keap1). Oxidative stress causes the dissociation of Nrf2 from Keap1 and is found to bind with antioxidant response elements (ARE) in the nucleus, promoting the transcription of a number of endogenous protective genes including antioxidant genes, phase II detoxification enzyme genes and molecular chaperones. Nrf2 endorses cellular redox homeostasis via its downstream activation of several intracellular antioxidants such as  $\gamma$ -glutamine cysteine synthase, SOD, CAT, glutathione reductase, glutathione peroxidase, peroxiredoxin and thioredoxin reductase, and phase II detoxifying enzymes such as HO-1, glutathione S-transferase, NAD(P)H quinone oxidoreductase 1; and also proteins involved in detoxifying xenobiotics and neutralising ROS<sup>(243,244)</sup>.

Several protein kinases such as MAPK, PKC, PI3K, glycogen synthase kinase 3 beta (GSK3b) and casein kinase 2 are reported to be involved in Nrf2 regulation, playing a role in its phosphorylation<sup>(245)</sup>. In addition, the cross talk between Nrf2 and ATF6 has been demonstrated in endothelial cells<sup>(246)</sup>. The Nrf2/ARE pathway regulates DDAH in order to reduce ADMA and PPAR- $\gamma$  to increase eNOS and its phosphorylation<sup>(247)</sup>. Several studies have reported that Nrf2 acts as a defence mechanism against diabetes-mediated complications. Zhong *et al.*<sup>(248)</sup> reported impairment of Nrf2-Keap1 in endothelial cells that were exposed to high glucose and also in retinas from donors with diabetic retinopathy. *In vivo* models of high-fat-diet Nrf2<sup>-/-</sup> and Nrf2<sup>+/+</sup> mice explained the increase of vascular ROS in Nrf2<sup>-/-</sup> than in Nrf2<sup>+/+</sup> mice and the adaptive activation of the Nrf2-ARE pathway confirming the endothelial protection under diabetes<sup>(249)</sup>. It has also reported that patients with diminished Nrf2 activity had a higher incidence of T2D and cardiovascular problems<sup>(250)</sup>. In STZ-induced diabetic Nrf2 knockdown mice, the diminished oxidative defence causes reduced renal function leading to diabetic nephropathy<sup>(251)</sup>. Activation of insulin signalling through the PI3K/Akt/mTOR (mechanistic target of rapamycin)/Nrf2/GCLC (glutamate-L-cysteine ligase; catalytic subunit) pathway affords significant protection in human brain endothelial cells against hyperglycaemia by maintaining cellular redox balance<sup>(252)</sup>. In addition, the Nrf2 knockout mouse model confirmed the role of Nrf2 in hyperglycaemia-induced ROS production and in cardiomyocytes and renal tissue injury<sup>(253)</sup>. Attention is being drawn to uncover the protective mechanism of polyphenolic compounds against diabetes-mediated endothelial dysfunction via Nrf2 activation.

Resveratrol confers a protective effect against high-glucose-induced oxidative stress in endothelial cells and vasoprotective effect in high-fat-diet mice, through the Nrf2 pathway<sup>(254)</sup>. Sulforaphane decreases the ROS production and restores

myogenic response in T2D mesenteric arterioles through the Nrf2 pathway<sup>(255)</sup>. HUVEC treated with eriodictyol showed the up-regulation of HO-1 through ERK/Nrf2/ARE-dependent pathways, in which ERK aids in the translocation of Nrf2 in the nucleus for HO-1 activation<sup>(256)</sup>.

Moreover, Nrf2 activation inhibits NF- $\kappa$ B through reduced ROS-mediated IKK activation and inhibits the degradation of I $\kappa$ B. Interaction between these two pathways maintains the homeostasis, in which oxidative stress causes the imbalance in the Nrf2-NF- $\kappa$ B axis<sup>(257)</sup>. Sulforaphane also suppressed hyperglycaemia-induced ROS and metabolic dysfunction in human microvascular endothelial cell via Nrf2 activation<sup>(258)</sup>. Sulforaphane has been also reported to enhance Nrf2-mediated antioxidant expression in experimental models of diabetic neuropathy through Nrf2 and NF- $\kappa$ B modulation<sup>(259)</sup>.

The molecular mechanisms of polyphenol antioxidants with emphasis on their potential in improvement of endothelial function are summarised in Table 1.

### Conclusion

Endothelial dysfunction is one of the key contributors to the development of diabetic complications, and it characterised by reduced endothelium-mediated vasorelaxation, impaired fibrinolytic capacity, overproduction of growth factors, increased expression of adhesion molecules and inflammatory genes, excessive generation of free radicals, increased oxidative stress and enhanced permeability of the cell layer. Under hyperglycaemia, the ultrastructure of the extracellular matrix of the endothelial cell tends to alter glucose metabolism and impair insulin signalling, ER stress and inflammation, the major risk factors that contribute to endothelial dysfunction. Numerous therapeutic strategies have been developed for restoring normal endothelial function in diabetic conditions. One such promising approach is the antioxidant therapy; in particular, polyphenols are reported not only to alleviate the status of oxidative stress but also to act on cellular signalling pathways including NO $\cdot$  signalling, VEGF-mediated angiogenesis, ER stress and Nrf2-antioxidant pathway, thereby preventing the vascular complications in diabetes. Hence, future research needs to focus on clinical acceptance of potent polyphenols along with the risk assessment and safety evaluation.

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D. V. L. S. and K. M. R. conceived and designed the study. N. S. and E. B. participated in the development of the database and drafted the paper. All the authors read and approved the final version of the manuscript.

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