Journal of Clinical & Experimental Pathology

Doina Popov, J Clin Exp Pathol 2018, 8:1
DOI: 10.4172/2161-0681.1000334

Review Article Open Access

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

Received date: December 18, 2017; **Accepted date:** January 9, 2018; **Published date:** January 12, 2018

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 Ca^{2+} into the cytosol, where accumulate within microdomains close to the inner aspect of ECs plasmalemma. As mitochondria are anchored in this particular location, Ca^{2+} enter their matrix, and activate the key Ca^{2+}-sensitive enzymes (e.g. Ca^{2+}-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 ($O_2^{\bullet -}$) and with a diminished formation of nitric oxide ($^{\bullet}NO$) an endothelium-dependent vasorelaxant [14]. The low $^{\bullet}NO$ levels are caused also by the rapid reaction with $O_2^{\bullet -}$ and formation of the potent oxidant peroxinitrite anions ($OONO^-$) 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 $O_2^{\bullet -}$ (half-life in the range of nano-to milliseconds) and hydroxyl radicals

(•OH) (half-life of few nanoseconds) and (ii) the hydrogen peroxide (H_2O_2), 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 $O_2^{\bullet-}$ into the mitochondrial matrix, where manganese superoxide dismutase (MnSOD or SOD2) rapidly convert $O_2^{\bullet-}$ to H_2O_2; furthermore, the polypeptide complex III releases the $O_2^{\bullet-}$ both into the matrix and the intermembrane space; in the latter location, copper-zinc superoxide dismutase (CuZnSOD or SOD1) convert the $O_2^{\bullet-}$ into H_2O_2 [17,18]. The H_2O_2 molecules either diffuse into the cytosol or interact with mitochondrial proteins; in the presence of Fe^{2+} or Cu^{2+} ions, H_2O_2 molecules are transformed into •OH (a potent ROS) *via* a Fenton reaction. In an additional pathway, local glutathione peroxidase decomposes H_2O_2 to H_2O and O_2. An earlier report demonstrated the involvement of H_2O_2 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 distinctive mechanisms [2,20]: (i) generation of moderate levels of mtROS, and regulation of their conversion and/or scavenge during cells response to shear stress [3,8,11,21], (ii) operation of mtROS as signaling molecules, (iii) involvement in eNOS activation and Ca^{2+} 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 Ca^{2+} 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 malfunctions 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, glycocalyx, 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 Src 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 H_2O_2 generation, augmenting mtROS levels [40]. The excessive mitochondrial $O_2^{\bullet-}$ generation may alter histone methylation in ECs leading to chronic upregulation of nuclear factor kappa B (NF-κB) 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 FFAs 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 glucotoxicity 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 IMMs 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 inductors, such as dynamin-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 arterioles 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 inflammasome, 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 $^{\bullet}$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 (CysNO) 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 diminishment 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 Endeavours towards Correction of Mitochondrial Malfunction in Dysfunctional ECs

An outlook on dysfunction inductors (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 (H_2S) 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 of IMM [94-96]. Encouraging results were reported for mitoquinone (MitoQ 10) [97,98] and plastoquinonyl decyltriphenylphosphonium1 (SkQ1) [99] that ameliorate antioxidant status, and for several other 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 ROS overproduction [49,58,104,105].

Among the modulators/suppressors that target different intracellular pathways, the recent reports mention metformin that 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-kB 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^+ (K_V) channel pore subunit $K_V1.5$ (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-treat 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 mitochondriotropics, 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 alleviators 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 PPARγ agonist Rosiglitazone [112] and the launch of Imeglimin as an oral glucose-lowering agent that completed a phase 2b clinical trial (US/EU EudraCT number 2012-004045-33) [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	[68*,69]
	Heme oxygenase-1 upregulation	[78]
	ACEIs, ARBs	[75]
	Metformin	[82]*
Acute glucose fluctuations	insulin	[73]*
Low glucose concentration	Metformin, Mito-TEMPOL	[81]*
	DRP1	[52]*
Free fatty acids/ Palmitic acid	corosolic acid	[53]
	exogenous H_2S	[15]
	Kv1.5 suppression	[108]
AGEs	Activation of Nrf2-dependent antioxidant gene expression	[78]
	Anemarrhena asphodeloides polyphenols	[86]
Decreased H_2S level	exogenous H_2S	[15]
	Overexpression of CSE	[74]
From mitochondria		
Excessive mtROS	*Mitochondria-targeted antioxidants*	[94*,95,96]
	MitoQ(10)	[83,97,98]
	SkQ1	[99]
	Resveratrol	[80]*
	paroxetine	[117]
	Mitochondrial ROS scavengers	
	MitoTEMPOL	[49*,71]
	Metallothioneins	[100,101]
	Mitochondrial uncoupling agents	
	CCCP	[49]*
	2,4-DNP	[104]*
	UCP2	[58,105]
	Regulation of mitochondrial antioxidant defense system	
	Metformin	[84]*
	PGC-1α overexpression	[25]*
	Diallyl trisulfide	[76]
	FIS1 or DRP1 expression silencing	[38]*
	Pin1 gene silencing	[107]*
	Nitrones spin traps	
	alpha-phenyl N-tertiary-butyl nitrone, 5,5-dimethyl-pyrroline N-oxide	[70]
PTP opening	Metformin	[113]*
Mitochondrial dynamics alteration	*Inhibitors of mitochondrial fission*	
	Metformin	[55]
	corosolic acid	[53]
	d-chiro inositol	[103]
	C-peptide activation of AMPKα	[109]*

	FIS1 or DRP1 expression silencing	[38]
Reduced mitochondrial biogenesis	*Mitochondrial biogenesis stimulation*: SIRT 1 Metformin Thiazolidinediones Resveratrol Esculetin C1q/TNF-related protein9 5-Aminoimidazole-4-carboxamide ribonucleotide	 [1], [89]* [84]* [106]* [34,102*,114] [57] [90] [34]
From endothelial cell metabolism		
eNOS uncoupling	eNOS activation inhibition of Wnt5a and JNK signaling	[70,91] [72]*
Oxidative stress – ROS/RNS	*Antioxidant defense stimulation* corosolic acid d-chiro inositol isoflavone Kakkalide C-peptide activation of AMPKα	 [53] [54] [54]* [109]
Acetylation of p66Shc under HG conditions and ROS production	Sirt1-regulated p66Shc deacetylation on lysine 81 Pin1 gene silencing	[40] [107]*
HBP	AMPK	[92]
gene expression of lysyl oxidase and endothelin-1	NF-kB, AP-1	[67]*
autophagy	natural flavonol Ampelopsin	[87]*
apoptosis	C-peptide activation of AMPKα Kv1.5 suppression	[109] [108]
nuclear translocation of NF-κB p65	Pin1 gene silencing	[107]*

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 Ca^{2+} 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 H_2S 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.

References

1. Tang X, Luo Y X, Chen H Z, Liu D P (2014) Mitochondria, endothelial cell function, and vascular diseases. Front Physiol 5: 175.
2. Kadlec AO, Beyer AM, Ait-Aissa K, Gutterman DD (2016) Mitochondrial signaling in the vascular endothelium: beyond reactive oxygen species. Basic Res Cardiol 111: 26.
3. Groschner LN, Waldeck-Weiermair M, Malli R, Graier WF (2012) Endothelial mitochondria-less respiration, more integration. Pflugers Arch 464: 63-76.
4. Broniarek I, Koziel A, Jarmuszkiewicz W (2016) The effect of chronic exposure to high palmitic acid concentrations on the aerobic metabolism of human endothelial EA.hy926 cells. Pflugers Arch 468: 1541-1554.
5. Teuwen LA, Draoui N, Dubois C, Carmeliet P (2017) Endothelial cell metabolism: an update anno 2017. Curr Opin Hematol 24: 240-247.
6. Lahera V, de Las Heras N, López-Farré A, Manucha W, Ferder L (2017) Role of mitochondrial dysfunction in hypertension and obesity. Curr Hypertens Rep 19: 11.
7. Missiaen R, Morales-Rodriguez F, Eelen G, Carmeliet P (2017) Targeting endothelial metabolism for anti-angiogenesis therapy: A pharmacological perspective. Vascul Pharmacol 90: 8-18.
8. Song P, Zou MH (2014) Redox regulation of endothelial cell fate. Cell Mol Life Sci 71: 3219-3239.
9. Li X, Fang P, Yang WY, Chan K, Lavallee M, et al. (2017) Mitochondrial ROS, uncoupled from ATP synthesis, determine endothelial activation for both physiological recruitment of patrolling cells and pathological recruitment of inflammatory cells. Can J Physiol Pharmacol 95: 247-252.
10. Kluge MA, Fetterman JL, Vita JA (2013) Mitochondria and endothelial function. Circ Res 112: 1171-1188.
11. Panieri E, Santoro MM (2015) ROS signaling and redox biology in endothelial cells. Cell Mol Life Sci 72: 3281-3303.
12. Marín-García J (2013) Endothelial mitochondria: Contribution to cardiovascular function and disease. Mitochondria and their role in cardiovascular disease 147-155.
13. Harjes U, Kalucka J, Carmeliet P (2016) Targeting fatty acid metabolism in cancer and endothelial cells. Crit Rev Oncol Hematol 97: 15-21.
14. Konukoglu D, Uzun H (2017) Endothelial dysfunction and hypertension. Adv Exp Med Biol 956: 511-540.
15. Liu J, Wu J, Sun A, Sun Y, Yu X, et al. (2016) Hydrogen sulfide decreases high glucose /palmitate-induced autophagy in endothelial cells by the Nrf2-ROS-AMPK signaling pathway. Cell Biosci 6: 33.
16. Jia G, Sowers JR (2014) Endothelial dysfunction potentially interacts with impaired glucose metabolism to increase cardiovascular risk. Hypertension 64: 1192-1193.
17. Muller FL, Liu Y, Van Remmen H (2004) Complex III releases superoxide to both sides of the inner mitochondrial membrane. J Biol Chem 279: 49064-49073.
18. Gutteridge JM, Halliwell B (2000) Free radicals and antioxidants in the year 2000. A historical look to the future. Ann N Y Acad Sci 899: 136-147.
19. Liu Y, Zhao H, Li H, Kalyanaraman B, Nicolosi AC, et al. (2003) Mitochondrial sources of H2O2 generation play a key role in flow-mediated dilation in human coronary resistance arteries. Circ Res 93: 573-580.
20. Widlansky ME, Gutterman DD (2011) Regulation of endothelial function by mitochondrial reactive oxygen species. Antioxid Redox Signal 15: 1517-1530.
21. Scheitlin CG, Crestanello JA, Zweier JL, Alevriadou BR (2014) Fluid mechanical forces and endothelial mitochondria: A bioengineering perspective. Cell Mol Bioeng 7: 483-496.
22. Guo R, Si R, Scott BT, Makino A (2017) Mitochondrial connexin 40 regulates mitochondrial calcium uptake in coronary endothelial cells. Am J Physiol Cell Physiol 312: C398-C406.
23. Katakam PV, Wappler EA, Katz PS, Rutkai I, Institoris A, et al. (2013) Depolarization of mitochondria in endothelial cells promotes cerebral artery vasodilation by activation of nitric oxide synthase. Arterioscler Thromb Vasc Biol 33: 752-759.
24. Widlansky ME, Malik MA (2015) Vascular endothelial function, in PanVascular Medicine, P. Lanzer (ed.), Chapter 4, Springer-Verlag Berlin Heidelberg 2015, pp. 89-120.
25. Valle I, Alvarez-Barrientos A, Arza E, Lamas S, Monsalve M (2005) PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells. Cardiovasc Res 66: 562-573.
26. Burwell LS, Nadtochiy SM, Tompkins AJ, Young S, Brookes PS (2006) Direct evidence for S-nitrosation of mitochondrial complex I. Biochem J 394: 627-634.
27. Erusalimsky JD, Moncada S (2007) Nitric oxide and mitochondrial signaling: from physiology to pathophysiology. Arterioscler Thromb Vasc Biol 27: 2524-2531.
28. Dedkova EN, Ji X, Lipsius SL, Blatter LA (2004) Mitochondrial calcium uptake stimulates nitric oxide production in mitochondria of bovine vascular endothelial cells. Am J Physiol Cell Physiol 286: C406-C415.
29. Bailey J, Shaw A, Fischer R, Ryan BJ, Kessler BM, et al. (2017) A novel role for endothelial tetrahydrobiopterin in mitochondrial redox b alance. Free Radic Biol Med 104: 214-225.
30. Smith RA, Hartley RC, Cochemé HM, Murphy MP (2012) Mitochondrial pharmacology. Trends Pharmacol Sci 33: 341-352.
31. Parikh S, Goldstein A, Koenig MK, Scaglia F, Enns GM, et al. (2015) Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genet Med 17: 689-701.
32. Viscomi C, Bottani E, Zeviani M (2015) Emerging concepts in the therapy of mitochondrial disease. Biochim Biophys Acta 1847: 544-557.
33. Karaa A, Goldstein A (2015) The spectrum of clinical presentation, diagnosis, and management of mitochondrial forms of diabetes. Pediatr Diabetes 16: 1-9.
34. Komen JC, Thorburn DR (2014) Turn up the power - pharmacological activation of mitochondrial biogenesis in mouse models. Br J Pharmacol 171: 1818-1836.
35. Guzman-Villanueva D, Weissig V (2017) Mitochondria-targeted agents: Mitochondriotropics, Mitochondriotoxics, and Mitocans. Handb Exp Pharmacol 240: 423-438.
36. Hsieh HJ, Liu CA, Huang B, Tseng AH, Wang DL (2014) Shear-induced endothelial mechanotransduction: the interplay between reactive oxygen

species (ROS) and nitric oxide (NO) and the pathophysiological implications. J Biomed Sci 21: 3.

37. Chien S (2007) Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. Am J Physiol Heart Circ Physiol 292: H1209-H1224.
38. Shenouda SM, WidlanskyME, Chen K., Xu G, HolbrookM, et al. (2011) Altered mitochondrial dynamics contributes to endothelial dysfunction in diabetes mellitus. Circulation 124: 444-453.
39. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, et al. (2005) Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. Cell 122: 221-233.
40. Kumar S, Kim YR, Vikram A, Naqvi A, Li Q, et al. (2017) Sirtuin1-regulated lysine acetylation of p66Shc governs diabetes-induced vascular oxidative stress and endothelial dysfunction. Proc Natl Acad Sci U S A 114: 1714-1719.
41. Keating ST, El-Osta A (2012) Chromatin modifications associated with diabetes. J Cardiovasc Transl Res 5: 399-412.
42. Alvarado-Vásquez N (2015) Circulating cell-free mitochondrial DNA as the probable inducer of early endothelial dysfunction in the prediabetic patient. Exp Gerontol 69: 70-78.
43. Stefano GB, Challenger S, Kream RM (2016) Hyperglycemia-associated alterations in cellular signaling and dysregulated mitochondrial bioenergetics in human metabolic disorders. Eur J Nutr 55: 2339-2345.
44. Makino A, Scott BT, DillmannWH (2010) Mitochondrial fragmentation and superoxide anion production in coronary endothelial cells from a mouse model of type 1 diabetes. Diabetologia 53: 1783-1794.
45. Zecchin A, Brajic A, Carmeliet P (2015) Targeting endothelial cell metabolism: new therapeutic prospects? Front Biol 10: 125-140.
46. Ballinger SW, Patterson C, Knight-Lozano CA, Burow DL, Conklin CA, et al. (2002) Mitochondrial integrity and function in atherogenesis. Circulation 106: 544-549.
47. Pircher A, Treps L, Bodrug N, Carmeliet P (2016) Endothelial cell metabolism: A novel player in atherosclerosis? Basic principles and therapeutic opportunities. Atherosclerosis 253: 247-257.
48. Ren X, Ren L, Wei Q, Shao H, Chen L, et al. (2017) Advanced glycation end-products decreases expression of endothelial nitric oxide synthase through oxidative stress in human coronary artery endothelial cells. Cardiovasc Diabetol 16: 52.
49. Kizhakekuttu TJ, Wang J, Dharmashankar K, Ying R, Gutterman DD, et al. (2012) Adverse alterations in mitochondrial function contribute to type 2 diabetes mellitus-related endothelial dysfunction in humans. Arterioscler Thromb Vasc Biol 32: 2531-2539.
50. Daumke O, Roux A (2017) Mitochondrial homeostasis: How do dimmers of mitofusins mediate mitochondrial fusion? Curr Biol 27: R353-R356.
51. Cao YL, Meng S, Chen Y, Feng JX, Gu DD, et al. (2017) MFN1 structures reveal nucleotide-triggered dimerization critical for mitochondrial fusion. Nature 542: 372-376.
52. Tanner MJ, Wang J, Ying R, Suboc TB, Malik M, et al. (2017) Dynamin related protein 1 mediates low glucose-induced endothelial dysfunction in humanarterioles. Am J Physiol Heart Circ Physiol 312: H515-H527.
53. Li Y, Zhou ZH, Chen MH, Yang J, Leng J, et al. (2016) Inhibition of mitochondrial fission and NOX2 expression prevent NLRP3 inflammasome activation in the endothelium: The role of corosolic acid action in the amelioration of endothelial dysfunction. Antioxid Redox Signal 24: 893-908.
54. Zhang B, Guo X, Li Y, Peng Q, Gao J, et al. (2017) d-Chiro inositol ameliorates endothelial dysfunction via inhibition of oxidative stress and mitochondrial fission. Mol Nutr Food Res 61.
55. Wang Q, Zhang M, Torres G, Wu S, Ouyang C, et al. (2017) Metformin suppresses diabetes-accelerated atherosclerosis via the inhibition of Drp1-mediated mitochondrial fission. Diabetes 66: 193-205.
56. Che R, Yuan Y, Huang S, Zhang A (2014) Mitochondrial dysfunction in the pathophysiology of renal diseases. Am J Physiol Renal Physiol 306: F367-F378.
57. Karnewar S, Vasamsetti SB, Gopoju R, Kanugula AK, Ganji SK, et al. (2016) Mitochondria-targeted esculetin alleviates mitochondrial dysfunction by AMPK-mediated nitric oxide and SIRT3 regulation in endothelial cells: potential implications in atherosclerosis. Sci Rep 6: 24108.
58. Xiong S, Wang P, Ma L, Gao P, Gong L, et al. (2016) Ameliorating endothelial mitochondrial dysfunction restores coronary function via Transient Receptor Potential Vanilloid 1-mediated Protein Kinase A/Uncoupling Protein 2 pathway. Hypertension 67: 451-460.
59. Mikhed Y, Daiber A, Steven S (2015) Mitochondrial oxidative stress, mitochondrial DNA damage and their Rrole in age-related vascular dysfunction. Int J Mol Sci 16: 15918-15953.
60. Incalza MA, D'Oria R, Natalicchio A, Perrini S, Laviola L, et al. (2017) Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. Vascul Pharmacol S1537-1891: 30113-1.
61. Mozos I, Luca CT (2017) Crosstalk between oxidative and nitrosative stress and arterial stiffness. Curr Vasc Pharmacol 15: 446-456.
62. Diers AR, Broniowska KA, Hogg N (2013) Nitrosative stress and redox-cycling agents synergize to cause mitochondrial dysfunction and cell death in endothelial cells. Redox Biol 1: 1-7.
63. Galán M, Kassan M, Kadowitz PJ, Trebak M, Belmadani S, et al. (2014) Mechanism of endoplasmic reticulum stress-induced vascular endothelial dysfunction. Biochim Biophys Acta 1843: 1063-1075.
64. Choy KW, Lau YS, Murugan D, Mustafa MR (2017) Chronic treatment with paeonol improves endothelial function in mice through inhibition of endoplasmic reticulum stress-mediated oxidative stress. PLoS One 12: e0178365.
65. Dong Y, Fernandes C, Liu Y, Wu Y, Wu H, et al. (2017) Role of endoplasmic reticulum stress signaling in diabetic endothelial dysfunction and atherosclerosis. Diab Vasc Dis Res 14: 14-23.
66. Nirala BK, Perumal V, Gohil NK (2015) Glycated serum albumin stimulates expression of endothelial cell specific molecule-1 in human umbilical vein endothelial cells: Implication in diabetes mediated endothelial dysfunction. Diab Vasc Dis Res 12: 290-297.
67. Adamopoulos C, Piperi C, Gargalionis AN, Dalagiorgou G, Spilioti E, et al. (2016) Advanced glycation end products upregulate lysyl oxidase and endothelin-1 in human aortic endothelial cells via parallel activation of ERK1/2-NF-κB and JNK-AP-1 signaling pathways. Cell Mol Life Sci 73: 1685-1698.
68. Monti LD, Casiraghi MC, Setola E, Galluccio E, Pagani MA, et al. (2013) L-arginine enriched biscuits improve endothelial function and glucose metabolism: a pilot study in healthy subjects and a cross-over study in subjects with impaired glucose tolerance and metabolic syndrome. Metabolism 62: 255-264.
69. Popov D, Costache G, Georgescu A, Enache M (2002) Beneficial effects of L-arginine supplementation in experimental hyperlipemia-hyperglycemia in the hamster. Cell Tissue Res 308: 109-120.
70. Headley CA, DiSilvestro D, Bryant KE, Hemann C, Chen CA, et al. (2016) Nitrones reverse hyperglycemia-induced endothelial dysfunction in bovine aortic endothelial cells. Biochem Pharmacol 104: 108-117.
71. Cho YE, Basu A, Dai A, Heldak M, Makino A (2013) Coronary endothelial dysfunction and mitochondrial reactive oxygen species in type 2 diabetic mice. Am J Physiol Cell Physiol 305: C1033-1040.
72. Bretón-Romero R, Feng B, Holbrook M, Farb MG, Fetterman JL, et al. (2016) Endothelial dysfunction in human diabetes is mediated by Wnt5a-JNK signaling. Arterioscler Thromb Vasc Biol 36: 561-569.
73. Horová E, Mazoch J, Hiigertová J, Kvasnička J, Skrha J, et al. (2012) Acute hyperglycemia does not impair microvascular reactivity and endothelial function during hyperinsulinemic isoglycemic and

hyperglycemic clamp in type 1 diabetic patients. Exp Diabetes Res 851487.

74. Suzuki K, Olah G, Modis K, Coletta C, Kulp G, et al. (2011) Hydrogen sulfide replacement therapy protects the vascular endothelium in hyperglycemia by preserving mitochondrial function. Proc Natl Acad Sci U S A 108: 13829-13834.
75. Chen F, Chen B, Xiao FQ, Wu YT, Wang RH, et al. (2014) Autophagy protects against senescence and apoptosis via the RAS-mitochondria in high-glucose- induced endothelial cells. Cell Physiol Biochem 33: 1058-1074.
76. Liu LL, Yan L, Chen YH, Zeng GH, Zhou Y, et al. (2014) A role for diallyl trisulfide in mitochondrial antioxidative stress contributes to its protective effects against vascular endothelial impairment. Eur J Pharmacol 725: 23-31.
77. Beyer AM, Freed JK, Durand MJ, Riedel M, Ait-Aissa K, et al. (2016) Critical role for telomerase in the mechanism of flow-mediated dilation in the human microcirculation. Circ Res 118: 856-866.
78. He M, Siow RC, Sugden D, Gao L, Cheng X, et al. (2011) Induction of HO-1 and redox signaling in endothelial cells by advanced glycation end products: a role for Nrf2 in vascular protection in diabetes. Nutr Metab Cardiovasc Dis 21: 277-285.
79. He M, Nitti M, Piras S, Furfaro AL, Traverso N, et al. (2015) Heme oxygenase-1-derived bilirubin protects endothelial cells against high glucose-induced damage. Free Radic Biol Med 89: 91-98.
80. Zhou X, Chen M, Zeng X, Yang J, Deng H, et al. (2014) Resveratrol regulates mitochondrial reactive oxygen species homeostasis through Sirt3signaling pathway in human vascular endothelial cells. Cell Death Dis. 5: e1576.
81. Wang J, Alexanian A, Ying R, Kizhakekuttu TJ, Dharmashankar K, et al. (2012) Acute exposure to low glucose rapidly induces endothelial dysfunction and mitochondrial oxidative stress: role for AMP kinase. Arterioscler Thromb Vasc Biol 32: 712-720.
82. Kinaan M, Ding H, Triggle CR (2015) Metformin: An old drug for the treatment of diabetes but a new drug for the protection of the endothelium. Med Princ Pract 24: 401-415.
83. Mackenzie RM, Salt IP, Miller WH, Logan A, Ibrahim HA, et al. (2013) Mitochondrial reactive oxygen species enhance AMP-activated protein kinase activation in the endothelium of patients with coronary artery disease and diabetes. Clin Sci (Lond) 124: 403-411.
84. Kukidome D, Nishikawa T, Sonoda K, Imoto K, Fujisawa K, et al. (2006) Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. Diabetes 55: 120-127.
85. Kristensen JM, Larsen S, Helge JW, Dela F, Wojtaszewski JFP (2013) Two weeks of Metformin treatment enhances mitochondrial respiration in skeletal muscle of AMPK Kinase dead but not wild type mice. PloS one 8: e53533.
86. Zhao Q, Sun Y, Ji Y, Xu L, Liu K, et al. (2014) Total polyphenol of Anemarrhena asphodeloides ameliorates advanced glycation end products-induced endothelial dysfunction by regulation of AMP-Kinase. J Diabetes 6: 304-315.
87. Liang X, Zhang T, Shi L, Kang C, Wan J, et al. (2015) Ampelopsin protects endothelial cells from hyperglycemia-induced oxidative damage by inducing autophagy via the AMPK signaling pathway. Biofactors 41: 463-475.
88. Brenmoehl J, Hoeflich A (2013) Dual control of mitochondrial biogenesis by sirtuin 1 and sirtuin 3. Mitochondrion 13: 755-761.
89. Yuan Y, Shi M, Li L, Liu J, Chen B, et al. (2016) Mesenchymal stem cell-conditioned media ameliorate diabetic endothelial dysfunction by improving mitochondrial bioenergetics via the Sirt1/AMPK/PGC-1α pathway. Clin Sci (Lond) 130: 2181-2198.
90. Cheng L, Li B, Chen X, Su J, Wang H, et al. (2016) CTRP9 induces mitochondrial biogenesis and protects high glucose-induced endothelialoxidative damage via AdipoR1 -SIRT1- PGC-1α activation. Biochem Biophys Res Commun 477: 685-691.
91. Li C, Reif MM, Craige SM, Kant S, Keaney JF Jr (2016) Endothelial AMPK activation induces mitochondrial biogenesis and stress adaptation via eNOS-dependent mTORC1 signaling. Nitric Oxide 55-56: 45-53.
92. Zibrova D, Vandermoere F, Göransson O, Peggie M, Mariño KV, et al. (2017) GFAT1 phosphorylation by AMPK promotes VEGF-induced angiogenesis. Biochem J 474: 983-1001.
93. Scott JW, Oakhill JS (2017) The sweet side of AMPK signaling: regulation of GFAT1. Biochem J 474: 1289-1292.
94. Smith RA, Murphy MP (2011) Mitochondria-targeted antioxidants as therapies. Discov Med 11: 106-114.
95. Escribano-Lopez I, Diaz-Morales N, Rovira-Llopis S, Bañuls C, Lopez-Domenech S, et al. (2016) Atherosclerosis, mitochondrial dysfunction and oxidative stress: Mitochondria-targeted antioxidants as potential therapy in Medicinal Chemistry. Front Med Chem 8: 96-135.
96. Feniouk BA, Skulachev VP (2017) Cellular and molecular mechanisms of action of mitochondria-targeted antioxidants. Curr Aging Sci 10: 41-48.
97. Graham D, Huynh NN, Hamilton CA, Beattie E, Smith RA, et al. (2009) Mitochondria-targeted antioxidant MitoQ10 improves endothelial function and attenuates cardiac hypertrophy. Hypertension 54: 322-328.
98. Gioscia-Ryan RA, LaRocca TJ, Sindler AL, Zigler MC, Murphy MP, et al. (2014) Mitochondria-targeted antioxidant (MitoQ) ameliorates age-related arterial endothelial dysfunction in mice. J Physiol 592: 2549-2561.
99. Zakharova VV, Pletjushkina OY, Galkin II, Zinovkin RA, Chernyak BV, et al. (2017) Low concentration of uncouplers of oxidative phosphorylation decreases the TNF-induced endothelial permeability and lethality in mice. Biochim Biophys Acta 1863: 968-977.
100. Ruttkay-Nedecky B, Nejdl L, Gumulec J, Zitka O, Masarik M, et al. (2013) The role of metallothionein in oxidative stress. Int J Mol Sci 14: 6044-6066.
101. Fujie T, Murakami M, Yoshida E, Yasuike S, Kimura T, et al. (2016) Transcriptional induction of metallothionein by Tris(pentafluorophenyl)stibane in cultured bovine aortic endothelial cells. Int J Mol Sci 17: E1381.
102. Ungvari Z, Labinskyy N, Mukhopadhyay P, Pinto JT, Bagi Z, et al. (2009) Resveratrol attenuates mitochondrial oxidative stress in coronary arterial endothelial cells. Am J Physiol Heart Circ Physiol 297: H1876-H1881.
103. Zhang D, Gao X, Wang Q, Qin M, Liu K, et al. (2013) Kakkalide ameliorates endothelial insulin resistance by suppressing reactive oxygen species-associated inflammation. J Diabetes 5: 13-24.
104. Xie Z, Zhang J, Wu J, Viollet B, Zou MH (2008) Upregulation of mitochondrial uncoupling protein-2 by the AMP-activated protein kinase in endothelial cells attenuates oxidative stress in diabetes. Diabetes 57: 3222-3230.
105. Szewczyk A, Jarmuszkiewicz W, Koziel A, Sobieraj I, Nobik W, et al. (2015) Mitochondrial mechanisms of endothelial dysfunction. Pharmacol Rep 67:704–710.
106. Fujisawa K, Nishikawa T, Kukidome D, Imoto K, Yamashiro T, et al. (2009) TZDs reduce mitochondrial ROS production and enhance mitochondrial biogenesis. Biochem Biophys Res Commun 379: 43-48.
107. Paneni F, Costantino S, Castello L, Battista R, Capretti G, et al. (2015) Targeting prolyl-isomerase Pin1 prevents mitochondrial oxidative stress and vascular dysfunction: insights in patients with diabetes. Eur Heart J 36: 817-828.
108. Du JY, Yuan F, Zhao LY, Zhu J, Huang YY, et al. (2017) Suppression of Kv1.5 protects against endothelial apoptosis induced by palmitate and in type 2diabetes mice. Life Sci 168: 28-37.
109. Bhatt MP, Lim YC, Kim YM, Ha KS (2013) C-peptide activates AMPKalpha and prevents ROS-mediated mitochondrial fission and endothelial apoptosis in diabetes. Diabetes 62: 3851-3862.
110. Vasquez A (2014) Mitochondrial medicine arrives to prime time in clinical care: Nutritional biochemistry and mitochondrial hyperpermeability ("Leaky mitochondria") meet

disease pathogenesis and clinical interventions. Integr Med (Encinitas) 13: 44-49.

111. Edeas M, Weissig V (2013) Targeting mitochondria: strategies, innovations and challenges: The future of medicine will come through mitochondria. Mitochondrion 13: 389-390.
112. Strum JC, Shehee R, Virley D, Richardson J, Mattie M, et al. (2007) Rosiglitazone induces mitochondrial biogenesis in mouse brain. J Alzheimers Dis 11: 45-51.
113. Detaille D, Guigas B, Chauvin C, Batandier C, Fontaine E, et al. (2005) Metformin prevents high-glucose-induced endothelial cell death through a mitochondrial permeability transition-dependent process. Diabetes 54: 2179-2187.
114. Csiszar A, Labinskyy N, Pinto JT, Ballabh P, Zhang H, et al. (2009) Resveratrol induces mitochondrial biogenesis in endothelial cells. Am J Physiol Heart Circ Physiol 297: H13–H20.
115. Valero T (2014) Mitochondrial biogenesis: pharmacological approaches. Curr Pharm Des 20: 5507-5509.
116. Scholpa NE, Schnellmann RG (2017) Mitochondrial-based therapeutics for the treatment of spinal cord injury: mitochondrial biogenesis as a potential pharmacological target. J Pharmacol Exp Ther 363: 303-313.
117. Gerö D, Szabo C (2016) Glucocorticoids suppress mitochondrial oxidant production via upregulation of Uncoupling Protein 2 in hyperglycemic endothelial cells. PLoS One 11: e0154813.
118. Kim HK, Han J (2017) Mitochondria-targeted antioxidants for the treatment of cardiovascular disorders. Adv Exp Med Biol 982: 621-646.
119. Duann P, Lin PH (2017) Mitochondria damage and kidney disease. Adv Exp Med Biol 982: 529-551.
120. Brenza TM, Ghaisas S, Ramirez JEV, Harischandra D, Anantharam V, et al. (2017) Neuronal protection against oxidative insult by polyanhydride nanoparticle-based mitochondria-targeted antioxidant therapy. Nanomedicine 13: 809-820.
121. Babizhayev MA, Yegorov YE (2016) Reactive Oxygen Species and the aging eye: Specific role of metabolically active mitochondria in maintaining lens function and in the initiation of the oxidation-induced maturity onset cataract-A novel platform of mitochondria-targeted antioxidants with broad therapeutic potential for redox regulation and detoxification of oxidants in eye diseases. Am J Ther 23: 98-117.
122. Spahis S, Borys JM, Levy E (2017) Metabolic syndrome as a multifaceted risk factor for oxidative stress. Antioxid Redox Signal 26: 445-461.
123. Wang CH, Wei YH (2017) Role of mitochondrial dysfunction and dysregulation of Ca2+ homeostasis in the pathophysiology of insulin resistance and type 2 diabetes. J Biomed Sci 24: 70.
124. Camara AK, Lesnefsky EJ, Stowe DF (2010) Potential therapeutic benefits of strategies directed to mitochondria. Antioxid Redox Signal. 13: 279-347.
125. Rovira-Llopis S, Bañuls C, Diaz-Morales N, Hernandez-Mijares A, Rocha M, et al. (2017) Mitochondrial dynamics in type 2 diabetes: Pathophysiological implications. Redox Biol 11: 637-645.
126. Lyakhovich A, Graifer D (2015) Mitochondria- mediated oxidative stress: old target for new drugs. Curr Med Chem 22: 3040-3053.
127. Apostolova N, Victor VM (2015) Molecular strategies for targeting antioxidants to mitochondria: therapeutic implications. Antioxid Redox Signal 22: 686-729.
128. Sivitz WI, Yorek MA (2010) Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. Antioxid Redox Signal 12: 537-577.
129. Fetterman JL, Holbrook M, Westbrook DG, Brown JA, Feeley KP, et al. (2016) Mitochondrial DNA damage and vascular function in patients with diabetes mellitus and atherosclerotic cardiovascular disease. Cardiovasc Diabetol 15: 53.
130. Li L, Wang C, Yang H, Liu S, Lu Y, et al. (2017) Metabolomics reveal mitochondrial and fatty acid metabolism disorders that contribute to the development of DKD in T2DM patients. Mol Biosyst 13: 2392-2400.
131. Goveia J, Stapor P, Carmeliet P (2014) Principles of targeting endothelial cell metabolism to treat angiogenesis and endothelial cell dysfunction in disease. EMBO Mol Med 6: 1105-1120.
132. de Zeeuw P, Wong BW, Carmeliet P (2015) Metabolic adaptations in diabetic endothelial cells. Circ J 79: 934-941.
133. Gimbrone MA Jr, García-Cardeña G (2016) Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ Res 118: 620-636.
134. Pircher A, Treps L, Bodrug N, Carmeliet P (2016) Endothelial cell metabolism: A novel player in atherosclerosis? Basic principles and therapeuticopportunities. Atherosclerosis 253: 247-257.
135. Galán M, Kassan M, Kadowitz PJ, Trebak M, Belmadani S, et al. (2014) Mechanism of endoplasmic reticulum stress-induced vascular endothelial dysfunction. Biochim Biophys Acta 1843: 1063-1075.
136. Daiber A, Oelze M, Daub S, Steven S, Schuff A, et al. (2014) Vascular redox signaling, redox switches in endothelial Nitric Oxide Synthase and endothelial dysfunction in I. Laher (ed.), Systems Biology of Free Radicals and Antioxidants, Springer-Verlag Berlin, Heidelberg 1177-1211.
137. Takamiya R, Ohtsubo K, Taniguchi N (2015) Glycoredox: new paradigm for glycosylation and redox signaling research, N. Taniguchi et al. (eds.), Glycoscience: Biology and Medicine, Springer Japan 1275-1281.