Mitochondrial Dysfunction Signature in Diabetic Vascular Endothelium

Lucia-Doina Popov

Nicolae Simionescu Institute of Cellular Biology and Pathology of the Romanian Academy, 8, B. P. Hasdeu Street, Bucharest, 050568, Romania

*Corresponding author: Lucia-Doina Popov, Nicolae Simionescu Institute of Cellular Biology and Pathology of the Romanian Academy, 8, B. P. Hasdeu Street, Bucharest, 050568, Romania, Tel: +40213192737; Fax: +40213194519; E-mail: doina.popov@icbp.ro

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Abstract

Mitochondrial dysfunction/malfunction is an important risk factor for the onset and progression of endothelial cells dysfunction, a key contributor to diabetes mellitus associated cardiovascular disease. The topics discussed here are: (i) The regulatory role of mitochondrial ROS in normal, healthy vascular endothelium, (ii) The adverse alteration of mitochondrial function in diabetes-related endothelial dysfunction, (iii) The endeavors towards correction of mitochondrial malfunction in dysfunctional endothelium, (iv) The key points of mitochondria-targeted interventions in diabetic patients, and (v) The pending, incompletely understood issues that open new basic research directions and may conduct to innovative therapeutic strategies in diabetes.

Keywords: Endothelial dysfunction; Mitochondrial dynamics; Mitochondrial medicine

Introduction

Endothelial cells (ECs) cover the lumen of all blood vessels and ensure homeostasis preservation. In physiologic conditions, ECs mitochondria have unique functional traits: (i) a reduced engagement in generation of the energy-rich molecule adenosine-5’-triphosphate (ATP) [1,2]; instead, the source of ATP required for the development of the normal metabolic activity of ECs is the anaerobic glycolysis [3-7]; (ii) a commitment to sense the local environmental signals, and to respond accordingly by regulating the intracellular signaling pathways that assure ECs survival; (iii) a rather modest content per endothelial cell (less than 5% of cytosol volume), a level compatible to the low energy requirements imposed by the ECs normal, quiescent phenotype [3]. ECs mitochondria perform also functions in common with those exerted in cells with higher energy demands (e.g. the cardiomyocytes), such as: the generation of mitochondria-derived reactive oxygen species (mtROS) and the regulation of their level by antioxidant mechanisms [8,9], the dynamic shape changes via fusion and fragmentation (fission) events, and the specific autophagic degradation of the malfunctioning mitochondria by "mitophagy", associated with the execution of the "quality control" activity [10,11].

In diabetes mellitus, hyperglycemia triggers mitochondrial dysfunction/malfunction characterized by the decrease in membrane potential, the dysfunction of the respiratory chain, and the overproduction of mtROS that signal the switch of ECs phenotype from quiescent to "activated" [10]. This change engages the luminal aspect of ECs plasmalemma (where novel molecules are expressed), the cytosol (where AMP kinase becomes upregulated), and mitochondria, where the glycolytic ATP production is replaced by lipid-derived free fatty acids (FFA) β-oxidation [4,12]. The latter pathway provides the carbons for de novo nucleotide biosynthesis, linked to ECs proliferation [7,13]. The "activated" ECs are constantly challenged by the high glucose concentration and the circulating oxidative stress that stimulate the influx of extracellular Ca²⁺ into the cytosol, where accumulate within microdomains close to the inner aspect of ECs plasmalemma. As mitochondria are anchored in this particular location, Ca²⁺ enter their matrix, and activate the key Ca²⁺-sensitive enzymes (e.g. Ca²⁺-sensitive dehydrogenases, ATP synthases) involved in ATP synthesis via oxidative phosphorylation pathway (OXPHOS) [3,12]. The aerobic mitochondrial metabolism provides the ATP essential for the biosynthesis of vasoconstrictor molecules, inflammatory cytokines, adhesion molecules, and growth factors. Diabetes induces also the uncoupling of endothelial NO synthase (eNOS) associated with increased levels of superoxide anions (O₂⁻) and with a diminished formation of nitric oxide (•NO) that can damage proteins, lipids and nucleic acids. Moreover, OONO generates reactive nitrogen species (RNS) that induce modification of mitochondrial Complexes I and III, and exacerbate mtROS production [8]. Ultimately, the overproduction of mtROS along with the intracellular oxidative stress (ROS and RNS) overwhelms the antioxidant defense mechanisms, the cytochrome c and mitochondrial DNA (mtDNA) are released, and finally ECs apoptosis is installed [6,15]. The interrelated processes exemplified above endorse mitochondrial dysfunction/ malfunction as an important risk factor for the onset and progression of endothelial dysfunction, a key contributor to diabetes-associated cardiovascular disease [16]. The details of these relationships are examined in the following sections: (i) The regulatory role of mtROS in normal, healthy vascular endothelium, (ii) The adverse alteration in mitochondrial function in correlation with diabetes-related ECs dysfunction, (iii) The endeavors towards correction of mitochondrial malfunction in dysfunctional ECs, and (iv) The key points of mitochondria-targeted interventions in diabetic patients. The review is concluded by the pending, incompletely understood issues that may open promising new directions in the diabetes treatment.

Mitochondrial ROS Regulate the Normal Functions of Endothelium

The major mtROS in vascular endothelium are: (i) the free radicals of short half-life that do not diffuse across membranes, such as O₂•⁻ (half-life in the range of nano-to milliseconds) and hydroxyl radicals.
(*)OH (half-life of few nanoseconds) and (ii) the hydrogen peroxide (H₂O₂), a stable, freely diffusible molecule [8].

Within the healthy ECs, mtROS are generated during electron transport chain (ETC) embedded within the inner mitochondrial membrane (IMM). The polypeptide complexes I, II, and III of ETC release O₂⁺ into the mitochondrial matrix, where manganese superoxide dismutase (MnSOD or SOD2) rapidly convert O₂⁺ to H₂O₂; furthermore, the polypeptide complex III releases the O₂⁺ both into the matrix and the intermembrane space; in the latter location, copper-zinc superoxide dismutase (CuZnSOD or SOD1) convert the O₂⁺ into H₂O₂ [17,18]. The H₂O₂ molecules either diffuse into the cytosol or interact with mitochondrial proteins; in the presence of Fe²⁺ or Cu²⁺ ions, H₂O₂ molecules are transformed into *OH (a potent ROS) via a Fenton reaction. In an additional pathway, local glutathione peroxidase decomposes H₂O₂ to H₂O and O₂. An earlier report demonstrated the involvement of H₂O₂ in shear stress-induced vasodilatation of coronary resistance arteries [19].

In physiologic conditions, mitochondria exert a rigorous control on ECs functional integrity and homeostasis by distinct mechanisms [2,20]; (i) generation of moderate levels of mtROS, and regulation of their conversion and/or scavenging during cells response to shear stress [3,8,11,21], (ii) operation of mtROS as signaling molecules, (iii) involvement in eNOS activation and Ca²⁺ homeostasis regulation, with participation of mitochondrial connexin 40 and mitochondrial interaction with ER [22]; moreover, eNOS activation is engaged in the balanced production of ECs vasodilators/vasoconstrictors during vascular tone regulation [23,24] and (iv) execution of mitochondrial quality control, that ensure organelle’s health maintenance; the coordinated processes of fusion, fission, and mitophagy contribute either to prevention of ECs dysfunction, safeguarding the survival of the cells [10], or to ECs death [8,21].

Besides mitochondria, ECs redox homeostasis is regulated by several other key controllers: (i) the peroxisome proliferator activated receptor-gamma coactivator 1α (PGC-1α), that transcriptionally regulates the mitochondrial antioxidant enzymes [25], (ii) the NO, that S-nitrosylates and inhibits the activity of mitochondrial complex I [26,27], (iii) the Ca²⁺ bioavailability, that stimulates *NO generation by eNOS [28], and (iv) the tetrahydrobiopterin (BH4), a redox-dependent modulator of mitochondrial signaling [29].

Taken together, the implications outlined above ascertain the regulatory and controller role of moderate mtROS levels in the normal and healthy ECs. In contrast, the overproduction of mtROS uncovers traits of mitochondrial dysfunction installment, with multiple consequences on the pathology of the vascular endothelium, as discussed next.

The Adverse Alterations in Mitochondrial Function Contribute to Type 2 Diabetes Mellitus-Related Endothelial Dysfunction

Mitochondrial dysfunction is biochemically described by ETC malfunction characterized by impaired OXPHOS, reduced ATP generation, and overproduction of mtROS. Based on its triggers, mitochondrial dysfunction is classified into two types: (a) the “primary mitochondrial dysfunction”, caused by mutations in either nuclear or mitochondrial DNA and central for the largest group of inborn errors of metabolism known as “mitochondrial disorders” and (b) the “secondary/acquired mitochondrial dysfunction”, caused by mutations that originate outside mitochondria, and are shared by the common forms of diabetes mellitus, inflammation, neoplasia, neurodegenerative and ischemic diseases, as well as by the ageing process [30-35].

In endothelium, mitochondrial dysfunction is installed under the influences of several circulating risk factors. An important endanger is the shear stress, defined as the tangential frictional force of blood flow acting on the vessel wall [36]. The perturbation of local hemodynamics engages the ECs mechano-sensors (such as G protein-coupled receptor), mechano-activated ion channels, growth factor receptors, glycoalyx, caveolae, membrane lipids, junction proteins, cytoskeleton network, integrins, focal adhesion kinase, etc. that all trigger the mecano-transduction process [37]. Among the biochemical consequences of the latter process, the literature quotes the modification of redox balance [11], activation of eNOS leading to increased NO bioavailability, and S-nitrosation of redox-sensitive cysteine-containing ECs proteins [36]. Interestingly, the steady laminar or the pulsatile shear stress and the flow subsequent a period of ischemia causes enhanced production of mtROS and RNS, mitochondrial fission and ECs propensity to apoptosis [21].

Other risk factors for mitochondrial dysfunction installment are the unusual biochemical compounds present in the blood in pathophysiologic circumstances. In diabetes mellitus, circulating hyperglycemia upregulates mtROS production, inhibits the intracellular buffering system of ROS, damages the mtDNA [1,38] and stimulates mitochondrial apoptosis via ROS generated from electrons transfer between cytochrome c and the 66-kDa Scc homology 2 domain-containing protein (p66Shc) [39]. The recent data show that high glucose concentration additionally induces p66Shc lysine acetylation, followed by its phosphorylation on serine 36 and the subsequent translocation to the mitochondria; at this specific location, acetylated p66Shc promotes H₂O₂ generation, augmenting mtROS levels [40]. The excessive mitochondrial O₂⁺ generation may alter histone methylation in ECs leading to chronic upregulation of nuclear factor kappa B (NF-kB) and to vascular inflammation [41]. Interestingly, the pre-diabetic condition is pro-inflammatory and associated with the release of mtDNA (a probable inducer of early endothelial dysfunction) and activation of the endothelial toll-like receptor 9 (TLR9) [42]. Furthermore, overproduction of mtROS shunts glucose to the hexosamine biosynthetic pathway (HBP); enhanced HBP activity inhibits protein subunits of mitochondrial respiratory complexes I, III, and IV and results in ATP depletion, along with the long-term activation of pro-inflammatory signaling pathways [43]. Published reports indicate that hyperglycemia causes defective mitochondrial biogenesis, mitochondrial fragmentation, excessive autophagy, and accumulation of toxic waste (such as ubiquitin and irreversibly depolarized mitochondria) within ECs [15,44,45]. The high concentration of FFA (like palmitic acid) or oxidized lipoproteins (like oxLDL) are also reported as risk factors for mitochondrial dysfunction induction. Thus, the growth of ECs under high palmitic acid conditions leads to a shift of cells metabolism toward the intensified oxidation of FFA associated with mitochondrial oxidative dysfunction [4]. Other reports on diabetic retinopathy show that lipotoxicity induced by high palmitic acid damages mtDNA, decreases cytochrome b transcripts, and augments glutotoxicity effects [40]. Furthermore, oxLDL promotes accumulation of mtROS and induces damage to the mtDNA, thus contributing to ECs dysfunction [46,47].

Based on the recent findings, it is important to update the latest data on the biomarkers of mitochondrial and endothelial dysfunction. It is generally admitted that mitochondrial dysfunction biomarkers consist
in excessive mtROS production, altered IMM potential (Δψm), an indicator of mitochondrial respiratory chain function/malfunction [48], mitochondrial DNA (mtDNA) damage [10], and ATP depletion; the latter process activates AMP-activated protein kinase (AMPK) signaling pathway leading to excessive autophagy and to ECs apoptosis [15]. Such adverse alterations have an important pathophysiologic role in Type 2 diabetes mellitus in humans [49].

An additional biomarker of mitochondrial dysfunction is the modification of morphology through dynamic fusion/fission events [6]. In ECs, mitochondrial tethering and fusion of their outer membranes (OMM) are mediated by the GTPase dynamin-like proteins mitofusin 1 (MFN1) and 2 (MFN2) [50]. The fusion of the IMM involves optic atrophy protein 1 (OPA1), another member of the GTP-ase dynamin-like proteins. The fusion roles are the preservation of mitochondrial network by complementation of damaged mtDNAs with healthy ones and the maintenance of membrane potential [51]. In diabetic conditions, mitochondrial dynamic changes are associated with increased abundance of fission inducers, such as dynamins-related protein-1 (DRP-1) and fission-1 protein (FIS1), and by mtROS overproduction [21,38].

The mitochondrial fission role consists in the removal of malfunctioning segment within fused mitochondria, preserving a population of healthy mitochondria. Recent reports emphasize that exposure of the human aorterios to low-glucose concentration activates the mitochondrial fission process [52]. In ECs, mitochondrial fission was found to be associated with oxidative stress, activation of the NLRP3 inflamasome, diabetes [38,53,54], and diabetes-accelerated atherosclerosis [55]. According to the novel developments, the occurrence of mitochondrial dysfunction in ECs is one of the causative factors in the pathophysiology of cardiovascular diseases [6,56-58].

Endothelial dysfunction was traditionally described by reduced *NO bioavailability, and impairment of the vascular wall relaxation in response to blood flow, agonists (acetylcholine, bradykinin), and several diseases such as diabetes mellitus, atherosclerosis, hypertension, heart failure, ischemia-reperfusion injury [2]. Moreover, ECs dysfunction is the first event in cardiovascular disease [21] and is a major stimulus of vascular aging [59].

The contemporary data point out to several factors/processes associated with endothelial dysfunction, as follows:

(i) The oxidative stress developed under pathophysiologic circumstances. In these conditions, besides mitochondria, a considerable number of enzymatic sources are engaged in endothelial ROS production, such as the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase isoforms (Nox) 2 and 4, the uncoupled eNOS, the cytosolic xanthine oxidase, and the decreased levels and/or activities of antioxidant superoxide dismutase (SOD) and catalase (CAT) [21,36,48]. The imbalance between aberrant ROS generation and antioxidant defense systems represents the primary cause of endothelial dysfunction, leading to vascular damage [60].

(ii) The nitrosative stress, characterized by increased RNS levels, the mechanisms involved consist in uncoupling of the NOS and oxidative damage to lipids, proteins and DNA [61]. At the crossroad between S-nitrosation and ROS, the S-nitrosating agent S-nitrosocysteine (CysNOS) was found to induce mitochondrial dysfunction in ECs, to cause nearly complete depletion of ATP and NAD+ and to trigger cells death [62].

(iii) The ER stress induces endothelial dysfunction by enhancement of NADPH oxidase activity along with oxidative stress and by the diminishing of eNOS activity associated with the impairment of endothelial-dependent vasorelaxation [63-65].

(iv) Endothelial dysfunction mobilizes also the coupling factor 6 (CF6, a component of mitochondrial ATP synthase), the Humanin (a 24-amino-acid peptide encoded by the 16s rRNA region of the mitochondrial genome that specifically influences mtROS generation), the cardiolipin (a phospholipid located at the IMM); when shifted to the OMM, cardiolipin activates the autophagic degradation of mitochondria [2].

(v) The advanced glycation end products (AGEs) promote the formation of Endothelial cell Specific Molecule-1 (ESM-1) (endocan), an endothelial dysfunction marker in diabetes [66]. The recent data show that AGEs trigger NF-κB- and activator protein-1 (AP-1)-mediated upregulation of Lysyl oxidase and Endothelin-1 via the AGE/RAGE/MAPK signaling cascade, being an aggravating factor for endothelial dysfunction [67].

(vi) The elevated expression of pro-inflammatory and pro-thrombotic factors [16] and the generation of microparticles with pro-adhesive and pro-coagulant properties [14] are other traits of pathology-related ECs dysfunction.

Taken together, the risk factors and the biomarkers outlined above emphasize the close relationship between mitochondrial and endothelial dysfunction, important triggers for the development and progression of organ damages [14].

The Endevours towards Correction of Mitochondrial Malfunction in Dysfunctional ECs

An outlook on dysfunction inducers (ordered according to their sources, i.e. the circulating blood, the ECs mitochondria, and ECs metabolism) in parallel with the specific modulators/suppressors involved in correction of each distinctive harmful effect is reviewed in Table 1. The insight into the mechanisms beyond alleviation of hyperglycemic mitochondrial and endothelial dysfunction outlined in Table 1 emphasizes two main correcting approaches: to point a distinct endothelial pathway and/or to target simultaneously several intracellular mechanisms employing a single modulator/suppressor. The distinct endothelial pathways targeted are:

(i) The improvement of eNOS activity by oral supplementation with L-arginine (the substrate of eNOS) [68,69], by nitrones that reverse eNOS dysfunction [70], or by intervention on posttranslational modifications, decreasing SOD2 ubiquitinilation [71] and signaling via wingless-type family member 5a (Wnt5a) and c-Jun N-terminal kinase (JNK) [72].

(ii) The counteraction of acute glucose fluctuations by insulin [73], the modulation of endogenous hydrogen sulfide (H2S) catabolism by overexpression of cystathionine-γ-lyase (CSE) [74],

(iii) The correction of renin-angiotensin system-mitochondrial damage by angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II type 1 receptor blockers (ARBs) [75],

(iv) The activation of the mitochondrial defense system [25,58,76,77],
(v) The activation of antioxidant genes expression by nuclear factor erythroid 2-related factor (Nrf2), a redox-sensitive transcription factor [78,79] and

(vi) The enrichment of mitochondria in silent mating-type information regulator 3 (SIRT3) deacetylase, leading to increased complex I activity and ATP synthesis [80].

A major role in dysfunction modulation is played by AMP kinase activation [81-87], a metabolic stress sensor implicated in mitochondrial fission (by DRP1 phosphorylation at Ser 637) [53-55], potentiation of mitochondrial biogenesis (by SIRT1, that activates PGC-1α-mediated transcription of nuclear and mitochondrial genes) [88-90], modulation of ‘NO generation [57] and stress adaptation via signaling through eNOS-dependent mammalian target of rapamycin complex 1 (mTORC1) pathway [91]. Moreover, the physiological substrate of AMPK is glutamine: fructose-6-phosphate amidotransferase 1 (GFAT1), the rate-limiting enzyme in the HBP that controls the post-translational modification of proteins by O-GlcNAcylation [92,93].

An ongoing endeavor points toward the amelioration of mitochondrial ROS overproduction by delivery of antioxidants that selectively target mitochondria, and concentrate at the matrix surface (MitoQ 10) [97,98] and plastocyanin decyltriphenylphosphonium compounds and strategies aiming regulation of the mitochondrial antioxidant defense system (Table 1).

Efficient are also the scavengers against the free radicals, such as the mitochondria-targeting SOD mimetic MitoTEMPOL [49,71], metallothioneins (low molecular weight, cysteine-rich and heavy metal binding proteins) [100,101], polyphenols, like resveratrol [102] and the isoflavone kakkalide, that additionally restore mitochondrial membrane potential [103]. Furthermore, the mitochondrial uncoupling agents, carbonyl cyanide m-chlorophenyl hydrazone (CCCP), 2,4-dinitrophenol (2,4-DNP), and Uncoupling protein 2 (UCP2) are examples of alleviators directed towards amelioration of mitochondrial antioxidant defense system (Table 1).

Among the modulators/suppressors that target different intracellular pathways, the recent reports mention metformin, which promotes mitochondrial biogenesis by activation of PGC-1α [84,106], and several genetic manipulations. The silencing of Prolyl-isomerase 1 (Pin 1) gene suppresses mitochondrial translocation of pro-oxidant adaptor p66 (Shc) and the subsequent organelle disruption. This procedure is also effective in restoring ‘NO release by ECs, and in blunting NF-κB p65 nuclear translocation known to trigger expression of adhesion molecules, e.g. the Vascular cell adhesion protein 1 (VCAM-1), Intercellular adhesion molecule 1 (ICAM-1) and Monocyte chemoattractant protein-1 (MCP-1) [107].

A recent example is the silencing of FIS1 or DRP1 protein expression (with siRNA) that conducts to inhibition of mitochondrial fission, of high glucose-induced alterations in mitochondrial networks, and of ROS generation; these effects were concurrent with eNOS activation and cGMP production [38].

Another strategy consists in suppressing the expression of endogenous voltage-gated K+ (Kv) channel pore subunit Kv1.5 (Kv1.5); the outcome is inhibition of two intracellular pathways: mitochondria-mediated ROS generation and the apoptotic signaling pathway [108]. Furthermore, activation of AMPKα by C-peptide corrects mitochondrial dynamics alterations (by inhibiting mitochondrial fission and mitochondrial membrane potential collapse), prevents ROS generation induced by high glucose concentration, and impedes ECs apoptosis [109].

The targets and the intracellular mechanisms described above and in Table 1 emerge from studies on ECs in culture and in vivo (animal models or human vessels). However, for a target-to-treatment strategy clinicians should be aware that mitochondrial dysfunction signature may vary according to the vascular bed under study, heterogeneity of endothelium, and diversity of (patho) physiological risk factors involved. Consequently, the current efforts to prevent and/or to correct the mitochondrial malfunction in dysfunctional ECs are still an ongoing endeavor.

The Key Points of Mitochondria-Targeted Interventions in Diabetic Patients

Initially, mitochondrial medicine anticipated the application of mitochondrial-directed interventions in the treatment of patients affected by “primary mitochondrial dysfunction” [31,110]. The use of mitochondrialtopics, mitochondriotoxics, mitocancerotropics, and mitocans (mitochondria-targeted anticancer drugs) to treat “mitochondrial diseases” has been discussed in excellent reviews [35,111].

However, besides the management of symptoms, there are no effective therapies for “mitochondrial disorders” mitigation [34].

The “secondary mitochondrial dysfunction” associated with diabetes mellitus and insulin resistance is a distinct topic that requires appropriate allocators able to slow down the associated cardiovascular pathology and their symptoms (Table 1).

Further insight into this topic is an urgent task, according to the prediction of a huge number of diabetic patients (439 million) by 2030 and of the higher frequency of this pathology at people aged between 40 and 59 years [42,59].

What are the key points of mitochondria-targeted interventions in diabetic patients? The current strategies are based on data obtained by in vitro investigation of human cells; their translation to Type 2 diabetic patients concluded with FDA-approved mitochondrial biogenic agents, such as the AMPK activator metformin [85] and the PPARY agonist Rosiglitazone [112] and the launch of Imliglomin as an oral glucose-lowering agent that completed a phase 2b clinical trial (US/EU EudraCT number 2012-00404S-33) [113].

Other strategies considered in the diabetes treatment target the disturbed intracellular events, such as suppression of augmented mitochondrial fission [21,38,55], activation of mitochondrial biogenesis [112,114-116], mitochondrial Sirtuins [1,88], and the UCP2 pathway [117].

A caution related to the mitochondria-targeted therapeutic interventions emphasizes that some diabetes-related pathologies might not have solely a mitochondrial origin, but mitochondria might interfere either at their inception or during their development [115].

Thus, mitochondria-targeted antioxidants have been found efficient in all the major vascular beds disturbed by diabetes, i.e. myocardium (preventing cardiomyocytes loss and the reduced energy supply), kidney, neurodegenerative and eye diseases [56,118-121].
Other mitochondrial-based potential treatments of renal diseases include genetic therapies, thiazolidinediones, Sirtuins, and resveratrol [34,56]. However, the therapeutic procedures directed specifically towards dysfunctional mitochondria of diabetic vascular endothelium in humans are still waiting to be uncovered.

| Dysfunction inductors | Modulators/Suppressors aiming dysfunction alleviation | References |
|-----------------------|------------------------------------------------------|------------|
| **From circulating blood** | | |
| High glucose concentration | L-arginine, Heme oxygenase-1 upregulation, ACEIs, ARBs, Metformin | [68,69], [78], [75], [82] |
| Acute glucose fluctuations | insulin | [73] |
| Low glucose concentration | Metformin, Mito-TEMPOL, DRP1 | [81], [52] |
| Free fatty acids/ Palmitic acid | corosolic acid, exogenous H$_2$S, Kv1.5 suppression | [53], [15], [108] |
| AGEs | Activation of Nrf2-dependent antioxidant gene expression, Anemarrhena asphodeloides polyphenols | [78], [86] |
| Decreased H$_2$S level | exogenous H$_2$S, Overexpression of CSE | [15], [74] |
| **From mitochondria** | | |
| Excessive mtROS | **Mitochondria-targeted antioxidants**<br>MitoQ(10), SkQ1, Resveratrol, paroxetine, **Mitochondrial ROS scavengers**<br>MitoTEMPOL, Metallothioneins, **Mitochondrial uncoupling agents**<br>CCCP, 2,4-DNP, UCP2, **Regulation of mitochondrial antioxidant defense system**<br>Metformin, PGC-1α overexpression, Diallyl trisulfide, FIS1 or DRP1 expression silencing, Pin1 gene silencing, Nitrones spin traps, alpha-phenyl N-tertiary-butyl nitrone, 5,5-dimethyl-pyrroline N-oxide | [94,95,96], [83,97,98], [99], [80], [117], [49,71], [100,101], [49], [104], [58,105] |
| PTP opening | Metformin | [113] |
| Mitochondrial dynamics alteration | **Inhibitors of mitochondrial fission**<br>Metformin, corosolic acid, d-chiro inositol, C-peptide activation of AMPKα | [55], [53], [103], [109] |
FIS1 or DRP1 expression silencing

Reduced mitochondrial biogenesis

Mitochondrial biogenesis stimulation:

- SIRT 1
- Metformin
- Thiazolidinediones
- Resveratrol
- Esculetin
- C1q/TNF-related protein9
- 5-Aminimidazole-4-carboxamide ribonucleotide

From endothelial cell metabolism

eNOS uncoupling

- eNOS activation
- inhibition of Wnt5a and JNK signaling

Oxidative stress – ROS/RNS

- Antioxidant defense stimulation
  - corosolic acid
  - d-chiro inositol
  - isoflavone Kakkalide
  - C-peptide activation of AMPKα

Acetylation of p66Shc under HG conditions and ROS production

- Sir1-regulated p66Shc deacetylation on lysine 81
- Pin1 gene silencing

HBP AMPK

gene expression of lysyl oxidase and endothelin-1

- NF-kB, AP-1

autophagy

- natural flavonol Ampelopsin

apoptosis

- C-peptide activation of AMPKα
- Kv1.5 suppression

nuclear translocation of NF-κB p65

Pin1 gene silencing

| Table 1: Mitochondrial and endothelial dysfunction in diabetes (*results from human endothelial cells). |

From Current Pending Issues to New Research Directions and Further Innovative Therapeutic Strategies in Diabetes

The two main malfunctioning targets in diabetes, mitochondria, and endothelium are currently addressed by strategies aiming both dysfunction alleviation and a potential therapeutic benefit. An urgent call is directed towards mitochondrial interventions, which have “the momentum” now [110].

Among the mitochondria-associated pending issues, the following have been quoted:

1. The still unknown percentage of mtROS in ECs in vivo, the less exploited mitochondria networking and crosstalk with other organelles, and the mitochondrial signaling pathways [3,10,122]. Recently, it was mentioned that disturbance of Ca²⁺ homeostasis deserves advanced studies to support the development of novel therapeutics aiming prevention and medication of insulin resistance and Type 2 diabetes [123].

2. Pharmacological therapies aiming inhibition of excessive mitochondrial fission and promotion of the fusion process [6,10,124,125]. Nowadays, mitochondrial pharmacology emerges as a distinct area of great expectations in improving/chemoprevention of mitochondrial dysfunction [126,127]. Precautions should be taken in therapeutic translation of the pharmacologic drugs; these must be previously checked for the specific delivery to ECs mitochondria, for the effects on mtDNA (avoiding its damage), for local efficacy (while preserving the viability of other vascular cells), for their safety, and potential side effects [30,117,124,127-130].

3. The metabolomic approach on mitochondrial metabolites outlines a promising novel direction in diabetes early detection and treatment [1,34,130].

Related to dysfunctional endothelium, the incompletely understood issues that may stress promising innovative directions in the diabetes treatment are:

1. The characterization of endothelial metabolism [131,132] including by metabolomic approaches [133]. Treatments targeting perturbations of ECs metabolism are not available now [134]. In this context, inhibition of ER stress [135] and the use of H₂S to balance the
cells redox system [8,15] are promising directions towards dysfunction modulation.

2. The necessity to measure endothelial function, focusing on oxidative stress impact, heme synthesis and heme oxygenase activity [10,136].

3. The still unclear mechanisms behind the novel coined “glycoredox” interaction, i.e. the association between the redox responses and the glycan function [137].

Conclusion

This review discusses the latest insights on diabetes mellitus-associated vascular endothelial cell dysfunction and highlights the novel regulatory and controller function of mitochondria. The basic knowledge provided here may encourage the teamwork of bench side and bedside scientists to advance into the domain of mitochondrial medicine, with significant potential for treatment of diabetic vascular dysfunctions.

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