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REVIEW

Insights into the molecular mechanisms of diabetes-induced endothelial dysfunction: focus on oxidative stress and endothelial progenitor cells

Mohamed I. Saad[1,2] · Taha M. Abdelkhalek[3] · Moustafa M. Saleh[3] ·
Maher A. Kamel[1] · Mina Youssef[4] · Shady H. Tawfik[5] · Helena Dominguez[6]

Abstract Diabetes mellitus is a heterogeneous, multifactorial, chronic disease characterized by hyperglycemia owing to insulin insufficiency and insulin resistance (IR). Recent epidemiological studies showed that the diabetes epidemic affects 382 million people worldwide in 2013, and this figure is expected to be 600 million people by 2035. Diabetes is associated with microvascular and macrovascular complications resulting in accelerated endothelial dysfunction (ED), atherosclerosis, and cardiovascular disease (CVD). Unfortunately, the complex pathophysiology of diabetic cardiovascular damage is not fully understood. Therefore, there is a clear need to better understand the molecular pathophysiology of ED in diabetes, and consequently, better treatment options and novel efficacious therapies could be identified. In the light of recent extensive research, we re-investigate the association between diabetes-associated metabolic disturbances (IR, subclinical inflammation, dyslipidemia, hyperglycemia, dysregulated production of adipokines, defective incretin and gut hormones production/action, and oxidative stress) and ED, focusing on oxidative stress and endothelial progenitor cells (EPCs). In addition, we re-emphasize that oxidative stress is the final common pathway that transduces signals from other conditions—either directly or indirectly—leading to ED and CVD.

Keywords Diabetes · Endothelial dysfunction · Endothelial progenitor cells · Oxidative stress

✉ Mohamed I. Saad
  m.ibrahim1988@hotmail.com

1 Department of Biochemistry, Medical Research Institute, Alexandria University, Alexandria, Egypt

2 Hudson Institute of Medical Research, School of Clinical Sciences, Monash University, Melbourne, VIC, Australia

3 Department of Human Genetics, Medical Research Institute, Alexandria University, Alexandria, Egypt

4 Department of Human Genetics, McGill University, Montreal, QC, Canada

5 Department of Molecular Medicine, University of Padova, Padua, Italy

6 Department of Biomedical Sciences, Copenhagen University, Copenhagen, Denmark

Abbreviations

ACh	Acetylcholine
ADMA	Asymmetric dimethylarginine
AMPK	Adenosine monophosphate-dependent kinase
AGEs	Advanced glycation end products
AG II	Angiotensin II
AR	Aldose reductase
ARI	Aldose reductase inhibitors
BH_4	Tetrahydrobiopterin
BAD	Bcl-2-associated death promoter
CRP	C-reactive protein
CXCR-4	Chemokine receptor type-4
CVD	Cardiovascular disease
cGMP	Cyclic guanosine monophosphate
COX	Cyclooxygenase
CMKLR1	Chemokine-like receptor 1
DPP-4	Dipeptidyl-peptidase-4
DAG	Diacyl glycerol
ET-1	Endothelin-1

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ED	Endothelial dysfunction	NIK	NF-kB inducing kinase
DDAH	Dimethylarginine dimethylaminohydrolase	Nampt	Nicotinamide phosphoribosyltransferase
ECs	Endothelial cells	PTEN	Phosphatase and tensin homolog
EPCs	Endothelial progenitor cells	PK	Protein kinase
ERK	Extracellar signal-regulated kinases	PI$_3$K/Akt	Phosphatidylinositol triphosphate kinase/Akt (protein kinase B)
EDHF	Endothelium-derived hyperpolarizing factor	PKC	Protein kinase C
eNOS	Endothelial NO synthase	PTPs	Protein tyrosine phosphatases
FAD	Flavin adenine dinucleotide	PON-1	Paraoxonase-1
FMN	Flavin mononucleotide	PGI$_2$	Prostacyclin
FOXO3a	Forkhead transcription factor3a	PSGL	P-selectin glycoprotein ligand
GTP	Guanosine triphosphate	PPARγ	Peroxisome proliferator activated receptor
GC	Guanylate cyclase	PARP	Poly (ADP-ribose) polymerase
GSH	Glutathione	PDGF	Platelet-derived growth factor
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	PLC	Phospholipase C
		PAI	Plasminogen activator inhibitor
GA-3P	Glyceraldehyde-3-phosphate	PGC	Peroxisome proliferator activated receptor γ coactivator
G-6-Pase	Glucose-6-phosphatase		
GIP	Glucose-dependent insulinotropic polypeptide	PEPCK	Phosphoenolpyruvate carboxykinase
		ROS	Reactive oxygen species
GLP-1	Glucagon-like peptide-1	RAGE	Receptors for advanced glycated end products
HDL	High density lipoprotein		
HIF-1α	Hypoxia-inducible factor-1α	STAT	Signal transducer and activator of transcription
HFD	High fat diet		
HUVECs	Human umbilical vein endothelial cells	SDF-1	Stromal cell-derived factor-1
HO-1	Heme oxygenase-1	S1P	Sphingosine-1-phosphate
HSP	Hexosamine synthetic pathway	SK	Sphingosine kinase
HSL	Hormone sensitive lipase	SOD	Superoxide dismutase
IR	Insulin resistance	SDH	Sorbitol dehydrogenase
IKK	Inhibitor of κB kinase	SOCS-3	Supressor of cytokine signaling 3
IRS	Insulin receptor substrate	SHP	Src-homology-2 domain-containing phosphatase
IGF-I	Insulin growth factor		
ICAM-1	Intercellular adhesion molecule-1	T1DM	Type 1 diabetes mellitus
JNK	c-Jun N-terminal kinases	T2DM	Type 2 diabetes mellitus
JAM-A	Junction adhesion molecule A	TNF-α	Tumor necrosis factor-α
LDL	Low density lipoprotein	TNFR	Tumor necrosis factor receptor
L-NAME	Nitro-L-arginine methyl ester	TRAF	TNFR-associated factor
LOX-1	Lecithin-like ox-LDL receptor-1	TRADD	TNF receptor-associated death domain
LC	Lactosyl ceramide	TGF	Transforming growth factor
MMP-9	Matrix metallopeptidase-9	TXA$_2$	Thromboxane A$_2$
MAPK	Mitogen activated protein kinase	tPA	Tissue plasminogen activator
MCP-1	Monocyte chemotactic protein-1	UDP-GlcNAc	UDP-N-acetylglucosamine
MnSOD	Mn superoxide dismutase	UCP	Uncoupling protein
MPO	Myeloperoxidase	VLDL	Very low density lipoprotein
MDA	Malondialdehyde	VSMCs	Vascular smooth muscle cells
NF-κB	Nuclear factor κB	VEGF	Vascular endothelial growth factor
NO	Nitric oxide	VCAM	Vascular cell adhesion molecule
NEFA	Non-esterified fatty acids	XRCC1	X-ray repair cross-complementing protein-1
Nox	NADPH oxidase		
NADPH	Reduced nicotinamide adenine dinucleotide phosphate	ZO	Zucker obese rats
		ZL	Zucker lean rats

Introduction

Diabetes mellitus is a heterogeneous, multifactorial, chronic disease characterized mainly by hyperglycemia owing to insulin insufficiency (due to partial or complete β-cell defect) and insulin resistance (IR) (inability of insulin to exert its actions on tissues). Type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are the most common forms of diabetes [1]. Recent epidemiological studies showed that the diabetes epidemic affects 382 million people globally in 2013, and this figure is expected to be 600 million people by 2035 [2].

There are many key players which contribute to diabetes pathophysiology: (1) β-cell dysfunction, (2) IR (defects of insulin signaling) in brain, skeletal muscles, and liver, (3) accelerated lipolysis resulting in increased production of non-esterified fatty acids (NEFA), (4) dysregulated production of adipokines, (5) dysregulated action/production of incretin and gut hormones, (6) hyperglucagonemia, (**7**) subclinical inflammation through production of proinflammatory mediators, (8) increased glucose production and reabsorption from the kidneys, and (9) oxidative stress and excessive production of reactive oxygen species (ROS) [1, 3–5] (Fig. 1).

Diabetes is associated with microvascular complications (e.g., retinopathy, nephropathy, and neuropathy) and macrovascular complications, culminating in accelerated endothelial dysfunction (ED), atherosclerosis, and cardiovascular disease (CVD) e.g., coronary artery, cerebrovascular, and peripheral vascular diseases [6, 7]. CVD accounts for more than 65 % of all deaths in T2DM [8]. The CVD mortality risk is almost two fold as higher in T2DM cases compared with age-matched control. T2DM increases the risk of coronary and peripheral artery diseases by 2–4 fold and increases the risk of stroke by 10 fold in individuals younger than 55 years old [8]. Unfortunately, the complex pathophysiology of diabetic cardiovascular damage is not completely understood.

The endothelium

The endothelium is a monolayer of endothelial cells (ECs) lining the lumen of all body vasculature. It is now considered a pivotal metabolically active, homeostatic organ which regulates the tone and structure of the blood vessels in response to different stimuli such as shear stress and agonists [e.g., acetylcholine (ACh) and insulin]. Disturbing
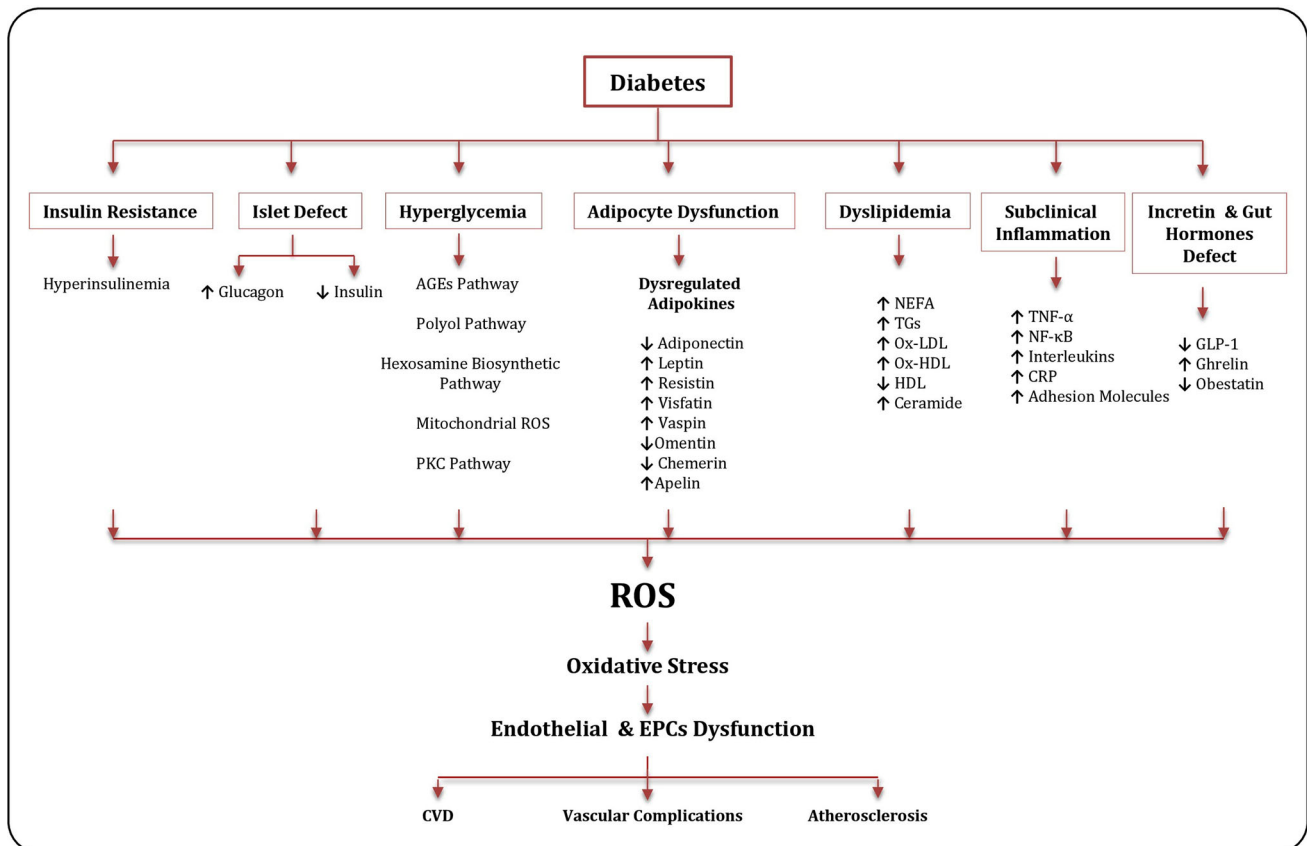

Fig. 1 General pathophysiological pathways contributing to the development of diabetic cardiovascular diseases

the tightly controlled balance between the vasodilating [e.g., nitric oxide (NO) and prostacyclin (PGI$_2$)] and vasoconstricting [e.g., endothelin-1 (ET-1) and thromboxane A$_2$ (TXA$_2$)] factors leads to ED. ED is the fundamental trigger of pathophysiological pathways which eventually culminates in atherosclerosis, CVD, and vascular diabetic complications [9–11].

The most important autacoid produced by the ECs is NO. Impaired NO bioavailability is the hallmark of ED. NO is generated from L-arginine by endothelial NO synthase (eNOS), an enzyme which is constitutively expressed in ECs (Fig. 2). NO bioavailability depends on the balance between the rate of its generation by eNOS and its inactivation particularly by ROS [9, 10]. Moreover, NO-mediated signaling pathways could stimulate mobilization of EPCs from bone marrow stem cell niches to the blood circulation [12].

The exact definition of EPCs is controversial due to the absence of unique marker or combination of markers to identify and characterize progenitor cells that differentiate into ECs in vitro and in vivo [13]. A myriad of cells with complex surface markers and different phenotypes has

been referred to as EPCs including colony forming unit-Hill (CFU-Hill) EPCs, circulating angiogenic cells (CACs), and endothelial colony forming cells (ECFCs) [14]. EPCs maintain endothelial integrity through mediating the processes of repair and regeneration for ECs injury [15]. Characterization of EPCs is done by three methods: (1) functional assays that evaluate their adhesion to fibronectin, secretion of growth factors, and ability to correct ischemia in animal models, (2) expression of EPCs-specific surface markers CD34+, KDR+ (Flk-1+ in mice), and CD133, and (3) the ability to form colonies in vitro (stemness) [16]. EPCs contribute to vascular repair by two main mechanisms: (1) EPCs proliferate to regenerate new ECs and (2) EPCs release cytokines and growth factors to stimulate proliferation of resident ECs and other EPCs [17]. The vasculogenic function of EPCs under hypoxic conditions is mediated by hypoxia-inducible factor-1α (HIF-1α) activation of chemokine receptor type-4 (CXCR-4) expression [18]. Moreover, HIF-1α induces expression of stromal cell-derived factor-1 (SDF-1) in ischemic ECs in a hypoxia-dependent manner. The gradient of SDF-1 concentration in ischemic tissue and its interaction with CXCR-
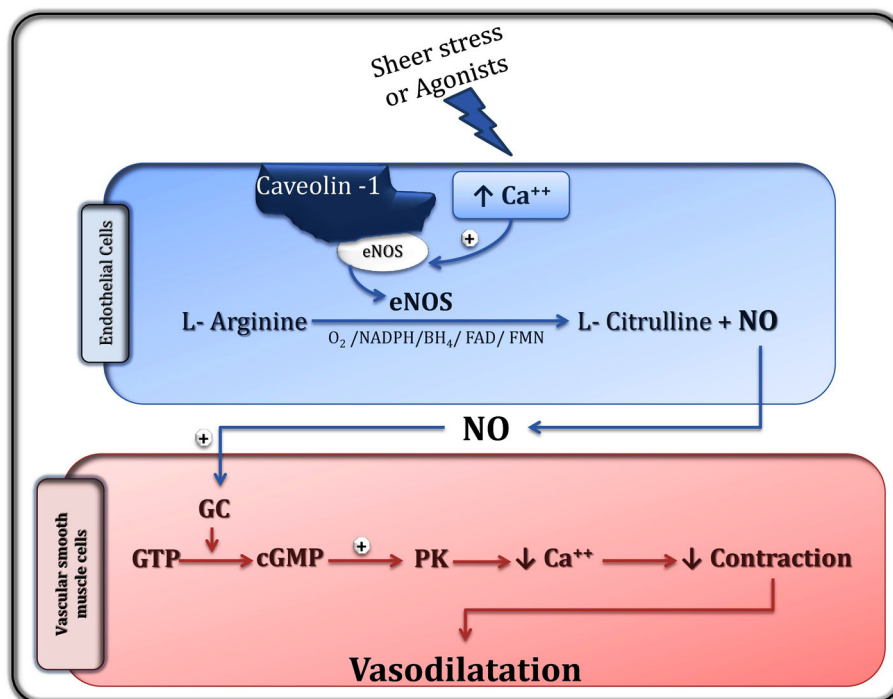

Fig. 2 Biosynthesis and physiological role of nitric oxide in endothelial and vascular smooth muscle cells. NO is generated from L-arginine by endothelial NO synthase (eNOS), an enzyme which is constitutively expressed in ECs. O$_2$, reduced nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and tetrahydrobiopterin (BH$_4$) are required as cofactors for this reaction. Under normal physiological conditions, stimulation of ECs induces elevation of intracellular Ca^{2+} concentration which binds to calmodulin, thereby releasing eNOS from the caveolar protein caveolin-1, which inhibits eNOS activity. NO diffuses to the surrounding cells, exerting its cardiovascular protective action. NO activates guanylate cyclase (GC) enzyme which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). cGMP enhances the activity of a cGMP-dependent protein kinase (PK), leading to decreased intracellular Ca^{2+} concentration, which eventually inhibits the contractile machinery in the vascular smooth muscle cells (VSMCs) followed by vasodilatation

4 receptors on EPCs drives migration, homing, and incorporation of EPCs in ischemic tissue for vascular repair [19].

Low levels of circulating EPCs are associated with ED [20]. Decreased EPCs levels in diabetic patients (both T1DM and T2DM) are also implicated in the pathogenesis of vascular complications [21, 22]. Therefore, EPCs could independently predict endothelial function and, consequently, be used for assessing microvascular ED [23]. However, Lombardo et al. demonstrated that T2DM affects the maturation and commitment of EPCs rather than affecting their production and mobilization processes [24].

Oxidative stress and endothelial dysfunction

A common feature of all free radicals is destruction of macromolecules such as proteins and DNA, the latter being of utmost importance because introducing permanent mutations in genetic material stymies normal function of the cell [25].

Oxidative stress in endothelial cells

Oxidative stress activates forkhead transcription factor (FOXO1) that increases the transcription of inducible NOS and disturbs eNOS function [26]. FOXO1 is essential for expression of cell adhesion molecules and activation of inflammatory mediators in ECs. Furthermore, FOXO1 promotes ECs senescence and significantly stymies their proliferation by its effect on pro-apoptotic proteins (Bim) and cell cycle inhibitors such as p53 and p21 [27]. Moreover, H_2O_2 induces p53 expression in Human umbilical vein endothelial cells (HUVEC), which is known to inhibit cellular proliferation.

The effect of ROS on the activation of certain pathways by transforming growth factor (TGF-β) was demonstrated in two types of cultured ECs. The use of anti-oxidants and their subsequent downstream effects attenuates the activation/ phosphorylation of p38 mitogen activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) pathways by TGF-β, thus inhibiting ECs apoptosis [28]. Uncouplers of oxidative phosphorylation decrease ROS production in mitochondria which inhibit inhibitor of kappa B (IκB) phosphorylation, inciting a state of resistance to tumor necrosis factor-α (TNF-α) activation of Nuclear factor κB (NF-κB) signaling pathway and inflammation in ECs [29]. Moreover, H_2O_2 and peroxynitrite-mediated activation of poly (ADP-ribose) polymerase (PARP-1) depletes cellular ATP and NAD^+ that is associated with ECs apoptosis. PARP-1 prevents phosphorylation of Bcl-2-associated death promoter (BAD), thereby inhibiting its pro-apoptotic effects by hindering its association with the multifunctional phosphoserine-binding protein 14-3-3 [30]. Hyperglycemia-induced

Reactive nitrogen species (RNS) promote apoptosis, which is mediated by JNK phosphorylation that increases pro-apoptotic Bax and increases cleavage of caspase-3, leading to ECs apoptosis [31]. H_2O_2 induces dephosphorylation of β-catenin mediated by p66shc, leading to activated downstream Wnt signaling. This leads to ED via decreased NO bioavailability, decreased vasorelaxation induced by ACh, increased TNF-α expression, and monocyte adhesion to ECs [32].

Oxidative stress in endothelial progenitor cells

Despite the discrepancy regarding the anti-oxidant capacity of EPCs among researchers, there is a consensus on the pivotal damaging effect of oxidative stress on EPCs functions [33]. Early-stage EPCs do not exhibit increased ROS in diabetes, and this may be due to increased expression of anti-oxidant enzymes such as catalase in diabetic EPCs [34]. H_2O_2 significantly decreases EPCs viability, increases apoptosis, and impairs tube formation. These effects are mediated by the increased expression of FOXO3a, which has high basal expression in EPCs and the subsequent activation of its downstream targets such as the pro-apoptotic protein Bim [35]. H_2O_2 induces EPCs apoptosis in a dose-dependent manner, which is inhibited by SIRT1-induced deacetylation of FOXO3a, thereby increasing FOXO3a ubiquitination and proteasomal degradation [35]. Oxidized low density lipoprotein (ox-LDL) induced activation of Akt/p53/p21 signaling pathway is associated with accelerated EPCs senescence in diabetic patients [36].

H_2O_2 induces oxidation of sulfhydryl groups of multiple anti-oxidant proteins such as glutaredoxin and thioredoxin, leading to activation of apoptosis signal-regulating kinase 1 [37], which induces EPCs apoptosis [38]. Furthermore, H_2O_2 induces oxidation of pivotal EPCs proteins such as the T-complex protein 1 subunit α, isoform A of prelamin-A/C, cofilin-1, peroxiredoxin-4, and actin [39]. Despite the relevance of these data in elucidation of ROS-induced impairment of EPCs function, no studies have explored their mechanistic roles in diabetes EPCs dysfunction.

Insulin resistance (IR) and endothelial dysfunction

Insulin resistance-induced ROS

Insulin, a hormone with pleiotropic metabolic and vascular effects such as vasodilation through generation of NO by activation of phosphatidylinositol triphosphate kinase/protein kinase B (PI$_3$K/Akt) [40], as well as vasoconstriction via MAPK-induced production of ET-1 [41]. IR is characterized by pathway specific impairment of insulin signaling where PI$_3$K/Akt signaling is impaired with reduced NO formation [42], meanwhile MAPK/extracellular signal-

regulated kinase (ERK) signaling is intact [42] and even enhanced due to hyperinsulinemia, which leads to increased ET-1 production [43] and enhanced proliferation of vascular smooth muscle cells (VSMCs) [44].

Insulin-induced eNOS-Ser1177 phosphorylation/activation is diminished in Zucker Obese (ZO) compared to Zucker Lean (ZL) rats, which explains reduced NO synthesis in ZO rats [45]. Therefore, reduced NO generation will hypothetically lessen NO-mediated ROS neutralization. Inhibition of eNOS activity by nitro-L-arginine methyl ester (L-NAME) paradoxically decreases ROS generation, implicating the role of insulin in induction of eNOS uncoupling, which enhances superoxide production and contributes to oxidative stress [46].

Of note here, superoxide is produced by eNOS as a natural intermediate under physiological conditions but is not released and coupled to NADPH consumption [47]. This effect is mediated by BH_4, which stabilizes eNOS dimers and prevents the dissociation of ferrous dioxygen complex of the enzyme's oxygenase domain. Uncoupled eNOS mainly generates superoxide due to the dissociation of ferrous dioxygen complex [48]. Interestingly, ROS produced by uncoupled eNOS oxidize BH_4 into dihydrobiopterin (BH_2), reducing BH_4 bioavailability and competing with it on active sites. This induces eNOS uncoupling and further production of ROS in a vicious cycle [49].

Insulin promotes the phosphorylation and translocation of p47^{phox} subunit of NADPH oxidase from cytosol to membrane, through activation of the PI_3K/protein kinase C (PKC) signaling pathway, hence provoking superoxide generation [50]. Moreover, inhibition of Nox2 in macrophages by RNA interference prevents insulin-stimulated superoxide production, while PKC inhibition prevents the insulin-induced superoxide production without affecting the phosphorylation of Akt [50].

Both Nox2 and Nox4 are expressed in ECs [51–53]. Indeed, insulin stimulates NADPH-dependent superoxide production in fibroblasts, smooth muscle cells, and adipocytes [54–56]. Insulin-treated Sprague–Dawley rats have increased superoxide production in vascular ECs of aortic segments as measured by lucigenin-enhanced chemiluminescence method [57].

Insulin effect on EPCs

Murphy et al. investigated the increased coronary artery disease risk in UK South Asians compared to Caucasians. They attributed this increased risk to the fact that South Asians were more insulin resistant, showing ED as well as reduced EPCs numbers and function [58].

The potential for insulin to affect the mobilization and differentiation of EPCs directly is not known. For example, a study of patients with T2DM has demonstrated that insulin stimulates the outgrowth of EPCs in vitro, and this effect was mediated by insulin growth factor (IGF-I) receptor stimulation of MAPKs and ERK1/2 but not insulin receptors [59]. Therefore, insulin presumably induces proliferation and differentiation of EPCs. However, recent study demonstrates that haploinsufficiency of IGF1-receptor enhances their phenotype despite producing fewer yields of EPCs. These angiogenic EPCs showed accelerated endothelial regeneration in vivo and enhanced tube forming and adhesive potential of angiogenic progenitor cells in vitro as well as overall enhanced vascular repair [60]. In a small study of patients with poorly controlled T2DM, insulin-mediated EPCs mobilization was significantly enhanced in subjects with SDF-1 3′-A/G allele, a polymorphism known to be associated with increased EPCs mobilization [61].

Mouse models heterozygous for insulin receptor knockout (IRKO) are non-diabetic but are characterized by ED and reduced EPCs numbers and function. When IRKO mice crossed with transgenic mice with Tie-2-driven human IR expression in ECs (HIRECO), the offspring is considered to have restored insulin signaling in ECs through insulin receptors. HIRECO × IRKO offspring show improved blood pressure, endothelial function, NO bioavailability, and vascular repair in the setting of global IR. This is not related to glucoregulation or EPCs abundance [62].

Insulin resistant, non-diabetic mice hemizygous for IRKO have a defect in EPCs mobilization manifested as lower numbers of circulating EPCs in peripheral blood but not in bone marrow compared with wild-type mice [63]. Moreover, after arterial injury, endothelial regeneration was delayed in IRKO mice and transfusion of mononuclear cells or c-kit + bone marrow cells from wild-type mice restored endothelial regeneration in IRKO mice [63]. EPCs in diabetic patients have reduced clonogenicity and uncoupled eNOS mediated by ROS, which further contribute to more oxidative stress and impaired vascular repair [22].

Inflammation and endothelial dysfunction

Subclinical inflammation is a hallmark sign of metabolic syndrome and T2DM. Systemic inflammation in these conditions is characterized by elevated levels of C-reactive protein (CRP), TNF-α, and many cytokines, most notable of them are interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-18 (IL-18) [64, 65]. These factors interact with different receptors that converge in their final mediators that increase oxidative stress and activate NF-κB in ECs, which eventually result in ED.

C-reactive protein (CRP)

CRP levels are significantly elevated in T1DM and T2DM patients [66, 67]. Furthermore, elevated CRP levels in

healthy individuals are highly correlated with markers of metabolic syndrome [66] and could be predictors for the risk of development of T2DM in various populations [68, 69]. As well, CRP has been proven to induce ED in cultured ECs [70]. The role of CRP in ED is related to the presence of other agents such as ox-LDL. In addition, CRP interacts with lecithin-like ox-LDL receptor-1 (LOX-1) and complement proteins to induce inflammation in cultured ECs [71].

The effects of CRP are also mediated by the IgG Fc receptor (FcγRIIB). CRP abolishes insulin activation of eNOS by inhibiting Akt phosphorylation at Ser473 [72]. Furthermore, CRP interaction with FcγRIIB receptor reduces protein nitrosylation in ECs and activates NF-κB pathway by reducing the inhibitory p65 S-nitrosylation. Those effects are also dependent on FcγRII receptors [73]. However, other studies have shown that NF-κB activation is mediated by activation of ERK1/2 pathway, but the exact receptors by which CRP mediates this effect is not elucidated. Also, CRP increases ROS generation by a mechanism which is independent on NADPH oxidase [74].

The effect of CRP on EPCs was also investigated by Chen and colleagues. CRP significantly disrupts EPCs migration, adhesiveness and proliferation. Furthermore, CRP abolishes eNOS expression in EPCs and increases EPCs apoptosis. Surprisingly, all these effects are shown to be mediated through receptors for advanced glycation end products (RAGE) [75]. Fujii et al. reported similar results where CRP instigates apoptosis and necrosis in EPCs. Furthermore, the culprit was shown to be the increase in mitochondrial ROS generation that was aggravated by CRP effect in modulating the expression of the anti-oxidant enzymes glutathione peroxidase and Mn superoxide dismutase (MnSOD) [76]. However, recent work by Fasing et al. showed no association between CRP plasma level and circulating EPCs [77].

Interleukins

IL-1, IL-6, and IL-18 are among the most notable interleukins in diabetic inflammation [65]. IL-1β decreases the expression of PI$_3$K, eNOS, paraoxonase-1 (PON-1) (antioxidant gene), and other genes involved in adenosine monophosphate-dependent kinase (AMPK) upstream signaling. Also, IL-1β increases expression of adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and monocyte chemotactic protein-1 (MCP-1) in cultured ECs. These effects are attenuated by β-carotene [78]. Vallejo et al. demonstrated that IL-1β induces ED in diabetic rat model by activating NADPH oxidase. This effect was partially prevented by incubation of the isolated blood vessels with Apocynin (NADPH oxidase inhibitor). Also, normal endothelial function was restored by incubation

with a NADPH oxidase, cyclooxygenase-2 (COX-2), eNOS inhibitors, and superoxide dismutase [79], proving the role of oxidative stress in IL-1β-induced ED.

Moreover, IL-1β induces murine EPCs viability, proliferation, and migration both in vivo and in vitro, an effect which is associated with ERK1/2 pathway activation [80]. Also, IL-1β increases mRNA and protein levels of vascular endothelial growth factor (VEGF)-A. Intriguingly, these observed effects of IL-1β on EPCs are mediated via the PI$_3$K/Akt signaling pathway [81]. On the contrary, Mao et al. showed that IL-1β significantly reduces the number and proliferation of pig EPCs. Moreover, IL-1β, through p38 MAPK pathway activation, reduces EPCs migration, adhesion, and angiogenic functions [82].

In addition, serum levels of IL-6, IL-10, and IL-17 are significantly elevated in T2DM patients [83]. Furthermore, IL-18 and IL-12 are associated with T2DM risk and ED in T2DM, respectively [84, 85]. IL-18 induces inflammation in ECs and increases the expression of vascular cell adhesion molecule (VCAM-1), a process that is mediated by PKCβ activity [86]. Moreover, endogenous inhibitor of IL-18 (IL-18BP) is downregulated in aortic ECs from diabetic animals by PKCβ heightened activity [86]. Furthermore, IL-18 suppresses Akt phosphorylation and activates IκB kinase (IKK), resulting in activation of NF-κB. [87]. Regarding the effect of IL-18 on EPCs, Kahlenberg et al. showed that IL-18 reduces the ability of EPCs from healthy individuals to differentiate into mature ECs [88].

IL-6 induces IR in ECs by inhibiting phosphorylation of Tyr612 in insulin receptor substrate-1 (IRS-1), thus inhibiting the IRS-1 downstream signals of PI$_3$K/Akt/eNOS [87, 89]. IL-6 inhibits phosphorylation/activation of AMPK at Thr172 in ECs regardless of insulin presence [90]. On the other hand, Andreozzi et al. presented compelling evidence that IL-6 inhibits IRS-1 via inhibitory phosphorylation on Ser312 and Ser616 through activation of JNK/signal transducer and activator of transcription-3 (STAT3) and ERK1/2 pathways using different experimental settings [91]. In addition, IL-6 enhances EPCs migration, proliferation, and differentiation in cell culture. It also activates both JNK/STAT3 pathway and ERK1/2 pathway [92].

Intriguingly, IL-10 exerts beneficial effects on ECs. IL-10 inhibits ox-LDL-induced ECs apoptosis via inhibition of ox-LDL-mediated activation of p38 MAPK and JNK. This effect was mediated by suppressor of cytokine signaling-3 (SOCS3), which is upregulated by IL-10 [93]. Moreover, IL-10 inhibits the insulting effects of TNF-α on ECs and reduces ICAM-1 expression as well as ROS generation through PI$_3$K activation [94]. Furthermore, IL-10 alone has no effect on EPCs migration and differentiation. However, it significantly increases the expression of VEGF and matrix metallopeptidase-9 (MMP-9) and potentiates the detrimental effects of TNF-α on EPCs [95].

Tumor necrosis factor-α (TNF-α)

TNF-α is a cytokine that has a myriad of functions such as mediating apoptosis, regulation of immunity, and hematopoiesis as well as contributing to the pathophysiology of many diseases. TNF-α exerts its effects among various cells via its action on its TNFR1 and TNFR2 receptors. TNFR1 is expressed ubiquitously and is present in different types of cells; on the other hand, TNFR2 is only expressed in few cell types including ECs [96]. TNFR1 activates NF-κB via the canonical pathway which is dependent on the activation of the IKK complex, which phosphorylates IκB and subsequently results in its proteasomal degradation. This is mediated by the downstream effectors of TNFR1: TNF receptor-associated death domain (TRADD) and TNFR-associated factor 2 (TRAF2) [97, 98]. Moreover, TNFR2 activates non-canonical NF-κB activation by preventing proteasomal degradation of NF-κB-inducing kinase (NIK), which activates IKKα, resulting in an activation of certain isoforms of NF-κB [99].

TNF-α serum level is elevated in T1DM and T2DM and is associated with various complications of diabetes [100, 101]. Studies conducted on Lepr diabetic mice and ZO diabetic rats showed that TNF-α mediates ED by increasing ROS generation via NADPH oxidase, an effect that was related to NF-κB activation [102–104]. Moreover, TNF-α increases the expression of genes encoding Nox2 subunits (p22^{phox} and p40^{phox}) in coronary artery ECs [104]. In cerebral microvascular ECs, TNF-α induces ROS generation mainly via Nox4 [105]. However, recent work conducted on cultured human microvascular ECs showed that TNF-α does not alter the expression of Nox2, p22, and p67, while inducing downexpression of Nox4 [106]. TNF-α mediates ROS production mainly via NADPH oxidase and to a lesser extent by the mitochondria [107]. Also, TNF-α stimulates ROS production in ECs by activating p47 [108] and enhancing its localization as well as the associated subunits p67 and Rac1 to the cell membrane [109, 110]. TNF-α activates p47 phosphorylation at Ser379 by PKC which induces a conformational change that allows binding with both p22 and TRAF4, and stimulates its membrane translocation as well as the downstream activation of ERK1/2, p38 MAPK, and JNK [111]. Furthermore, TNF-α antagonist (infliximab) was successful in alleviating ED in metabolic syndrome patients [112]. However, other studies using different TNF-α antagonist (etanercept) failed to show similar results [113].

TNF-α induces expression of IL-18 which has deleterious effects on EPCs differentiation. TNF-α reduces Akt phosphorylation mediated by insulin and increases EPCs apoptosis. These effects are mediated through NF-κB activation [114]. Additionally, TNF-α inhibits EPCs migration and proliferation in a dose and time-dependent manner. TNF-α mediates downexpression of VEGF-receptor 1 and SDF-1 as well as the inducible and endothelial forms of NOS [115]. Interestingly, some studies have shown that TNF-α enhances EPCs migration, adhesion, and tubule formation [95], but these results were not replicated by other researchers.

Nuclear factor kappa B (NF-κB)

NF-κB is a family of transcription factors highly conserved in several species. Cytoplasmic NF-κB molecules have an inhibitor component that hinders their nuclear localization required for their actions. These inhibitor components are termed inhibitor of kappa B (IκB) and include IκBα, β, and ε as well as the products of precursor proteins p105 and p100 termed IκBγ and IκBδ [116]. The canonical pathway is usually activated by pro-inflammatory cytokines and is dependent upon IKK complex. The non-canonical pathway is mediated solely by IKKα [117].

Sustained activation of NF-κB is a characteristic sign observed in diabetic patients and animal models [118]. Endothelial constitutive inhibition of NF-κB in mice models attenuates age-related changes in ECs as well as decreases and increases the expression of cellular adhesion molecules and eNOS, respectively [119]. Pharmacological inhibition of NF-κB by Salsalate significantly inhibits p65 nuclear translocation, p47 NADPH oxidase subunit expression, and nitrotyrosine levels in cultured HUVECs. Furthermore, Salsalate ameliorates ROS-inhibited vasodilation in a manner similar to that mediated by high dose of anti-oxidant (vitamin C) [120]. These experiments suggest that NF-κB mediates its effects via increasing ECs oxidative stress. However, some reports have demonstrated that NF-κB is activated by ROS [121] and protects ECs from TNF-α-induced apoptosis [122]. In experiments using constitutively inhibited NF-κB [123] and overexpression of IκBα [122], TNF-α stimulation of cultured ECs results in more apoptotic cells. Consistently, IκB decreases the activation of the anti-oxidant/anti-apoptotic heme oxygenase-1 (HO-1) enzyme in cerebral ECs [122].

Ephemeral but stable overexpression of NF-κB in murine embryonic EPCs does not impair migration or vasculogenesis. Moreover, NF-κB overexpression enhances EPCs adherence to endothelium through increasing the expression of adhesion molecules E-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) [124]. In insulin resistant EPCs isolated from ZO rats and stimulated by TNF-α, NF-κB induces apoptosis via caspase-3 [114]. Nonetheless, damage induced by some environmental toxins such as Benzo[a]pyrene on EPCs is mediated by activation of NF-κB which increases ROS production and impairs EPCs migration, proliferation, and vasculogenic activities [125].

Dyslipidemia and endothelial dysfunction

Cholesterol and modified cholesterol

Oxidized LDL (Ox-LDL) particles are LDL lipoproteins that have their protein and/or lipid component oxidatively modified [126]. Ox-LDL blood levels are elevated in T2DM patients and obese individuals [127]. Ox-LDL is considered a prerequisite for the development of atherosclerosis and therefore, is a major contributor to CVD [128]. The main culprit in the generation of ox-LDL is the oxidative stress generated by increased induction of iNOS by FOXO1 [129]. Oxidized and glycated oxidized LDL inhibit aortic dilatation induced by ACh, an effect that is induced by increased ROS generation [26]. Ox-LDL phosphorylates/activates p66^{shc}—an adaptor protein that functions as a redox enzyme—via its activation of LOX-1 receptor [130]. Ox-LDL increases ROS generation in human arterial ECs via activation of p66^{shc} by Ser36 phosphorylation and eNOS uncoupling [131]. Downstream effects of p66^{shc} activation involve PKCβ2 phosphorylation at Thr641 and Ser660, JNK phosphorylation, and increased expression of p47^{phox} [130].

Furthermore, recent work showed that ox-LDL mediates vascular dysfunction by allosteric activation of angiotensin I receptors in ECs. This allosteric activation is mediated by ox-LDL binding to LOX-1 receptor, resulting in increased phosphorylation/activation of ERK1/2, which is a known activator of NF-κB [132]. Ox-LDL obtained from human subjects increases ROS generation, TNF-α expression and NF-κB activation in cultured ECs through binding to LOX-1 [133].

Ox-LDL induces dysfunction of cultured EPCs isolated from healthy subjects [134]. Ox-LDL induces EPCs senescence and inhibits VEGF-mediated differentiation via LOX-1 receptors. Furthermore, ox-LDL increases expression of LOX-1 mRNA in EPCs [135]. Ox-LDL reduces the number of viable EPCs in culture and inhibits their migration and adhesion activities [136, 137]. These effects were shown to be either mediated by both NADPH oxidase and NF-κB activation, or activation of LOX-1 and its subsequent inhibition of Akt/eNOS pathway [136, 137].

Oxidized HDL (ox-HDL) particles exhibit dysfunction in their anti-inflammatory and anti-oxidant activities [138]. Blood levels of ox-HDL and myeloperoxidase (MPO) enzyme are elevated in T2DM patients [127]. Interestingly, HDL particles isolated from diabetic patients are dysfunctional in their ability to induce ECs migration, proliferation, and adhesion compared to HDL isolated from normal healthy patients [139]. Ox-HDL particles lose their ability to attenuate TNF-α inflammatory effect and, surprisingly, activate NF-κB in cultured ECs via activation of IKK complex [140]. Ox-HDL increases ROS generation in cultured ECs via its activation of NADPH oxidase. This effect is greatly attenuated by pretreatment of cultured cells with LOX-1 antibody, suggesting a possible interaction between LOX-1 and ox-HDL [141].

Administration of reconstituted HDL to T2DM patients ameliorates the decline in circulating EPCs [142]. In addition, treatment of cultured EPCs with HDL induces their proliferation and protects them from apoptosis. It also increases their migration, adhesion, and tube formation [143]. Furthermore, HDL protects EPCs from the deleterious effects of ox-LDL. However, high concentration of HDL (>400 μgm/ml) induces EPCs senescence and impairs their tube formation capability via activation of Rho kinase that inhibits Akt and p38 MAPK activation [144]. On the other hand, ox-HDL stimulates EPCs apoptosis in a dose-dependent manner, depending on the surface receptor CD36. In addition, ox-HDL interaction with CD36 increases the activity of NADPH oxidase, upregulates Nox2 mRNA (NADPH oxidase subunit), and activates MAPK/NF-κB pathway [145].

Ceramide and sphingolipids

Ceramide is a major sphingolipid that lies in the crossroad of various degradation and synthesis pathways of sphingolipids. Inhibition of ceramide de novo synthesis ameliorates IR in diabetic mice models [146].

Ceramide accumulation in arteries caused ED in high fat diet (HFD) mice, an effect that is mediated by enhancing the association of protein phosphatase 2A (PP2A) with eNOS, resulting in dephosphorylation of eNOS active form. Furthermore, the effect of ceramide on eNOS activity is not associated with AMPK, Akt, or ERK1/2 phosphorylation [147]. Therefore, inhibition of de novo synthesis of ceramide attenuates ED in HFD mice models [148, 149]. Ceramide increases NADPH oxidase activity and mitochondrial ROS generation in ECs [150]. Ceramide also potentiates the ROS-generating effect of palmitate in cultured bovine aortic ECs in a dose-dependent manner [147].

Additionally, palmitate-induced ED was shown to be mediated by ceramide accumulation in EPCs. Ceramide accumulation inhibits EPCs proliferation, migration, and tube formation via its inhibition of Akt/eNOS pathway [151].

Non-esterified fatty acids (NEFA)

NEFA induce apoptosis and decrease proliferation in cultured ECs [152]. Palmitic and linoleic acids inhibit phosphorylation/activation of eNOS both in basal and insulin-stimulated conditions. However, only palmitic acid inhibits PI$_3$K/Akt/eNOS pathway by inducing expression of PTEN

[153]. NEFA induce monocyte adhesion to cultured ECs, which is an essential step in endothelial inflammation [154].

Stearic and α-linoleic acids induce ECs apoptosis via downregulating X-ray repair cross-complementing protein 1 (XRCC1), c-myc, and transcription factor E2F-1 [155]. NEFA also induce apoptosis in cultured ECs via pathways that enhance acyl-coA generation and NF-κB activation [156]. Additionally, palmitic acid induces apoptosis via increasing ROS generation in mesenteric artery ECs, while the anti-oxidant N-acetyl cysteine partially mitigates this effect [157]. Palmitate also activates COX-2 and thromboxane prostanoid (TP) receptor, leading to further ROS generation in mice aortic ECs, possibly by activation of NF-κB [158]. In addition, the isoflavonoid tectorigenin (anti-oxidant) attenuates the palmitate-induced oxidative stress in cultured ECs [159].

Stearic, α-linolenic and arachidonic acids induce apoptosis of cultured EPCs via a caspase-8-dependent mechanism [160]. Palmitic and linoleic acids inhibit EPCs adhesion, migration, proliferation, and vasculogenesis capabilities of EPCs in a dose-dependent manner via inhibition of eNOS. However, only palmitic acid is shown to inhibit Akt phosphorylation [161]. Moreover, palmitic acid effect is shown to be mediated via its binding to peroxisome proliferator activated receptor (PPARγ) and subsequent inhibition of STAT5A expression. PPARγ and STAT5A form heterodimers that enhance transcription of cell cycle progression proteins specifically cyclin D1 and reduces the expression of cell cycle progression inhibitor p21. This decrease in STAT5A is responsible for the halted proliferation of EPCs induced by palmitic acid [160].

Hyperglycemia and endothelial dysfunction

Hyperglycemia is the major causal factor in the development of ED in diabetes. There are several pathways by which hyperglycemia could generate ROS and contribute to oxidative stress, and ultimately developing ED. Here, we briefly highlight these pathways to emphasize the role of glucose toxicity in oxidative stress and ED (Fig. 3).

Advanced glycation end products (AGEs) pathway

AGEs production is increased in diabetic mice Lepr^{db} compared to non-diabetic controls. Moreover, protein expression of RAGE is higher in diabetic mice than controls [162]. Carboxymethyl lysine (AGEs)-mediated activation of NF-κB was significantly diminished in double negative-RAGE transfected cells, suggesting that AGEs activate NF-κB through binding to RAGE [163]. The use of soluble form of RAGE to inhibit AGEs/RAGE interaction and signaling decreases NADPH oxidase expression (NOX-2, p22^{phox}, and

p40^{phox}) and oxidase activity in isolated coronary arterioles, suggesting the role of AGEs/RAGE in Nox activation. The study also suggests that AGEs/RAGE signaling activates TNF-α and induce ED through production of superoxide mediated by NF-κB [162]. AGEs/RAGE stimulates MAPK-mediated NF-κB upregulation and increases COX-2 expression, leading to increased expression of cleaved caspase-3 and cleaved PARP in a dose-dependent manner in pancreatic islet microvascular ECs [164].

Yoon et al. demonstrated that hydroxymethylglutaryl (HMG)-coa reductase inhibitors (statins) reduce intracellular ROS by inhibiting AGEs/RAGE interactions in a dose-dependent manner. Statins are capable of inhibiting all of the stimulatory effects of AGEs on NF-κB, p65, phosphorylated ERK, phosphorylated p38 MAPK, COX-2, and c-Jun [165]. AGEs increase the release of soluble dipeptidyl-peptidase 4 (DPP-4) in HUVECs, where DPP-4 increases RAGE expression and interacts with D-Mannose-6-phosphate/IGF II receptor to dose-dependently increase ROS generation and endothelial damage [166]. Exposure to high glucose AGEs in ECs and EPCs increased cyclin-dependent kinase inhibitors p27 and p57 expression due to down regulation of mir221/mir222 expression. Moreover, AGEs exposure is associated with impaired vessel formation in vivo mouse model [167].

The effect of AGEs on the number and functions of EPCs is profound and has been studied extensively. AGEs reduce NO production and eNOS expression while increasing caspase-3 and Bcl-2 expression as well as EPCs apoptosis in a time-dependent manner. These effects are hampered by MAPK inhibitors [168]. AGEs also significantly decrease anti-oxidant enzymes SOD and glutathione peroxidase expression and confirm the involvement of RAGE in AGEs-mediated ROS production in EPCs [169].

AGEs/RAGE interaction impairs late EPCs function but not proliferation by downregulation of Akt and Cox-2. Furthermore, increasing RAGE expression deteriorates EPCs migration and tube formation in a dose-dependent manner [170]. Even in apparently healthy subjects, serum levels of AGEs correlated with reduced numbers and migratory capacity of EPCs [171], which reflects the possible role of AGEs as independent markers for vascular disease prognosis. In another study, AGEs promoted EPCs apoptosis while decreasing the production of SDF-1, NO, PGI$_2$, and tissue plasminogen activator (tPA) [172]. AGEs modification of basement membrane proteins such as fibronectin in EPCs interfere with signaling through integrin receptor α5β1 by abolishing cell recognition motifs on fibronectin, leading to impaired adhesion of EPCs in diabetic retinopathy [173]. Inflammation is also an important component of RAGE effect on EPCs function as demonstrated by CRP-induced RAGE expression which diminishes eNOS expression and NO production in EPCs [75].
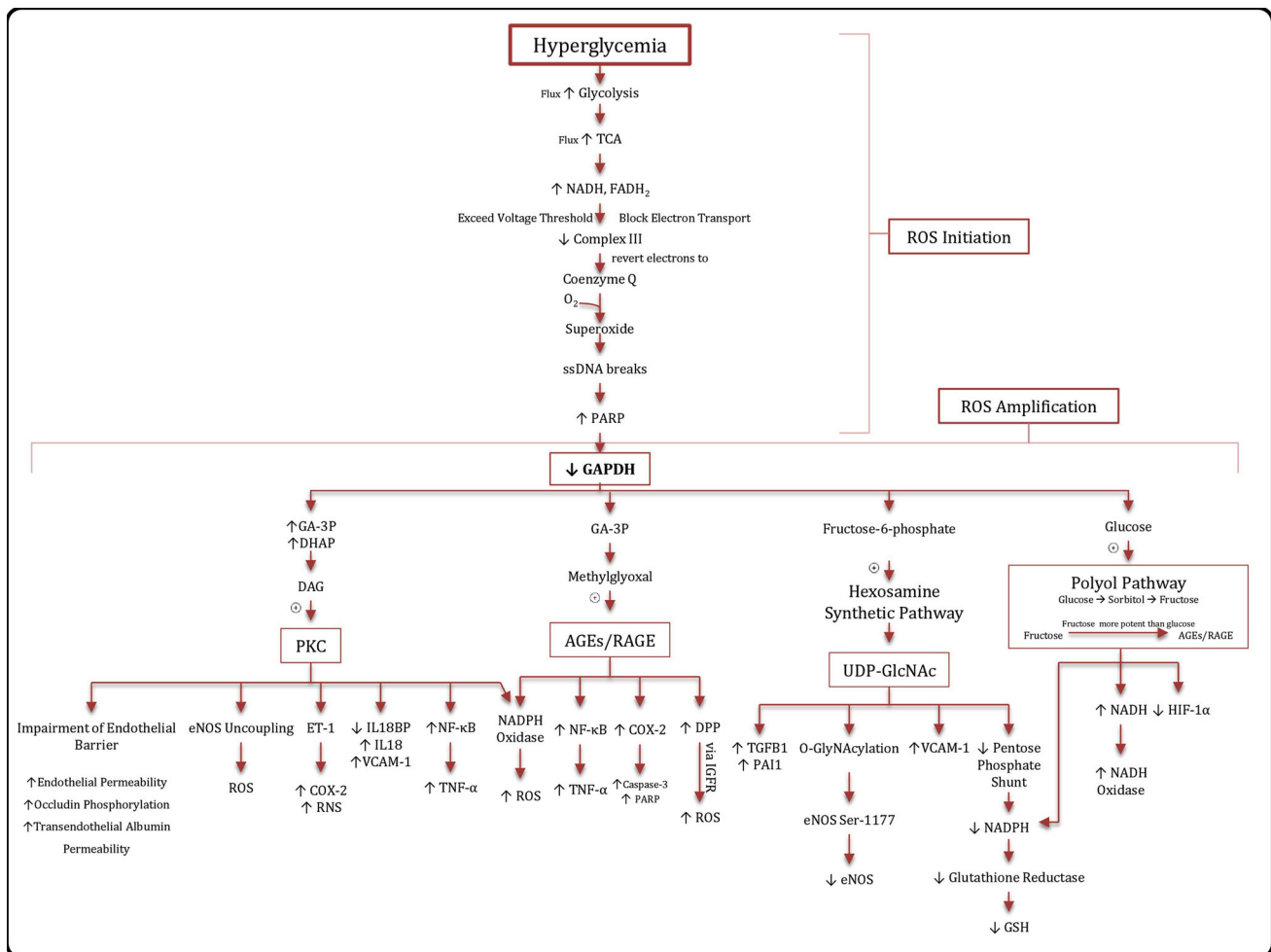
Fig. 3 The central role of mitochondrial ROS in hyperglycemia-induced endothelial dysfunction

Polyol pathway

The polyol pathway consists of two enzymes: aldose reductase (AR), which reduces glucose to sorbitol using NADPH as a co-factor, and sorbitol dehydrogenase (SDH), which converts sorbitol to fructose with its co-factor NAD^+ [174]. Polyol pathway is a major contributor to oxidative stress in states of hyperglycemia [175]. Several mechanisms have been proposed to explain the role of polyol pathway in hyperglycemia-induced oxidative stress. Firstly, the consumption of NADPH in the sorbitol reduction reaction by AR leads to its depletion and inefficient regeneration of glutathione (GSH) by glutathione reductase which uses NADPH as a co-factor [176]. This is supported by the fact that, in hyperglycemia, around 30 % of glucose is shuttled through polyol pathway [176]. Secondly, NADH on the other hand accumulates as a result of the reduction of NAD^+ upon oxidizing sorbitol to fructose by SDH [177], creating a state of pseudohypoxia [178]. NADH is the primary substrate for NADH oxidase that produces

ROS [179]. Thirdly, the polyol pathway results in oxidation of glucose into fructose. Fructose and its metabolites fructose-3-phosphate and 3-deoxyglucosone are more potent glycation agents than glucose [180]. Therefore, hyperglycemia-induced flux of glucose through polyol pathway increases the production of AGEs and their interaction with RAGE, thereby increasing ROS generation and inducing oxidative stress.

Aldose reductase inhibitors (ARI) improve diabetic complications such as diabetic retinopathy [181] and diabetic nephropathy [182], as well as regenerating GSH [183] in animal models, suggesting the role of AR in ROS production. However, ARI have intrinsic free-radical scavenger activity [184], which may account for restoring GSH levels regardless of ARI activity. Therefore, the use of genetic models to investigate the role of AR in hyperglycemia-induced ROS revealed important insights. Mice normally have low AR in their lenses and are resistant to diabetic cataract. Induction of diabetes in transgenic mice overexpresses AR, specifically in their lenses, leads to

cataract with significant decrease in GSH level and significant increase in the level of malondialdehyde (MDA), an oxidative stress marker. Moreover, introducing SDH deficient mutation in transgenic AR mice increases GSH levels and reduces MDA levels, implicating the role of SDH in generation of oxidative stress [175]. On the other hand, induction of diabetes in AR-knockout mice rendered them resistant to diabetic neuropathy with normal nerve conduction velocity and GSH levels [175].

Polyol pathway in hyperglycemic state is characterized by increased cytosolic NADH/NAD$^+$ ratio by enhancing SDH activity and increasing superoxide generation. Free radicals induce single strand breaks that activate PARP, thereby inhibiting the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and increasing concentrations of triose phosphate [185]. Fragmentation of triose phosphate compounds leads to increased formation of methylglyoxal [186], which forms AGEs with many cellular proteins including HIF-1α (methylgloxal modification of Arg17 and Arg23 in the bHLH domain) and P300 coactivator [187]. This modification impairs both HIF-1α/aryl hydrocarbon receptor nuclear translocator (ARNT) heterodimer formation as well as inhibiting the complex HIF-1α/ARNT/P300 from binding to consensus hypoxia response elements of target genes (such as SDF-1, VEGF, CXCR4 and eNOS) in EPCs required for neovascularization [187, 188]. This reflects the elaborate interplay between hyperglycemia-induced pathways in disrupting normal ECs and EPCs functions.

Protein kinase C (PKC) pathway

Hyperglycemia increases PKC activity in the membranous pool, most probably by translocation of PKC from cytoplasm to cell membrane [189]. In retinal ECs, high glucose concentration inhibits GADPH which leads to accumulation of glycolytic intermediates, dihydroxyacetone phosphate, and glycerol 3-phosphate that undergo acetylation to lysophosphatidic and phosphatidic acids and increase de novo synthesis of diacylglycerol (DAG) [190].

Hyperglycemia increases DAG levels in cultured ECs in a time-dependent manner, as well as increasing PKC-β2 isoform in aorta and heart of diabetic rats [191]. Additionally, hyperglycemia-mediated PKC activation has been shown to impair intercellular communications through gap junction transmembrane channels [192], as well as perturbing endothelial barrier and, thereby, increasing transendothelial albumin permeability [193]. For example, VEGF-induced endothelial permeability requires PKC-dependent phosphorylation of occludin (transmembrane tight junction protein) [194]. Hyperglycemia-induced PKC activation promotes eNOS gene expression and triggers eNOS uncoupling, thus increasing superoxide production and decreasing NO

bioavailability. PKC activation in hyperglycemia also increases expression of the NADPH oxidase subunits gp91^{phox} and p22^{phox} [195]. Intermittent high glucose is characterized by increased PKC. Moreover, PKC inhibitors reduce oxidative stress markers and HUVECs apoptosis [196]. Also, PKC activation increases peroxynitrite formation and tyrosine nitration of PGI$_2$ synthetase, thereby reducing PGI$_2$ release [195]. PKC-δ activation, secondary to hyperglycemia, leads to p38 MAPK phosphorylation that increases the expression of Src-homology-2 domain-containing phosphatase-1 (SHP-1), which dephosphorylates platelet-derived growth factor (PDGF) receptor-β and impairs its downstream signaling, resulting in vascular cell apoptosis [197]. PKC-β2 activation by high glucose upregulates p66^{Shc} in human aortic ECs, leading to mitochondrial ROS generation and ED [198].

Hyperglycemia-induced PKCβ activation decreases IL-18 binding protein (IL-18BP) expression (IL-18 inhibitor) which leads to increased IL-18 and VCAM-1, resulting in monocyte adhesion to ECs and ED [86]. High glucose concentration increases NF-κB activation through PKC pathway in ECs [199].

PKC is an essential mediator in EPCs signaling. CXCR2-mediated activation of PLC forms DAG, which activates PKC that triggers abnormal Ca^{2+} release. Calcium signaling is critical for mobilization and recruitment of EPCs at sites of vascular remodeling and repair [200].

Hexosamine biosynthetic pathway (HSP)

Hexosamine synthetic pathway (HSP) diverges from glycolysis and serves as a cellular nutrient sensor. HSP involves converting fructose-6-phosphate and glutamine into glucosamine-6-phosphate catalyzed by the rate-limiting enzyme glutamine/fructose-6-phosphate amidotransferase (GFAT) and ends with UDP-N-acetylglucosamine (UDP-GlcNAc). O-GlcNAc transferases use UDP-GlcNAc in post-translational modification of proteins by O-GlcNAcylation as well as glycolipids, proteoglycans, and gangliosides [201].

HSP is involved in many diabetic vascular complications, inflammation and oxidative stress. Hyperglycemia-induced ROS inhibits GADPH, thereby inhibiting flux of glyceraldehyde-3-phosphate and upstream metabolites (e.g., fructose-6-phosphate) from flow through glycolysis and inducing their shuttling through HSP. Hyperglycemia-induced HSP stimulates TGFβ1 expression by increasing the expression of upstream stimulatory factor proteins and their binding to glucose-response element in TGFβ1 promoter [202]. Increased HSP activity leads to increased O-GlcNAcylation and activation of transcription factor specificity protein-1 and enhances its binding to multiple genes involved in diabetic complications, such as increasing expression of TGFβ1 and plasminogen activator inhibitor 1 (PAI-1) in ECs [203].

HSP activation induces oxidative stress by inhibiting pentose phosphate shunt, thereby diminishing NADPH and the anti-oxidant GSH [204]. Reciprocal relation between phosphorylation and O-GlcNAcylation on the same or contiguous amino acid residues plays a pivotal role in the regulation of protein activity. HSP-induced O-GlcNAcylation of eNOS at ser1177 decreases phosphorylation at this site and prevents eNOS activation in ECs [205].

Mitochondrial ROS

Hyperglycemia increases the formation of electron donors such as NADH and $FADH_2$ from glycolysis and TCA cycle. In ECs, increased flux of NADH and $FADH_2$ through electron transport chain increases voltage gradient across inner mitochondrial membrane to a critical threshold, thereby, hindering electron transport in complex III and reverting electrons back to coenzyme Q, which catalyze superoxide generation by reducing molecular oxygen [206, 207]. It has been established that hyperglycemia-induced mitochondrial ROS generation is the unifying mechanism, which underlies all previously discussed pathways in hyperglycemia [208]. Supporting this idea, dissipating voltage gradient across mitochondrial membrane by uncoupling protein 1 (UCP-1) or neutralizing superoxide by MnSOD prevents hyperglycemia-induced ROS or even activation of other pathways [209]. Moreover, hyperglycemia fails to promote superoxide generation in rho zero ECs that have been depleted of their mitochondrial DNA thus having non-functional mitochondrial electron transport chain [208].

Mitochondrial ROS acts as initiating event in hyperglycemia-induced cellular damage, since these free radicals promote DNA single-strand breaks, which activate PARP. Activated PARP then adds poly-ADP polymers to nuclear proteins and to GADPH (which shuttles in and out of the nucleus to participate in DNA repair), thereby inhibiting GADPH [185]. Inhibition of GADPH blocks glycolysis and leads to accumulation of glycolytic intermediates upstream of glyceraldehyde-3-phosphate (GA-3P), hence activating all the previously discussed pathways and amplifying the signal of ROS production initiated in mitochondria. Accumulated GA-3P non-enzymatically forms methylglyoxal that act as a potent AGEs and activates RAGE expression. GA-3P and DHAP also fragment into DAG, which activates PKC pathway [210].

Upstream of GA-3P, accumulation of fructose-6-phosphate activate HSP and induce formation of UDP–GlcNAc which modifies many cellular proteins posttranslationally. Ultimately, inactivation of GAPDH leads to accumulation of glucose, which activates polyol pathway through AR, consuming NADPH and generating NADH, thereby compromising inherent anti-oxidant capacity such as GSH reductase. Besides mitochondrial ROS, the activity of xanthine oxidase increases in plasma of diabetic animals due to increased release of the enzyme from hepatocytes. Xanthine oxidase binds to vascular ECs by sulfated glycosaminoglycan [211]. Xanthine oxidase is a superoxide-producing enzyme; therefore, allopurinol (xanthine oxidase inhibitor) or releasing xanthine oxidase from the surface of ECs significantly diminishes superoxide production in aortic rings from diabetic rats [211]. Also, hyperglycemia-induced mitochondrial ROS is inhibited by PPARγ coactivator-1α (PGC-1α), which increases the expression of VEGF in bovine retinal capillary ECs [212].

Diabetic EPCs have high ROS concentration, probably due to excessive activation of mitochondrial ROS generation [213]. Diminished anti-oxidant capacity of EPCs in diabetes could result from impaired AMPK activation secondary to elevated protein phosphatase 2A (PP2A), and subsequently downregulation of MnSOD expression (the mitochondrial isoform of SOD) [214]. High glucose-induced PKC in cell culture significantly increases EPCs apoptosis through upregulating p66[Shc] protein expression and inducing ROS generation by the mitochondria [215]. Moreover, EPCs are characterized by significantly diminished mitochondrial MnSOD expression (but not cytosolic CuZnSOD) in *db/db* mice. Interestingly, MnSOD gene therapy in diabetic EPCs before transplantation in *db/db* mice significantly augmented and accelerated wound healing [213]. Taken together, these findings imply the pivotal role of mitochondrial ROS in ECs and EPCs dysfunction in the setting of diabetes.

Adipokines and endothelial dysfunction

Adipose tissue is now widely recognized as not only a storage organ for lipids in the form of TGs but also an active endocrine organ which produces and secretes a wide range of protein hormones, cytokines, and mediators, collectively known as adipokines or adipocytokines. Adipokines are involved in many vital processes in different body organs including glucose and lipid metabolism, IR, inflammation, atherogenesis, apoptosis, and regulation of appetite and body weight [216]. Therefore, adipokines are relatively new and pivotal tools to understand the molecular pathophysiology of metabolic and cardiovascular diseases. Adipokines might exert their action on ECs and EPCs directly or indirectly, through modulation of metabolic pathways, inflammatory mediators, and even through modulating the effect of other adipokines, autacoids, and hormones. In this section, we will discuss recent research investigating the association between certain adipokines and ED.

Adiponectin

Generally, adiponectin exerts insulin-sensitizing, antidiabetic, antioxidant, anti-inflammatory, anti-atherogenic, vasoprotective, and anti-apoptotic actions on different cell types through adiponectin receptors, e.g., AdipoR1 and AdipoR2 [217]. Diabetes, IR, CVD, and metabolic syndrome are associated with reduced adiponectin level in animal models [218, 219] and in human patients [220, 221]. Also, hypoadiponectinemia is associated with ED and impaired vascular reactivity [222].

The association between adiponectin serum level and oxidative stress markers has been investigated in a cross-sectional study by Gustafsson et al. It has been shown that higher levels of adiponectin were associated with more favorable oxidative stress profile [223]. Adiponectin stimulates vascular NO production through phosphorylation of eNOS at Ser1177 and inhibits ROS production in ECs mainly through suppression of NADPH oxidase activity [221, 224]. In support with this, Antonopoulos et al. depict that reduced adiponectin level stimulates vascular NADPH oxidase in T2DM subjects [225]. In humans with CVD and atherogenic mice, adiponectin improves endothelial function by reducing ROS production, serum LDL-cholesterol, and TGs as well as increasing BH_4 bioavailability and restoring eNOS coupling [226–228]. Moreover, adiponectin prevents hyperglycemia-induced ROS production in ECs by suppressing ROS/p38 MAPK/p16^{INK4A} and/or activating cAMP/PKA signaling pathways [229, 230]. Also, adiponectin reduces ox-LDL, angiotensin II (AG II), and hyperglycemia-induced ROS production in cultured HUVECs, thus protecting ECs against ox-LDL, fluctuating glucose concentration, and AG II-induced endothelial damage and apoptosis [224, 231–233]. Interestingly, Chen et al. showed that the inflammatory effect of microRNA-221 (miR-221) on HUVECs is mediated by targeting AdipoR1 receptors and abolishing the anti-inflammatory effect of adiponectin [234].

Adiponectin promotes proliferation, survival, differentiation, migration, and vascular recruitment of EPCs through activation of both PI_3k/Cdc42/Rac1 and AMPK signaling pathways [235–237]. Also, it has been proposed that pretreatment of angiogenic stem cells with adiponectin before transplantation enhances their survival and proliferation activities [238]. Therefore, the number of circulating EPCs is reduced in adiponectin knockout mice and administration of adiponectin restores the number of EPCs [229]. Moreover, plasma levels of adiponectin were positively associated with EPCs number in patients with coronary artery disease [239] and atherosclerotic stroke in large arteries [240]. On the other hand, Li et al. showed that in diabetic patients, hypoadiponectinemia was not associated with reduced level of EPCs; however, they confirmed the beneficial effects of adiponectin on EPCs in vitro [241].

This controversy could be, in part, due to the variance in the degree of adiponectin deficiency, diabetic state and other disease conditions, and other concurrent metabolic dysregulations affecting EPCs activity, e.g., insulin and leptin sensitivity.

Collectively, diabetic hypoadiponectinemia may play a pivotal role in the development of ED and CVD, particularly through enhancing production of ROS and pro-inflammatory mediators, promoting IR, NO inactivation, eNOS uncoupling, and impairment of EPCs migration and function as well. Therefore, adiponectin could be used as a potential marker for predicting CVD and a measure for the risk of metabolic disorders [242]. In addition, adiponectin could be used as a target for treating metabolic disorders and its associated CVD via increasing endogenous production or mimicking the actions of adiponectin on its signaling pathways. For example, rosiglitazone (PPARγ agonist) has been shown to restore normal vascular function in diabetic and obese mice through adiponectin-mediated mechanism [243, 244].

Leptin

Leptin is involved in obesity, IR, CVD, inflammation, and diabetes, partly due to its regulatory action on satiety and energy homeostasis, production of inflammatory mediators, and on lipid and carbohydrate metabolism [216]. IR is associated with increased levels of leptin (hyperleptinemia), with exclusively augmented leptin effects on some organs (or pathways) and diminished actions on others, reflecting a state of leptin resistance [245], which may be analogous to IR in its selectivity.

Leptin is the adipokine of controversy, with a myriad of discordant results. Although several studies show positive association between leptin concentration and high incidence and severity of cardiovascular events [246], Martin et al. demonstrated that no such association exists in a multi-ethnic cohort study [247]. Also, the association between serum leptin concentration and vascular endothelial function in the setting of T2DM and obesity is controversial; while some results indicate positive association [248], others showed inverse relationship between leptin levels and endothelial function [249] and even enhanced vascular function in severe obese people with high levels of leptin compared with obese subjects [250]. While the difference in subject properties (e.g., age, sex, body mass index, disease status, drugs used, and degree of hyperleptinemia) and the technique used in assessing endothelial function could be possible explanations for these inconsistent data [248], it could be further explained in the context of leptin resistance. First, the effect of leptin on ECs and generally on cardiovascular system may depend on the degree of leptin sensitivity. Second, it is not clear

when leptin resistance starts in the context of metabolic disorders. Third, the exact cut-off value at which leptin sensitivity ends and resistance begins cannot be determined. Last but not least, the selective leptin resistance affecting certain signaling pathways could be another powerful perspective.

Endothelium-dependent vasodilatation induced by leptin is mediated by NO and endothelium-derived hyperpolarizing factor (EDHF). Also, leptin enhances endothelium-dependent relaxation through induction of endothelial neuronal NOS (nNOS) expression via JAK2/STAT3 in mice lacking eNOS, HFD mice, and in HUVECs [251]. Hyperleptinemia and obesity may result in impaired leptin-induced NO production but enhanced EDHR-induced action, and both actions are diminished by leptin antagonist [252].

Leptin upregulates eNOS expression in ECs and thereby enhances NO production [253]. Short-term exposure to leptin failed to enhance superoxide and peroxynitrite production in HUVECs. In contrast, long term exposure to leptin (or hyperleptinemia) enhances superoxide, peroxynitrite and ET-1 production, and consequently inactivates bioavailable NO, in spite of the overexpression of eNOS [253, 254]. This could partly explain that hyperleptinemia could trigger eNOS uncoupling from NO-synthesizing to superoxide-producing enzyme. Therefore, hyperleptinemia inversely correlates with NO bioavailability and cGMP in the aortic endothelium and the aortic wall, respectively, and directly correlates with ROS in ECs of obese mice [253]. This is also why hyperleptinemia in obese subjects is associated with enhanced ROS generation, poor oxidative stress profile and lower level of NO metabolites [255, 256], resulting in ED.

In addition, it has been shown that chronic leptin administration results in ED in mice via stimulation of the pressor effect of the sympathetic nervous system [257], and these effects were confirmed in healthy subjects [258]. Moreover, pretreatment of mice with TEMPOL (superoxide scavenger) completely diminishes the pressor effect of chronic leptin administration on endothelial function [257], re-emphasizing the role of ROS in mediating hyperleptinemia-induced ED.

It has been suggested that leptin plays an important role in mediating subclinical inflammatory state associated with obesity-related conditions. Hyperleptinemia is associated with low-grade inflammation and metabolic dysregulation manifested by high levels of CRP, TNF-α, IL-10, IL-12, glucose, insulin, and TGs and high IR values in obese mice and humans [259]. On the contrary, it has been shown that leptin does not underlie the pro-inflammatory condition in obese human subjects [260, 261]. Leptin enhances the secretion of IL-18 (implicated in T2DM complications) from monocytes via activation of caspase-1 inflammasome [262].

Leptin enhances ERK1/2 phosphorylation and NF-κB activation as well as the production of TNF-α in HUVECs [263].

Leptin through its receptors on EPCs stimulates tube formation of EPCs at physiological concentration, while in hyperleptinemic states, tube formation and EPCs migration are inhibited, with no effect in both conditions on EPCs proliferation [264]. It has been demonstrated that leptin stimulates the homing potential and adhesive activities of EPCs, thus enhancing its repair activity in vivo and in vitro [265]. Also, leptin enhances the mobilization of vascular angiogenic ECs (sca-1$^+$, flk-1$^+$) from bone marrow in mice through leptin-mediated activation of Nox2, enhanced ROS generation, and stimulation of MMP9 expression [266]. These effects could explain the enhanced vascular effects in hyperleptinemic obese subjects [250]. Taken together, we thought that the state of leptin resistance has no or little effect on the beneficial effects of leptin on EPCs.

In a nutshell, although there is an open debate over whether leptin possesses beneficial or detrimental effects on cardiovascular system, generally clinical studies support the notion that leptin in a cardiovascular risk factor, thus reducing leptin production could have beneficial effects in the clinical settings.

Resistin

Generally, resistin is a diabetogenic, inflammatory, and angiogenic adipokine which plays a pivotal role in the pathogenesis of diabetes and its complications. Although some studies indicate that resistin is not associated with IR and metabolic syndrome [267–270], large body of evidence supports the notion that elevated resistin levels are implicated in IR, diabetes, and metabolic syndrome and could be an important risk factor for CVD [271–276].

Endothelial function is shown to be inversely affected by resistin level [277]. Resistin impairs bradykinin-induced relaxation (but not ACh), and this effect is shown to be not mediated via modulating superoxide, NO, and PGI$_2$ production [278]. Also, resistin inhibits insulin-mediated vasodilatation via altering IRS-1 phosphorylation and subsequent inhibition of Akt/eNOS phosphorylation [279].

In ECs, resistin causes imbalance in redox enzymes and mitochondrial dysfunction; therefore, it enhances the generation of ROS and activation of p38 MAPK/JNK pathways, which eventually leads to eNOS downregulation and decreased NO bioavailability [280, 281]. Not surprisingly, hyperresistinemia interferes with endothelial PI$_3$K/Akt signaling pathway and consequently reduces eNOS phosphorylation/activation and NO production [282].

In obese children and hypertensive T2DM patients, resistin serum levels were correlated positively with markers of endothelial activation (ICAM-1, VCAM-1 and E-selectin), homocysteine, and oxidative/nitrosative stress

[283, 284]. Furthermore, serum resistin level is associated with CRP in T2DM patients [285].

Recently, Ying et al. have shown that the protective effects of miR-492 against IR/hyperglycemia-induced ED are mainly due to its downregulating effect on resistin production [286]. Therefore, we thought that decreasing resistin levels will be of great importance in the context of T2DM and its associated CVD. To the best of our knowledge, resistin effect on EPCs is still uncovered yet.

Visfatin

Another controversial adipokine is visfatin or extracellular nicotinamide phosphoribosyltransferase (Nampt), an essential enzyme in the pathway of NAD biosynthesis [287]. Although visfatin is mainly synthesized and secreted from visceral adipose tissue in rats [216], leukocytes are the major visfatin source in humans [288], suggesting an inflammatory role of visfatin in humans rather than a metabolic role.

Serum visfatin concentrations are increased in obesity, T2DM, metabolic syndrome, CVD, and gestational diabetes [289, 290]; however, these results are still controversial [291, 292]. Serum visfatin levels negatively correlate with endothelial function in T2DM [293], but this association do not appear in prediabetic obese patients [294]. Moreover, it has been shown that serum visfatin levels correlate with inflammatory markers rather than markers of IR [295]. This results also contradicts with other research which shows that serum visfatin positively correlates with systemic IR and hyperlipidemia [296], suggesting that visfatin levels could change primarily according to the degree of inflammation associated with IR, obesity, and T2DM. Therefore, visfatin could be used as a marker for systemic inflammation in T2DM [297].

Visfatin induces the expression of inflammatory and adhesion molecules in ECs, and this mechanism could be mediated through ROS-dependent NF-κB activation [298, 299]. Also, inflammed ECs (exposed to pro-inflammatory mediators, e.g., TNF-α, IL-1β, or AG II) are considered a source of visfatin [300].

Circulating visfatin concentrations are positively correlated with coronary artery disease in patients with T2DM [301], suggesting a deleterious effect of high concentrations of visfatin on ECs. In human endothelial and vascular smooth muscle cells, visfatin enhances NF-κB signaling pathways [302, 303], and these inflammatory activities are thought to be mediated by its intrinsic Nampt activity [303].

Consistently, Xia et al. demonstrated that visfatin induces ED in coronary ECs via stimulating p47^{phox} translocation, activation of NADPH oxidase, and therefore increases superoxide production [304]. Vallejo et al. reported similar visfatin activities in HUVECs and mesenteric microvessels of rats and humans; however, they showed that visfatin-induced ED through activation of NADPH oxidase is dependent on its Nampt activity [305]. Recently, Villalobos et al. further investigated the detrimental effects of visfatin on ECs and showed that visfatin-induced ROS production causes DNA destruction, p53 upregulation, and telomere dysfunction in HUVECs [306]. Nevertheless, recent study by Karataş et al. showed no such association between visfatin serum levels and ED [307].

Intriguingly, Borradaile et al. demonstrate that enhanced expression of visfatin in human aortic ECs protects the cells against ROS-induced senescence caused by hyperglycemia and thus enhance the replicative longevity and angiogenic capacity of ECs [308]. Consistent with this, Visfatin enhances the activity of eNOS as well as increase production of NO and cGMP in HUVECs and coronary artery ECs via activation of PI$_3$K/Akt pathway, consequently exerting vasodilating effect [309, 310]. These contradictions require further investigation focusing on the role of visfatin concentration and the disease context at which visfatin exerts its actions.

Regarding the effect of visfatin on EPCs function, Chen et al. have shown that increased visfatin levels along with enhanced oxidative stress reduce EPCs number in obese patients [311]. Also, visfatin adversely affects the migration and adhesion capacities of EPCs through activation of NF-κB [312]. These effects are consistent with visfatin-induced ED in the setting of metabolic syndrome.

Omentin

Omentin is an adipokine with insulin sensitizing, antidiabetic, anti-inflammatory, and anti-atherogenic activities. It positively correlates with HDL and adiponectin concentrations. Its serum concentration is decreased in IR, obesity, and T2DM [313]. Reduced circulating omentin level is independent risk factor for CVD in T2DM patients [314]. Administration of omentin in rats increases adiponectin levels, reduces IL-6 concentration, and enhances the production of NO [315]. Moreover, circulating omentin levels could be used as a useful marker for evaluation of endothelial function [316].

Interestingly, omentin enhances HUVECs survival and differentiation through activation/phosphorylation of Akt/eNOS pathway [317]. Moreover, omentin inhibits TNF-α-induced COX-2 activation and production of adhesion molecules via inhibition of the JNK activation and diminishing ERK/NF-κB pathway in HUVECs [318, 319]. To the best of our knowledge, the effect of omentin on EPCs has not been investigated yet.

Vaspin

Vaspin is one of the most recently discovered adipokines, exerting mainly insulin sensitizing and anti-atherogenic

actions. Circulating vaspin levels are significantly elevated in IR, obesity, and T2DM [320], suggesting a compensatory role of vaspin in the setting of IR.

In ECs, Jung et al. demonstrated that vaspin enhances NO production and alleviates NEFA-induced ED. Mechanistically, vaspin enhances eNOS activity mainly by reducing asymmetric dimethyl arginine [inhibitor of eNOS] (ADMA) level through induction of dimethylarginine dimethylaminohydrolase (DDAH II) expression—the enzyme responsible for ADMA degradation. These effects are mediated via STAT3 pathway [321]. Also, vaspin protects ECs against NEFA-induced apoptosis via activation of PI3k/Akt signaling pathway [322]. Consistently, vaspin diminishes methylglyoxal-induced apoptotic activity in ECs by inhibiting caspase-3 activation through inhibition of Nox-mediated ROS generation [323]. Taken together, vaspin could have powerful anti-apoptotic and anti-oxidant actions particularly on ECs.

While Fu et al. study showed that vaspin could not protect HUVECs from TNF-α-induced inflammatory damage [324], Jung et al. and Liu et al. experiments indicate that vaspin attenuates TNF-α/IL-1-induced NF-κB activation through stimulation of AMPK in ECs [325, 326], confirming the anti-inflammatory role of vaspin on ECs.

Treatment of EPCs with vaspin elevates the number and enhances proliferation and migration capacities of EPCs in a dose-dependent manner. Moreover, vaspin mitigates hyperglycemia-induced EPCs dysfunction. These beneficial effects are shown to be mediated through activation of PI3K/Akt/eNOS pathway [327].

Chemerin

Chemerin is involved in the regulation of glucose homeostasis and insulin sensitivity. High-plasma chemerin levels are associated with metabolic syndrome, IR, obesity, and T2DM [328, 329]. In obese patients, chemerin positively associates with oxidative stress and inflammation as well as leptin concentration, whereas it shows a negative association with anti-oxidant status and adiponectin level [330]. Interestingly, chemerin levels were not only elevated in metabolic syndrome patients with coronary artery disease compared with patients without coronary artery disease but also correlated positively with the severity of CVD [331]. Overexpression of chemerin and its receptors ChemR23 is associated with diabetic microvascular complications, e.g., diabetic nephropathy, and reducing the expression of chemerin, and ChemR23 could be a possible way to ameliorate diabetic complications [332]. Collectively, chemerin could be a possible link between T2DM and its cardiovascular complications.

Chemerin potentiates the vasoconstrictor responses to phenylephrine and ET-1 via activation of ERK1/2 pathway [333]. Also, chemerin decreases NO production, enhances

NO breakdown, and also decreases NO-dependent cGMP signaling, particularly via inducing eNOS uncoupling, enhancing superoxide production and diminishing GC activity [334, 335]. Moreover, treatment of human aorta ECs with chemerin induces ROS generation with enhanced expression of the autophagy-related genes. Knockdown of ChemR23 or treatment with the antioxidant (Mito-TEMPO) diminishes the chemerin-associated ROS generation and abrogates the upregulation of autophagy-related genes [336]. These results support the idea that chemerin induces ED and could be a cardiovascular risk factor, particularly in T2DM and obesity.

While chemerin levels are associated with systemic inflammation in obese and T2DM patients [337], Yamawaki et al. demonstrated the protective anti-inflammatory role of chemerin in ECs against TNF-α-induced inflammatory response, via inhibition of NF-κB pathway [338]. This discrepancy between in vivo and in vitro experiments needs further elucidation. Also, the effect chemerin on EPCs remains to be investigated.

Apelin

Apelin has a role in energy metabolism. Elevated apelin levels are associated with obesity, hyperinsulinemia, IR, and T2DM in humans and mice. Apelin exerts a powerful glucose-lowering effect concomitant with enhanced glucose disposal in skeletal muscle and adipose tissues [339], suggesting that elevated apelin levels could be a compensatory mechanism to enhance insulin sensitivity in case of IR-related disorders.

Apelin is a vasoactive peptide. In healthy volunteers, apelin exhibits arterial NO-mediated vasodilatation [340]. Apelin abolishes the vasoconstricting action of AG II and potentiates the vasodilator response to ACh via inducing phosphorylation of Akt and eNOS in diabetic mice [341].

Interestingly, single-nucleotide polymorphism at the apelin gene is associated with arterial stiffness. This observation led Liao et al. to overexpress miR-765—which interferes with apelin gene expression—in ECs to further elucidate the underlying mechanism of arterial stiffness. They found that overexpression of miR-765 reduces mRNA and protein levels of apelin, consequently inhibiting the phosphorylation of eNOS and ERK/Akt/AMPK signaling pathways [342].

However, Nagano et al. showed that apelin exerts a vaso-constricting effect in mice treated with L-NAME, suggesting that apelin induces ED under pathological conditions [343]. In addition, Lu et al. experiments depict that treatment of HUVECs with apelin induces the expression of adhesion molecules and chemokines via NF-κB/JNK signal pathway, implying a role of apelin in endothelial inflammation [344].

Role of incretins and gut hormones in endothelial dysfunction

Diabetes is associated with incretin defect; that is a reduction or even loss of incretin secretion/action [345]. Recently, Ravassa et al. showed that T2DM patients with decreased GLP-1 levels have enhanced oxidative stress profile, with increased risk for cardiovascular events [346].

In the last 15 years, incretins have been extensively studied in cell cultures, animals, and human, and now there are many incretin-based therapies used in the treatment of diabetes and associated obesity. Incretin-based therapies include (1) GLP-1 agonists or analogs such as exendin-4, liraglutide, and exenatide that mimics the action of GLP-1 on its receptors (GLP-1R) and (2) dipeptidyl peptidase-4 (DPP-4) inhibitors or gliptins such as sitagliptin, vildagliptin, and linagliptin which inhibit the catalytic activity of DDP-4, the enzyme that degrades incretins [347]. Herein, we will discuss the beneficial effects of GLP-1, incretin-based therapies and other gut hormones on endothelial function.

Glucagon-like peptide-1 (GLP-1)

Generally, GLP-1 exerts vasodilator, anti-inflammatory, anti-oxidant, and anti-apoptotic actions on ECs. GLP-1 and vildagliptin improve endothelial function in T2DM and T1DM patients [348–350]. In obese patients, alogliptin (DPP-4 inhibitor) improves postprandial ED [351].

In a clinical trial (NCT01181986), exenatide protects T2DM patients from hyperglycemia and lipemia-induced ED via GLP-1R and AMPK-dependent mechanism [352]. However, the clinical trial (NCT01931982) failed to show a beneficial effect of 10 weeks liraglutide treatment on coronary and peripheral microvascular function in T2DM patients [353]. More clinical research and meta-analysis of data are needed to confirm these results.

GLP-1 and its analogs enhance the phosphorylation/activity and the protein expression of eNOS in HUVECs through a GLP-1R-dependent mechanism and downstream PKA signaling pathway [354, 355]. GLP-1 and its analogs abolish TNF-α mediated inflammatory injury and thus exerting a potent anti-inflammatory effect on ECs, presumably via activation of PPARγ [356–358].

GLP-1 protects ECs from ROS-induced senescence via activation of PKA pathway and enhancing the expression of antioxidant genes [359]. GLP-1 protects HUVECs from apoptosis induced by AGEs mainly by inhibiting ROS generation and suppressing the expression of RAGE [360, 361]. GLP-1 protects ECs from ox-LDL-induced apoptosis via inhibiting PARP-1/iNOS pathway [362]. The DPP-4 inhibitor linagliptin and GLP-1R agonist exendin-4 improve endothelium-dependent relaxation of rat mesenteric arteries

pretreated with high glucose, partly by inhibition of super-oxide generation caused by hyperglycemia [363]. Liraglutide abolishes hyperglycemia-induced ROS in human aortic ECs by inhibiting PKC/NAD(P)H oxidase pathway and diminishing Endoplasmic reticulum stress [364, 365].

GLP-1 enhances EPCs proliferation and differentiation via increasing VEGF expression [366]. Sitagliptin increases circulating EPCs in T2DM patients and experimental animals through upregulation of SDF-1α, which is a target for DPP-4 action, via eNOS pathway [367, 368]. These results may further elucidate the improvement in endothelial function in patients treated with incretin-based therapies.

Ghrelin

Ghrelin is an orexigenic and hyperglycemic peptide. Ghrelin is post-translationally modified to exist in two forms: unacylated and acylated, where acylated form being in excess compared to unacylated one in conditions of IR [369].

Although there are some contradictory results, T2DM and obesity are associated with decreased levels of ghrelin, suggesting a compensatory mechanism in response to IR and may be a cause in inducing hyperinsulinemia [370]. Lower ghrelin concentrations are associated with oxidative stress in obese patients [371] and also could be a risk factor for cardiovascular events [372].

Ghrelin enhances endothelial function and restores the balance between ET-1 and NO in patients with metabolic syndrome [373, 374]. It diminishes homocysteine-induced ED via enhancing the eNOS expression, abolishing the ROS generation, and inhibition of NF-κB activation in porcine coronary artery cardiac microvascular ECs and human coronary artery ECs [375, 376]. Unacylated ghrelin protect against oxidative stress in ECs by increasing SIRT1 and SOD-2 expression in mouse model [377].

In addition, it diminishes AG II and NEFA-induced apoptosis via activation of the PI$_3$K/Akt pathway and inhibiting the NEFA-induced ROS generation in ECs [378, 379]. Also, it inhibits hyperglycemia, AG II, and TNF-α-induced endothelial injury in HUVECs via suppressing NF-κB activation, thus exerting anti-inflammatory and immunomodulatory effects [380–382]. Also, recent research shows that ghrelin protects HUVECs from hyperglycemia-induced apoptosis through activation of mTOR/P70S6K signaling pathway [383].

Regarding ghrelin effect on EPCs, Chen et al. have shown that ghrelin induces EPCs migratory capacity via activating PI$_3$K/Akt/eNOS/NO signaling pathway [384]. Consistently, Togliatto et al. showed that unacylated ghrelin enhances EPCs vasculogenic activity, stimulates EPCs mobilization through activating Akt/eNOS/NO pathway,

and inhibits EPCs senescence via modulating Rac1, the regulatory protein in NADPH oxidase [385].

Overall, ghrelin has been proved to exert many beneficial and protecting effects on ECs; however, its hyperglycemic and orexigenic effects might limit its clinical application. Therefore, developing ghrelin agonists which specifically mimic ghrelin effects on ECs and are devoid from its effects on food, insulin, and glucose metabolism would be a promising way to address ED.

Obestatin

Lower levels of the gut hormone obestatin are associated with obesity, T2DM, prediabetics and IR [386, 387]. Low level of plasma obestatin could be associated with early atherosclerosis in T2DM patients [388]. Obestatin exerts vascular relaxation via activation of specific endothelium-dependent NO signaling pathways [389]. Obestatin actions are still to be fully elucidated in many organ tissues.

Conclusion

In conclusion, the etiology of diabetic vascular dysfunction is complex. Furthermore, metabolic disturbances such as IR, compensatory hyperinsulinemia, inflammation, hyperlipidemia, hyperglycemia, as well as the defect in secretion/action of adipokines and incretins contribute independently to ED, at least in part, through activation of ROS generating mechanisms. The complex metabolic disturbances that characterize diabetes contribute to elevated oxidative stress to various extents. Hyperglycemia-induced ED mediated by multiple biochemical pathways converge into increasing ROS generation in ECs and EPCs. While in dyslipidemia, a sole pathway (Ox-LDL) is the major contributor to oxidative stress-induced ED. Moreover, some metabolic pathways increase oxidative stress by potentiating the effect of other pathways rather than having a direct effect. Furthermore, it appears that in the majority of metabolic disturbances discussed, an intricate interplay exists between metabolic intermediates of distinct pathways that lead to ED either directly or by promoting oxidative stress, making the distinction between causes and consequences somewhat blurred. Thus, given the current research methodologies that isolate the effect of a certain biochemical parameter, the elucidation of the complex interplay that characterizes the role of oxidative stress in ED will require novel research protocols that harness contemporary computational advances, which will allow the analysis of such complex experiments.

However, the amount of evidence on the detrimental effects of oxidative stress on endothelial function and its link to the pathophysiological pathways underlying diabetic complications is compelling. This notion led us and many other researchers to suggest that the oxidative stress is the common soil, which transmits signals from other metabolic conditions leading to ED, and consequently CVD. An improved understanding of the mechanisms of ED in this setting could provide new approaches for patient management. Intriguingly, a main source of discrepancy seen in many studies may be attributed to (1) differences in ECs and EPCs isolation methods, (2) culture techniques between different labs, and (3) differences between ECs from microvascular versus macrovascular tissues, which show wide variability in gene expression profiles.

The use of antioxidants as adjuvant to standard diabetic treatment has long yielded controversial results in clinical trials, probably due to emphasizing short-term effects of these agents. We discussed in this review the long-term effects of oxidative stress on ECs and EPCs; therefore, we suggest that a better assessment of long-term effects of antioxidants would elucidate the potential clinical benefits of these agents—if any. Finally, more research is needed to characterize targets and develop more powerful anti-oxidants, paving the way for efficient management of ED, and hopefully therefore, reducing the risk of diabetic cardiovascular events.

Compliance with ethical standard

Conflict of interest None declared.

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