Perspectives and Potential Applications of Mitochondria-Targeted Antioxidants in Cardiometabolic Diseases and Type 2 Diabetes

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Abstract: There is abundant evidence to suggest that mitochondrial dysfunction is a main cause of insulin resistance and related cardiometabolic comorbidities. On the other hand, insulin resistance is one of the main characteristics of type 2 diabetes, obesity, and metabolic syndrome. Lipid and glucose metabolism require mitochondria to generate energy, and when O_2 consumption is low due to inefficient nutrient oxidation, there is an increase in reactive oxygen species, which can impair different types of molecules, including DNA, lipids, proteins, and carbohydrates, thereby inducing proinflammatory processes. Factors which contribute to mitochondrial dysfunction, such as mitochondrial biogenesis and genetics, can also lead to insulin resistance in different insulin-target tissues, and its association with mitochondrial dysfunction can culminate in the development of cardiovascular diseases. In this context, therapies that improve mitochondrial function may also improve insulin resistance. This review explains mechanisms of mitochondrial function related to the pathological effects of insulin resistance in different tissues. The pathogenesis of cardiometabolic diseases will be explained from a mitochondrial perspective

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and the potential beneficial effects of mitochondria-targeted antioxidants as a therapy for modulating mitochondrial function in cardiometabolic diseases, especially diabetes, will also be considered. © 2013 Wiley Periodicals, Inc. Med. Res. Rev., 00, No. 0, 1–30, 2013

Key words: cardiometabolic disease; diabetes; insulin resistance; mitochondria; oxidative stress

1. INTRODUCTION

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Cardiometabolic diseases, which include multiple pathologies such as type 2 diabetes, metabolic syndrome, and coronary heart disease, are a growing health problem worldwide.[1] For example, diabetes is associated with numerous complications that severely affect the quality of life and life expectancy of patients, such as macro- and microvascular impairments. Currently, around 300 million people worldwide have diabetes, and this figure is expected to rise to 500 million over the following years. Diabetes is related to an increase in the risk of cancer and other deleterious conditions, with all their physical and clinical consequences. The number of people with cardiometabolic syndrome, a key precursor to metabolic diseases such as diabetes and subsequent cardiovascular complications, is also growing.[2] Furthermore, insulin resistance, the main characteristic of cardiometabolic syndrome, is associated with activation of the tissue renin–angiotensin system.[3] The principal metabolic action of insulin is to promote glucose uptake in skeletal muscle and to suppress glucose production in the liver, thereby maintaining glucose homeostasis. On the other hand, insulin resistance, defined as a decreased sensitivity to these metabolic actions, correlates with type 2 diabetes and cardiovascular diseases (CVDs).[4]

Mitochondria play a key role in the metabolism by regulating energy homeostasis through the metabolization of nutrients, producing ATP and generating heat (Fig. 1). Mitochondrial dysfunction is characterized by an inhibition of mitochondrial O_2 consumption, changes in the mitochondrial membrane potential ($\Delta\Psi_m$), and a reduction in ATP levels due to an imbalance between energy intake and expenditure.[5] (Fig. 2). In fact, changes in $\Delta\Psi_m$ may be due to both reduced activity in the electron transport chain (ETC) complexes and therefore reduced pumping of protons, or increased uncoupling produced by the activity of uncoupling proteins (UCPs) or the ADP/ATP translocator (also called adenine nucleotide translocase, ANT).

Different factors, both genetic and environmental (exercise, diet, and stress) can affect insulin sensitivity and regulate mitochondrial function.[6, 7] In addition, insulin resistance has been associated with mitochondrial dysfunction in several tissues, including lung, spleen, liver, heart, skeletal muscle,[8–10] and even in cells, such as leukocytes in type 2 diabetes.[11] Therefore, insulin resistance due in part by mitochondrial dysfunction may be a common pathophysiologic etiology of many widespread chronic diseases.

2. MITOCHONDRIA

Mitochondria play a key role in the life and death of cells, which make them a major target for cytoprotective pharmacological agents. Physiologically, mitochondria perform several fundamental regulatory processes in the cell (Fig. 1). They consume approximately 92–95% of cellular O_2 in the process of oxidative phosphorylation (OXPHOS). ETC is located in the inner membrane of the mitochondrion, and the production of ATP requires two steps: NADH (or $FADH_2$) oxidation and ADP phosphorylation, which produces ATP. NADH and $FADH_2$ are generated by glycolysis and β-oxidation of fatty acids (FAs) and are oxidized to FAD or NAD^+. Electrons from $FADH_2$ and NADH are transferred through different respiratory chain complexes to O_2, which generates H_2O. The driving force by which F_0F_1-ATPase
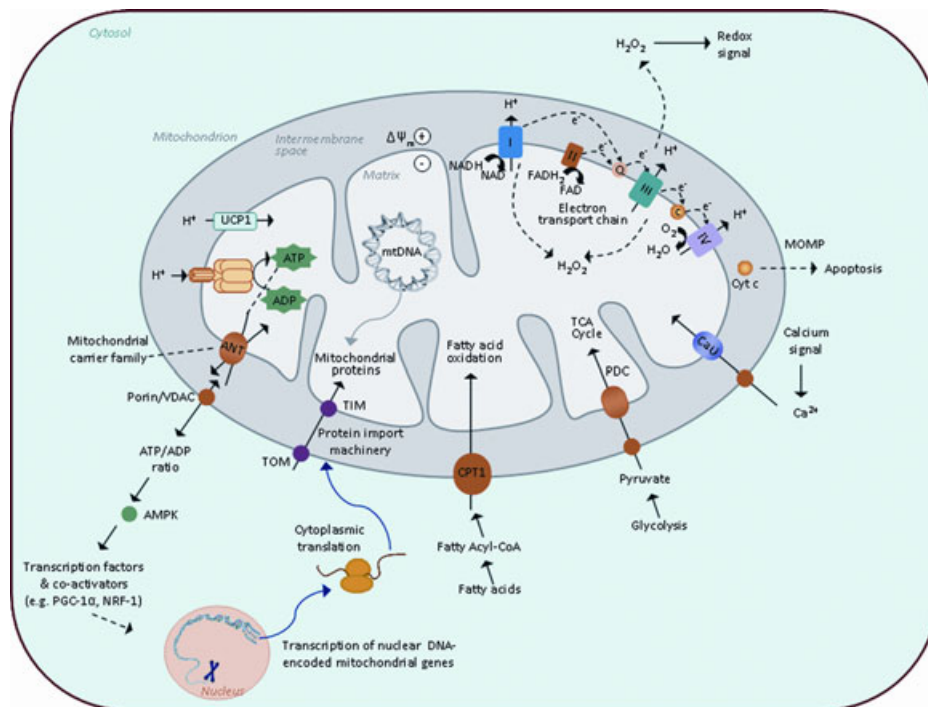
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Figure 1. Mitochondrial function in health. The mitochondrial proteome consists of mtDNA and nuclear DNA-encoded proteins. Cytosolic fatty acids and pyruvate fuel the catabolic processes in the mitochondrion (β-oxidation and the Krebs cycle), giving rise to oxidable susbstrates (succinate, NADH) of the electron transport chain. The respiratory chain complexes, associated with the IMM, transfer electrons from an electron donor to an electron acceptor, thus creating a transmembrane electrochemical gradient. The electrochemical energy from this transmembrane proton gradient is harnessed by ATP synthase (Complex V) to combine ADP and inorganic phosphate (P_i) and produce ATP, which is transferred through the IMM by ANT. The ADP/ATP ratio is sensed in the cytosol by AMPK, which modulates many metabolic pathways with the aim of preserving the energy of homeostasis. The electron flow along the respiratory chain generates moderate amounts of ROS, which act as signaling and regulatory molecules. UCPs are mitochondrial transporters located at the inner membrane that dissipate the proton gradient, thus, displaying metabolic and thermogenic activity. AMPK, AMP-activated protein kinase; ANT, ADP/ATP translocator or adenine nucleotide translocase; CaU, calcium uniporter; CPT1, carnitine palmitoyltransferase 1; MOMP, mitochondrial outer membrane permeabilization; mtDNA, mitochondrial DNA; NRF-1, nuclear respiratory factor 1; PDC, pyruvate decarboxylase; PGC1-α, peroxisome proliferator-activated receptor gamma coactivator-1-alpha; ROS, reactive oxygen species; TCA, tricarboxilic acid cycle; TIM, translocase of the inner membrane; TOM, translocase of the outer membrane; UCPs, uncoupling proteins; VDAC, voltage-dependent anion channel.

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(ATP synthase) produces ATP is the proton gradient across the membrane. A portion of the electrons at ETC can leak, thus causing generation of reactive oxygen species (ROS), molecules which play an important role in the development of several human diseases.[12–14]

There are different mechanisms by which mitochondria can produce heat, such as the proton leak, which undermines the proton-motive force, thus generating heat instead of ATP. This effect can be significant in different physiological and pathological situations. It has been demonstrated that UCPs (Fig. 1) can reduce the proton gradient.[15] UCPs are a family of inner mitochondrial membrane proteins that are thought to control several aspects of mitochondrial function, such as ROS generation, FA homeostasis, and regulate mitochondrial biogenesis. UCP1 is expressed mainly in brown adipose tissue, UCP2 is ubiquitous, and UCP3 is present in skeletal muscle.[16] UCP1, which counts for up to 10% of total membrane protein content, regulates adaptive thermogenesis, whereas UCP2 and UCP3 do not play a key role in
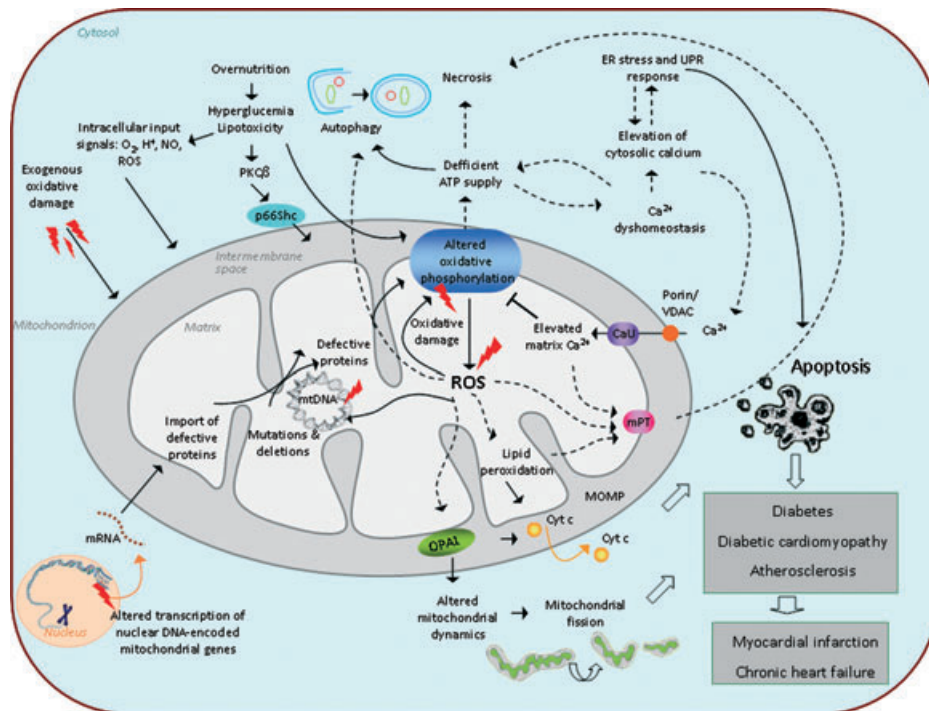
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Figure 2. Mitochondrial (dys) function in disease with a focus on cardiometabolic pathologies. Mutations in mtDNA and altered transcription of nuclear genes encoding mitochondrial proteins give rise to dysfunctional mitochondrial proteome. This leads to a defect in the mitochondrial OXPHOS process, culminating in a decreased level of ATP generation. The insufficient ATP supply can provoke necrotic cell death through a major "energetic catastrophe." Altered/impaired electron transport leads to augmented generation of ROS, which damages biological macromolecules including DNA (causing mtDNA mutations) and lipids (lipid peroxidation). Mitochondrial dysfunction can also be triggered by toxic exogenous stimuli and increased input of nutrients, and is influenced by various cytosolic signaling molecules. Impaired mitochondria present altered mitochondrial morphology and dynamics (through modification of the activity of OPA1). Damaged mitochondria can signal for autophagy (and the specific form, mitophagy) and induce programs of cell death, such as apoptosis (through mitochondrial permeability transition and release of mitochondrial proapoptotic proteins such as cyt *c*). Mitochondrial dysfunction also involves altered calcium handling in close relation with ER stress and activation of the UPR response. CaU, calcium uniporter; cyt *c*, cytochrome *c*; ER, endoplasmic reticulum; MOMP, mitochondrial outer membrane permeabilization; mPT, mitochondrial permeability transition; mtDNA, mitochondrial DNA; OPA1, optic atrophy gene 1; PKCβ, protein kinase C-beta; ROS, reactive oxygen species; UPR, unfolded protein response.

thermogenesis, as knockout mice for UCP2 and UCP3 have a normal basal proton conductance, adequate response to cold and body weight.[17] In general, abundant evidence suggests that UCPs play an important role in mitochondrial function by regulating generation of both ROS and heat, which underscores the fact the energy balance of mitochondria is key to cellular function. The role of UCPs in cardiometabolic diseases is still not fully clear. By hyperpolarizing the inner mitochondrial membrane, UCPs may protect the cell through decreasing the production of mitochondrial ROS and regulating mitochondrial apoptosis signals. Indeed, mice overexpressing UCP2 or UCP3 have been shown to exhibit decreased ROS production,[18] higher metabolic rate, and to be protected against weight gain and insulin resistance.[19] Also, there is abundant although discrepant evidence linking the common functional promoter polymorphism of *UCP2*, $-866\,\mathrm{G} > \mathrm{A}$ (rs659366) and changes in the risk of type 2 diabetes, obesity, and coronary artery disease.[20, 21]

The correlation between UCPs and lipid metabolism regarding cardiometabolic pathologies merits special mention. It has been described that in patients with heart disease there is

a high concentration of circulating free fatty acid (FFAs) and this is inversely correlated with myocardial phosphocreatine:ATP ratio, suggesting a cardiac energy deficit.[22] Several studies have pointed out that there is a positive correlation between the content of cardiac UCPs and the concentration of circulating FFAs, explaining the energy deficit observed in the damaged heart.[22] In addition to this, an increase in UCPs not always results in uncoupling of mito-chondrial respiration from ATP production, as measured by postdepletion phosphocreatine synthesis rate.[23]

There is also evidence that UCPs are involved in the translocation of FA anions away from the mitochondrial matrix. When not all FAs can enter mitochondria via carnitine palmi-toyltransferase as oxidizable fatty acyl-coenzyme A (acyl-CoA) esters, the excess can enter the mitochondrial matrix via a flip-flop mechanism in their unesterified (nonoxidizable) form where they become deprotonated. The resultant FA anions can neither be oxidized nor leave the matrix due to the proton gradient and are therefore locked in the matrix where they are harmful to mitochondria. UCPs can act as outward transporters of FAs, thereby protecting mitochondria in conditions characterized by an oversupply of FAs.[24] In line with this function, UCP content inversely relates to oxidative capacity. Hence, concentrations of UCP are 14-fold lower in cardiac muscle than in glycolytic muscle.[25]

Increased levels of fat loading in mitochondria by consumption of a high fat diet can upregulate UCPs in cardiac muscle.[26] Considering UCPs as an FA anion exporter, the positive association between cardiac UCPs content and plasma FFA concentrations can be considered a beneficial rather than an unfavorable-adaptive response, attempting to protect the damaged heart from lipotoxicity. In this context, inhibition of fat oxidation, which has been proposed as a treatment for heart disease, can result in the upregulation not downregulation of expression of UCPs. These results point out that increased UCPs levels are beneficial for the damaged human heart, although can be detrimental in β-cells due to less insulin secretion.

In addition, it is necessary to take into account that the nutrient oversupply, particularly that of FAs, can induce OXPHOS uncoupling. For example, feeding animals with a high-fat diet for several weeks is sufficient to reduce the mitochondrial rate of ATP synthesis.[27] In an-other study, it was demonstrated that high-fat diet downregulated genes coding for proteins involved in mitochondrial biogenesis and OXPHOS in human skeletal muscle.[28] In addition, increased levels of FA exposure, as for example in obesity, leads to intracellular accumulation of ceramides and diacylglycerol (DG), which reduces phosphatidylinositol 3-kinase (PI3K) signaling in muscle leading to increased insulin resistance.[29] Other studies have reported that peroxisome proliferator-activated receptors (PPARs) can bind FAs, particularly polyunsatu-rated acids,[30] and PPAR ligands may upregulate UCP2.[31] In line with this, Bugge et al. have demonstrated that peroxisome proliferator-activated receptor gamma (PPARγ) can activate both UCP2 and UCP3 expression via an enhancer located within the first intron of the *UCP3* gene.[32]

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Obesity, type 2 diabetes, and insulin resistance can produce a chronic elevation of circulating FAs which can become cytotoxic. The increased basal leakage of electrons and uncoupling in the mitochondria is a serious problem in these conditions because FAs can also cause oxidative stress and alterations in the mitochondrial structure and function. Namely, FA interaction with the membrane carriers can lead to mitochondrial membrane depolarization and result in opening of the permeability transition pore and initiation of apoptosis.

It is very important to point out that there is a transcriptional control of UCP2, for example, by glutamine and for this reason mRNA levels not always refer protein levels.[33] Thus, it is more worthy to assess the protein levels or perform functional measurements of UCP effects on mitochondria.

Type 2 diabetes and age-related insulin resistance are associated with mitochondrial dysfunction (Fig. 3),[34–36] and it has been shown that physical activity is one of the central
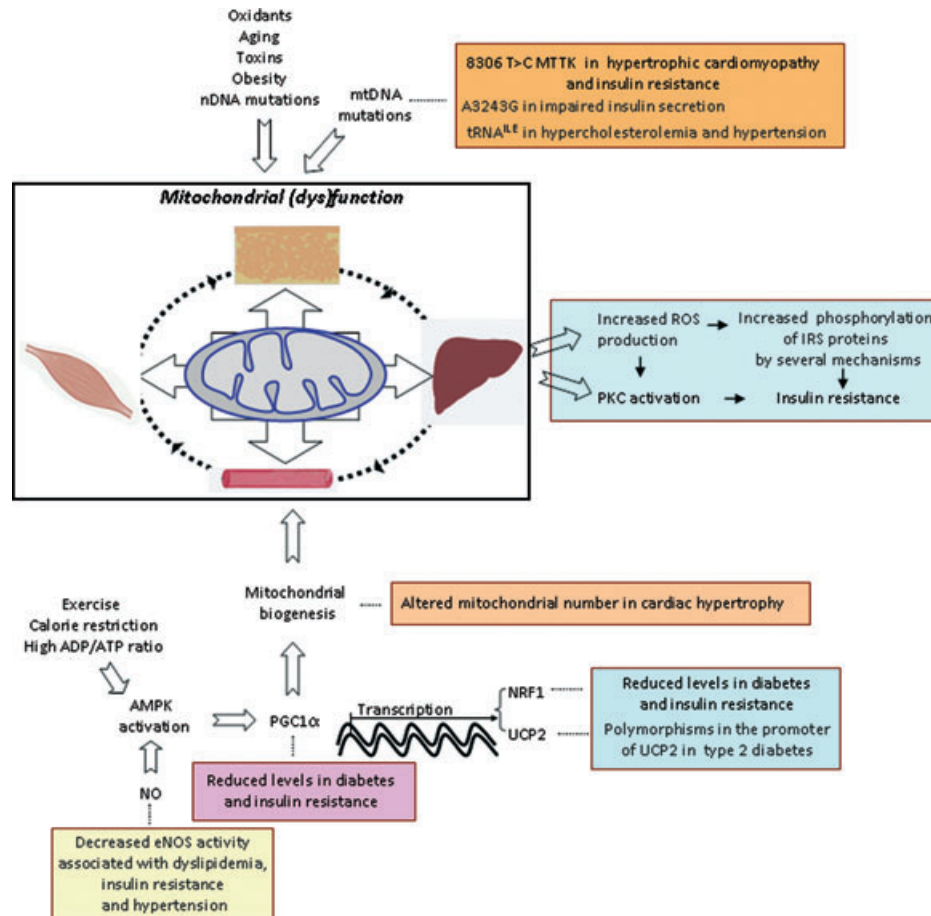
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Figure 3. (Patho)physiological factors and mechanisms affecting mitochondrial function with relevance in cardiometabolic diseases. Numerous extrinsic stimuli (oxidants, toxins) and intrinsic conditions (aging, obesity, nDNA, and/or mtDNA mutations, altered mitochondrial biogenesis) affect mitochondrial function. Several indicators associated with mitochondrial dysfunction, such as increased ROS, altered number of mitochondria, and mitochondrial biogenesis, have been documented in cardiometabolic diseases including insulin resistance, diabetes, hypertension, and cardiac hypertrophy. AMPK, AMP-activated protein kinase; eNOS, endothelial nitric oxide synthase; IRS, insulin receptor substrate; mtDNA, mitochondrial DNA; NO, nitric oxide; NRF1, nuclear respiratory factor 1; PGC1α, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PKC, protein kinase C; ROS, reactive oxygen species; UPC2, uncoupling protein 2.

determinants of muscle mitochondrial function in type 2 diabetes.[36] Therefore, mitochondria are emerging as a key target in cardiometabolic disease therapy. Furthermore, it has been hypothesized that there are several important parameters that affect mitochondrial function, including mitochondrial biogenesis, genetic factors, and oxidative stress (leading to insulin resistance).

A. Mitochondrial Biogenesis

Skeletal muscle of insulin-resistant, obese, or type 2 diabetic humans is characterized by impairment of mitochondrial function and fewer and smaller mitochondria.[37–39] Indeed,

mitochondrial oxidative capacity is fully correlated with the number and size of mitochondria.[6] A decreased OXPHOS is related with reduced expression of mitochondrial proteins encoded by both the nuclear (e.g., succinate dehydrogenase and pyruvate dehydrogenase) and the mitochondrial (e.g., cytochrome c oxidase subunit II) genome (Fig. 2).[37] In this sense, nuclear respiratory factors (NRFs) and PPARγ play a crucial role in cellular homeostasis.[40] Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1-α) is pivotal in transactivating genes essential for homeostasis maintenance[41] and modulates two fundamental enzymes, namely, sirtuin 1 (SIRT1) and AMP-activated protein kinase (AMPK). During energy depletion, in which there is an increase in the AMP:ATP ratio, PGC1-α activates AMPK.[42] Moreover, during caloric restriction or exercise there is an increase in NADH content, which upregulates SIRT expression and as a consequence PGC-1α.[43]

Nitric oxide (NO) is a homeostatic molecule that modulates mitochondrial O_2 consumption by inhibiting mitochondrial complex IV, which also regulates mitochondrial biogenesis.[44,45] A number of in vivo and in vitro studies have shown that nitrite activates AMPK to stimulate mitochondrial biogenesis independently of soluble guanylate cyclase, thus, providing evidence that nitrite is a versatile regulator of mitochondrial function, and that nitrite-mediated biogenesis plays a protective role in vascular injury.[46]

The overexpression of PGC-1α increases both the transcription of genes related to glucose uptake and transport, and β-oxidation.[47] PGC-1α also enhances OXPHOS and promotes the generation of type 1 muscle fiber.[48] Furthermore, it is involved in the pathogenesis of insulin resistance, and has been related with diabetes when its levels are reduced.[49]

PGC-1α transcriptionally regulates UCP, thus playing a role in thermogenesis in different tissues, including adipose tissue.[50] When ATP demand is high, such as during exercise, cold exposure, and fasting, the expression of PGC-1α is increased[51,52] (Fig. 3). PGC-1α is an activator of PPARγ and PPARα and of several transcription factors, including NRF-1.[40,53] This factor regulates the expression of different genes that are important for mitochondrial gene expression, such as ETC genes and mitochondrial transcription factor A (TFAM), and for replication of the mitochondrial genome.[53] Expression of PGC-1 and NRF-1 is reported to be undermined in diabetic and insulin-resistant human subjects, respectively.[54] Furthermore, PGC-1 expression decreases with age[55] and the fact that insulin-resistant human patients have small and fewer mitochondria in their skeletal muscle seems to be due to decreased expression of PGC-1α and PGC-1β.[56]

AMP-activated protein kinase (AMPK) is a major regulator of mitochondrial biogenesis,[57] which has led to its exploitation for the development of pharmacological agents, such as activators of AMPK [5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide, AICAR], which promote mitochondrial biogenesis through NRFs and PGC-1α.[58] AMPK is stimulated by exercise, leading to activation of PGC-1α by direct phosphorylation of key residues and, consequently, stimulating mitochondrial biogenesis.[59,60]

Several DNA microarray reports have demonstrated that expression of PGC-1α with its role in mitochondrial biogenesis is responsible for certain metabolic disorders (e.g., insulin resistance and type 2 diabetes)[53,57,61] (Fig. 3) in that a reduced number of mitochondria leads to insufficient mitochondrial function. Other studies have revealed that mitochondrial O_2 consumption is lower in obese and type 2 diabetes subjects than in lean active adults.[38] It has also been demonstrated that mRNA expression of TFAM, NRFs, PGC-1α, and PGC-1β is similar in insulin-resistant offspring of type 2 diabetes parents and controls, though mitochondrial function is significantly decreased in the former.[37] These findings suggest that mitochondrial dysfunction involves other components apart from an undermined mitochondrial biogenesis.

B. Genetic Factors

Mitochondrial proteins are encoded by mitochondrial and nuclear genes. The capacity of mitochondria is determined by their number, state, and size, and by the expression level of OXPHOS subunits.[6] In this regard, it is of relevance that 13 protein subunits of the mitochondrial ETC are encoded by mitochondrial genes.

The fact that the mitochondrial genome is proximal to sources of ROS gives strength to the idea of its high susceptibility to mutagenesis.[35] Mitochondrial respiratory chain deficiencies are associated with mutations of mitochondrial DNA (mtDNA), and such inherited dysfunction of the mitochondrial OXPHOS system is the hallmark of many mitochondrial diseases.[62,63] Several nuclear and mtDNA mutations have been identified as the cause of defects and isolated disorders of individual OXPHOS enzymes, including mitochondrial ATP synthase in patients with cardiovascular-metabolic diseases.[64] The diseases attributed to familial mtDNA mutations are less common than those related to nuclear DNA defects. This may be a result of mitochondria containing several copies of their genome, as the continuous fusion of mitochondria means that modified genes mix with normal genes, which is a process that characterizes many human diseases. Wilson et al.[65] associated a simple thymidine-to-cytidine mutation in the mitochondrial tRNA^{ILE} gene with different pathologies, including hypercholesterolemia and hypertension, while Cardaioli et al.[66] demonstrated that the mitochondrial 8306 T>C MTTK mutation induces sporadic myopathy, myoclonus, leukoencephalopathy, neurosensory deafness, hypertrophic cardiomyopathy, and insulin resistance (Fig. 3). Cardiomyopathy, neurological disorders, and liver dysfunction have been found in patients with defects in acyl-CoA dehydrogenase.[67] Another mtDNA mutation, that of A3243G, which encodes tRNA (Leu-UUR), has been shown to impair insulin secretion[68] (Fig. 3). In addition, Hirschey et al.[69] reported that SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of metabolic syndrome.

Moreover, type 2 diabetes, loss of weight, and reduced insulin secretion have been associated with the presence of polymorphisms in the promoter of UCP2.[20,21] It has also been demonstrated that nuclear genes that encode mitochondrial proteins play an important role in insulin resistance.[70] Thus, the pathogenesis of cardiometabolic syndrome and CVD, which occurs through functional impairment of mitochondria, is highly influenced by human genetic factors inherited through nuclear and/or mitochondrial genes. In addition, it is important to mention that the level of mitochondrial uncoupling is also an important determinant of mitochondria capacity.

C. Oxidative Stress

Mitochondria are the main source of ROS (Fig. 2), and these molecules are a fundamental factors in the development of diabetic complications.[62,63,71] Therefore, the use of specific compounds to eradicate mitochondrial ROS has become important to ameliorate complications related with diabetes.[72] For example, lipoic acid (LA) reduces inflammation and insulin resistance and improves mitochondrial function, thus, preventing CVD in humans.[72]

There are two main sites of electron leakage in the ETC: complex I and III.[62,63,73,74] Excess production of ROS has an important effect on mitochondrial $\Delta\Psi_m$ in diabetes, condition in which a high amount of substrates is supplied as a consequence of elevated levels of glucose. Specifically, it has been hypothesized that ETC dysfunction and its complications contribute to many diabetes-related pathologies, including nephropathy, neuropathy, and retinopathy, while deleterious genetic mutations involving a reduction in the activity of complex I lead to high rates of mitochondrial ROS production and mitochondrial impairment.[75] This evidence highlights mitochondrial impairment as a research priority for the future. Indeed, mitochondria-targeted

antioxidant therapy is already showing great promise in this respect. In fact, there are many enzymes in the mitochondria that are susceptible to damage by ROS, with complex I being the most vulnerable.

Mitochondrial dysfunction at complex I, together with an increment in ROS production, a reduction of $\Delta\Psi_m$, and an impairment of antioxidant defenses[11] have been described in diabetic patients. In other pathologies in which insulin resistance occurs, such as polycystic ovary syndrome (PCOS), impairment of mitochondrial complex I, and an increase in leukocyte-endothelium interactions have also been reported,[76,77] while diabetic nephropathy is very common among patients with mitochondrial dysfunction.[78] This damage eventually impairs the mitochondrial handling of calcium, alters $\Delta\Psi_m$, and diminishes ATP production. In this sense, it has been described that CoQ10 can prevent the mitochondrial morphology and function, proteinuria and glomerular hyperfiltration in db/db mice, highlighting the role of mitochondria in the pathogenesis of diabetic nephropathy.[79]

In a recent article, it has been also described that the increase in human leukocyte/endothelial cell interactions and mitochondrial dysfunction in type 2 diabetic patients correlate with the development of silent myocardial ischemia.[80]

Idebenone is a safe and efficient mitochondrial antioxidant that protects mitochondria from oxidative damage in Friedreich's ataxia patients.[81] Interestingly, idebenone also reduces cardiomyopathy in the same subjects, unlike traditional antioxidants, such as α-tocopherol or vitamin E.[81,82] MitoQ, another mitochondria-targeted antioxidant, is selectively uptaken by mitochondria due to a covalent attachment to the lipophilic triphenylphosphonium (TPP^+) cation, thus, accumulating 1000-fold in mitochondria.[62,63,83] The efficacy of these mitochondria-targeted antioxidants in the treatment of cardiometabolic diseases and diabetes remains to be determined, but their targeted specificity for mitochondria is reason enough to study their potential as agents of diabetes and CVD therapy.

3. INSULIN RESISTANCE AND MITOCHONDRIAL DYSFUNCTION

Glucose homeostasis is mediated by insulin in a controlled relation with glucose uptake and gluconeogenesis rate. In addition, there are other less well-known roles of insulin associated with renal, cardiovascular, and neural functions, which may explain why insulin resistance is a risk factor for hypertension, CVD, neuropathy, retinopathy, and nephropathy.[84]

Insulin resistance is defined by a diminished capacity of tissues or cells to respond to the levels of insulin. Many conditions can contribute to this phenomenon, such as obesity, stress, environmental factors, or altered lipid and glucose metabolism.[85] Indeed, the cellular and molecular mechanisms of insulin resistance are important to understanding the pathogenesis of several diseases with which it is associated.

Excess energy intake, lipodystrophy, or oxidative stress can increase circulating FFAs, which leads to accumulation of FFAs, triglycerides, and DG in different tissues, including skeletal muscle, liver, heart, and β-cells. In addition, the accumulation of lipids and a high-fat diet in mammals reduce insulin-stimulated glucose disposal.[86] Considered together, these data suggest that alterations in lipid metabolism leading to impairment of insulin signaling are key to the development of insulin resistance.[87,88] Moreover, the effects of impairment of insulin signaling can have a bearing on insulin-stimulated glucose metabolism in skeletal muscle and other tissues, such as heart, vasculature, liver, and adipose tissue.[89,90]

Insulin signaling constitutes a highly complex network composed of multiple pathways and signaling from heterologous receptors.[91] It is initiated when insulin binds to insulin receptors (IRs),[92] which causes an autophosphorylation of IR tyrosine residues and elevated tyrosine kinase activity of the receptor. Thus, the receptor can phosphorylate insulin receptor substrate

(IRS) family members and can activate PI3K, leading to the generation of phosphatidylinositol 3,4,5-triphosphate. Activation of PI3K stimulates several downstream serine kinases, including protein kinase B (Akt), protein kinase C (PKC), phosphoinositide dependent kinase-1, and other kinases, all of which eventually modulate the biological and pleiotropic metabolic actions of insulin. Multiple mechanisms have been proposed for insulin resistance:[92] (i) degradation of IRS proteins;[93] (ii) elevated activity of phosphatases;[94] (iii) increased serine phosphorylation of IRS proteins;[95] and (iv) decreased activation of IR downstream signaling molecules including Akt and PKC.[96]

A decrease in tyrosine phosphorylation of IRS has been demonstrated in human subjects and in different insulin-resistant animal models.[97, 98] Phosphorylation of IRS proteins at specific serine residues can inhibit the interaction of said proteins with IR, leading to a decrease in tyrosine phosphorylation of IRS and undermining of the activation of PI3K.[94] Increased proinflammatory signaling is other important mechanism of insulin resistance. In fact, FFAs can stimulate Toll-like receptors inducing proinflammatory signaling, which can activate IκB kinase (IKKβ) and c-Jun N-terminal kinase (JNK), stimulating the production of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β, and interleukin-6.[99, 100] Studies with anti-inflammatory drugs or gene silencing of IKKβ or JNK have shown an improvement of insulin sensitivity, with significant reductions in serine phosphorylation of IRS proteins.[101, 102]

Another important mechanism of insulin resistance based on activation of serine kinases is the endoplasmic reticulum (ER) stress. ER stress can activate JNK, thereby increasing serine phosphorylation of IRS proteins. In this sense, the use of chemical chaperones, such as 4-phenyl butyric acid (PBA) and taurine-conjugated ursodeoxycholic acids (TUDCAs), has therapeutic effects by improving insulin sensitivity and reducing ER stress.[103] Such treatment has been shown to reduce hepatic JNK activity, IRS-1 serine phosphorylation, and fatty liver in animals.[103] Therefore, ER stress appears to act both indirectly, by inducing lipid accumulation, and directly as a negative modulator of the insulin signaling pathway. For these reasons, ER stress is considered a key factor in the development of insulin resistance.[104]

One of the most plausible hypotheses concerning insulin resistance is that of mitochondrial dysfunction and consequent increases in ROS, which act as secondary messengers by activating the serine kinases that phosphorylate IRS proteins[37] (Fig. 3). Furthermore, ROS can stimulate inflammatory signaling through activation of IKKβ, which can phosphorylate IRS-1.[105] In this sense, mitochondrial function and insulin sensitivity have been demonstrated to improve by using antioxidants or following an increase in the expression of UCP2–UCP3 and a decrease in ROS levels. The increase of DG, FA metabolites, and long-chain fatty acyl-CoA can produce mitochondrial dysfunction.[106] In this sense, DG, an allosteric activator of PKCs, can increase serine phosphorylation of IRS proteins, inducing insulin resistance.[107] In fact, PKCθ-deficient mice are protected against fat-induced insulin resistance.[108] This suggests that the activation of PKCs as a result of mitochondrial dysfunction is another cause of insulin resistance.

Different studies of human biopsies, both in vivo and ex vivo, have highlighted the association of insulin resistance with impairment of mitochondrial function, including lower levels of mitochondrial oxidative enzymes, a decreased mitochondrial number, and abnormal mitochondrial morphology.[109, 110] In this sense, an increase in levels of plasma FFAs or lipids can induce insulin resistance in human liver and muscle.[111] In diabetes, the accumulation of intramyocellular lipids leads to reducing mitochondrial oxidative capacity, which correlates negatively with insulin sensitivity.[112]

Obesity leads to an increase of triglycerides in adipose tissue, and consequently can alter glucose metabolism and insulin resistance in different nonadipose tissues. Conversely, it has been shown that lipodystrophy induces type 2 diabetes and insulin resistance in human subjects.[113] Adipocytes can release adipokines, including leptin, adiponectin, resistin, and TNF-α, which

can modulate different metabolic pathways.[114, 115] In adipocytes from type 2 diabetic patients with obesity, the number of mitochondria and the expression of genes involved in mitochondrial biogenesis are significantly decreased.[116] In this way, insulin-resistant metabolic tissue is characterized by a reduced mitochondrial gene expression in adipocytes, a lower number and abnormal morphology of mitochondria, and abnormal OXPHOS.

Endothelial dysfunction and insulin resistance are very common in CVD, such as coronary artery disease, heart failure, hypertension, silent myocardial ischemia, and stroke.[117] In fact, high blood pressure is reported in over two-thirds of patients with type 2 diabetes, and its appearance coincides with hyperglycemia.[118] Increased levels of FFAs can contribute to insulin resistance by reducing mitochondrial oxidative capacity, ATP synthesis, and cardiac efficiency in insulin-resistant *ob/ob* and obese mice.[119] In addition, intramyocardial lipid accumulation can induce lipotoxic injury and cardiac dysfunction in different mouse models of obesity.[120, 121] The impairment of endothelium-dependent vasodilation and glucose intolerance can be related to intramyocardial lipid accumulation, and this effect can precede type 2 diabetes and heart failure.[122]

The heart, one of the tissues with the greatest caloric needs and most robust oxidation of FAs, contains low levels of endogenous antioxidants, which makes it particularly susceptible to oxidative stress and subsequent functional and structural abnormalities.[123] An association has been demonstrated between alterations of mitochondrial morphology/function and mitochondrial oxidative state in the myocardium of Zucker obese rats with insulin resistance.[124] Transmission electron microscopic analysis of myocardial tissue has revealed increased numbers of morphologically abnormal mitochondria in several insulin-resistant rat models.[125] Similarly, an increase in the number of mitochondria has been recorded in hypertrophied rat hearts displaying oxidative stress,[126] perhaps as a consequence of the energy requirements and of ROS production. In contrast, other studies have demonstrated that the number of mitochondria and their DNA content are reduced in animal models of pathological hypertrophy and patients.[127] In summary, the role of mitochondria in the heart is essential, and cardiac mitochondrial dysfunction seems to contribute to CVD, including cardiomyopathy, coronary heart disease, heart failure, hypertension, and silent myocardial ischemia.

An interaction between endothelial dysfunction and insulin resistance has been proposed,[128] though the details are still unclear. Endothelial cells are glycolitic cells.[129] Moreover, mitochondria in the endothelium can play a relevant role as sensors for local O_2 concentration and in signaling as regulators of intracellular $[Ca^{2+}]$.[130] In addition, it has been proposed that mitochondrial dysfunction and subsequent ROS production are the key factors in the development of macrovascular and microvascular damage.[8] Different studies have shown that it is possible to prevent the endothelial dysfunction associated with hyperglycemia by blocking the excess of mitochondrial ROS generation.[131, 132]

In addition, endothelial nitric oxide synthase (eNOS) in vessels can play an important role in vasodilation, mitochondrial biogenesis, and insulin-stimulated NO production.[133] Indeed, eNOS knockout mice are characterized by dyslipidemia, hypertension, and insulin resistance.[134] Thus, insulin resistance impairs NO synthesis and the mitochondrial dysfunction associated with it compromises several cardiac functions, which in turn leads to heart failure, coronary artery disease, and silent myocardial ischemia (Fig. 3).

Type 2 diabetes can appear in insulin-resistant patients when β-cells cannot sense glucose properly and fail to produce and secrete enough insulin to maintain normal levels of glucose. Mitochondrial function increases the ATP:ADP ratio and can modulate the inhibition of the potassium channel (K_{ATP}), which leads to secretion of insulin. In addition, mitochondrial function is related with β-cell function through the ATP:ADP ratio.[135] Han et al. have shown that taurine can enhance the glucose sensitivity of UCP2 overexpressing β-cells probably by

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enhancing mitochondrial Ca^{2+} influx through the Ca^{2+} transporter, which enhances mito-chondrial function and increases the ATP/ADP ratio as a result.[136] In addition, it has been demonstrated that insulin secretion is impaired in β-cells which are deficient in some mito-chondrial genes, and that when this situation is reversed, β-cells recover their capacity to secrete insulin.[137] Knockout of Tfam, a nuclear DNA-encoded mitochondrial protein, results in impaired insulin secretion, reduced β-cell mass, severe mtDNA depletion, and develop-ment of diabetes.[138] The results of these studies support the hypothesis that mitochondrial function is important for β-cell function and contributes to the pathogenesis of type 2 dia-betes by modulating insulin secretion and insulin action. Thus, lipid-induced mitochondrial dysfunction can impair insulin signaling due to the generation of ROS. For all these reasons, mitochondria should be considered a key target in therapy for insulin resistance and related diseases.

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4. STRATEGIES FOR MITOCHONDRIAL PHARMACOLOGY WITH SPECIAL FOCUS ON ANTIOXIDANTS

The delivery of drugs to specific subcellular compartments improves the therapeutic efficacy of the compounds and avoids the detrimental consequences of their accumulation in off-target subcellular territories. Mitochondrial drug targeting, a process of selective drug delivery to these organelles, is a complex action that depends on the presence (or more often lack) of specific transporters on the mitochondrial membrane. As it is particularly difficult to diffuse through the inner membrane, mitochondria-targeted molecules need to be encapsulated inside a carrier, a process which needs to guarantee the preservation and the control of the drug's pharmacological activity once the active molecule is inside the mitochondrion. Current strategies for delivering drugs to the mitochondria fall into two categories: active and passive targeting.[139] In the former case, specific interactions are provoked at mitochondrial sites, including antigen–antibody and ligand–receptor association, and in the latter, the physicochemical properties (electric charge, hydrophilicity, size, and mass) of the carrier are compatible with those of the mitochondrial compartment, thus, converting mitochondria into a specific pharmacological target. Small molecules have been successfully targeted to mitochondria in vivo in several ways; namely, through conjugation to lipophilic cations,[140] enclosure inside liposomes,[139] and incorporation into mitochondria-targeted peptides[141] (Fig. 4). To date, the molecules used in these targeting approaches involve coenzymes and substrates of the ETC, such as cytochrome c; succinate vitamin B1, B2; and proapoptotic proteins, such as the Bax/Bcl2 family and p53, as well as relevant antioxidants.[139]

Mitochondrial $\Delta\Psi_m$ is used by lipophilic cations for their selective accumulation within the mitochondrial matrix (Fig. 4).[142] This process is expressed by the Nernst equation, by which uptake increases tenfold for every 60 mV of plasma membrane potential, leading to uptake within mitochondria in vivo.[143,144] The use of lipophilic cations to deliver pharmacological agents to the cell interior was first demonstrated with the lipophilic cation rhodamine 123 in a complex with the anticancer compound cisplatin.[145] TPP$^+$ and its methylated form TPMP$^+$ are the most widely used lipophilic cations for mitochondrial accumulation of antioxidants.[140] An alternative spin trap with a lower molecular weight and bearing an N-arylpyridinium ion[146] has been employed with seemingly positive results, though its efficacy requires further confirmation. The TPP moiety, driven by the plasma membrane potential allows rapid cellular uptake of bioactive molecules, followed by specific mitochondrial matrix accumulation. A high number of antioxidants have been successfully targeted to mitochondria through their conjugation to TPP, including ubiquinone;[147,148] vitamin E;[149] resveratrol;[150] ebselen;[151] LA;[152] nitroxides,
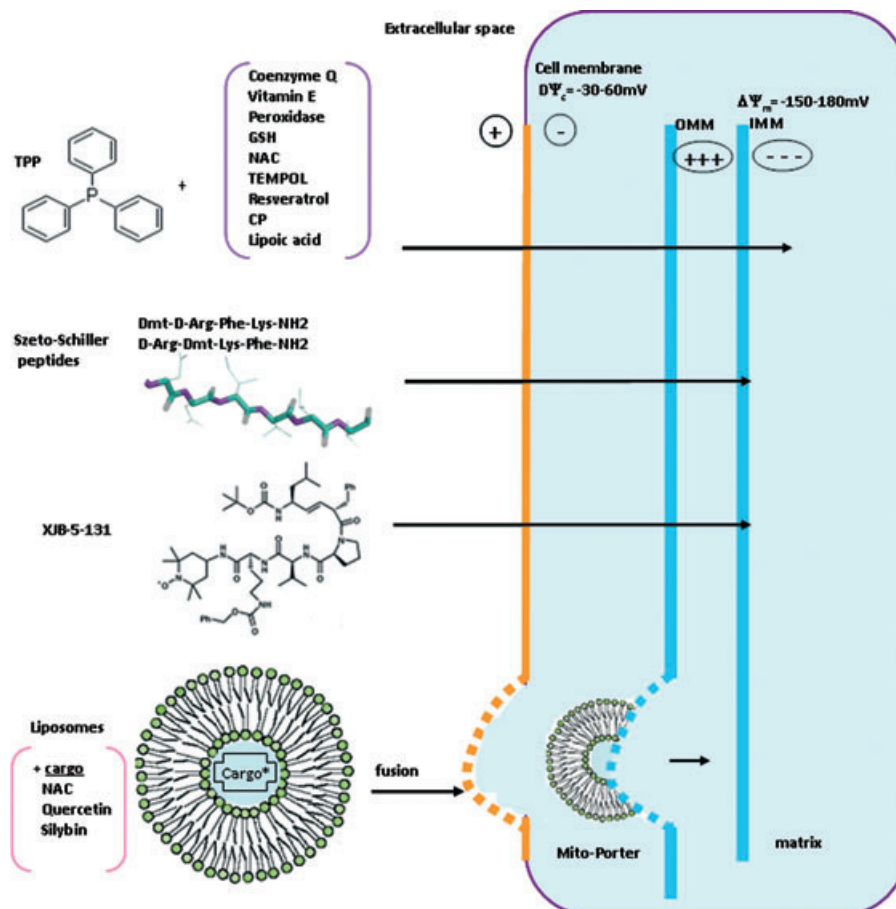
Figure 4. Mitochondrial delivery systems. A Lipophilic cations, such as TPP specifically enter mitochondria in a $\Delta\Psi_m$-dependent fashion. TPP has been conjugated with several antioxidants exemplified by vitamin E (mitoE) and coenzyme Q (MitoQ). B Szeto-Schiller peptides are cationic agents that localize mainly to the IMM (80%). Their antioxidant properties lie in the presence of tyrosine and dimethyltyrosine residues. C XJB-5–131 is targeted to the mitochondrion through its oligopeptidic fragment of the membrane-active antibiotic gramicidin S. A stable nitroxide radical portion of this compound renders it antioxidant. D Antioxidant liposomes carry lipid- or water-soluble cargo of antioxidants. A special type of liposome-based carrier is MITO-Porter, a mitochondria-targeted envelope-type nanodevice that delivers its cargo to the target compartment through fusion. Dmt, dimethyltyrosine; GSH, glutathione; IMM, inner mitochondrial membrane; NAC, N-acetyl cysteine; OMM, outer mitochondrial membrane; TEMPOL, 4-hidroxy-2,2,6,6-tetramethylpiperidine-1-oxy radical; TPP, triphenylphosphonium.

such as TEMPOL,[153] plastoquinone,[154] and nitrones (Fig. 4).[155] Lipophilic cations show great potential with respect to the accurate delivery of antioxidants to mitochondria; however, they have the following disadvantages: (i) their capacity (only electrically neutral and low molecular weight molecules can be successfully transferred); (ii) their sublocalization (these chemicals tend to localize to the mitochondrial matrix and the matrix-facing surface of the inner membrane, disabling the targeting of many important processes that take place on the outer surface of the inner membrane, the outer membrane, or the intermembrane space); and (iii) their toxicity (at high concentrations they can depolarize $\Delta\Psi_m$ and compromise cell viability).

MitoQ, which consists of a ubiquinone moiety linked to a TPP by a ten-carbon alkyl chain, is the most studied and widely used mitochondrial antioxidant.[140] Within mitochondria, MitoQ is adsorbed to the matrix-facing surface of the inner mitochondrial membrane (IMM),

where it can be recycled by complex II of the ETC into the active ubiquinol form.[149,156] This highly effective antioxidant reacts with ROS, such as peroxynitrite ($ONOO^-$), and also inhibits its formation[149] and mitochondrial lipid peroxidation.[156,157] In addition, the ubiquinone form is known to react directly with other ROS, such as superoxide.[158] MitoQ has been shown to have a beneficial role in several in vitro settings of mitochondrial oxidative stress. More importantly, its benefits have been reported in animal models of cardiometabolic pathologies, such as ischemia/reperfusion, sepsis, and diabetes (Table I), and in humans.[159] Of note, it has been demonstrated that intravenous and oral administration of MitoQ leads to a rapid uptake from blood into cells.[143] A growing number of studies confirms that antioxidants are capable of modulating mitochondrial survival/cell-death pathways, including apoptosis, mitophagy, mitoptosis, and necrosis. In this regard, mitochondria-targeted antioxidants merit a special attention. It has been demonstrated that MitoQ inhibits the mitochondrial fission induced in HeLa cells and fibroblasts by mitochondrial respiratory chain inhibitors (piericidin and myxothiazol), which points to the beneficial potential of MitoQ in novel mitochondrial contexts, such as mitochondrial dynamics.[160]

Although the therapeutic efficacy of mitochondria-targeted antioxidants in diabetes or CVD needs to be confirmed, several in vivo and in vitro studies have demonstrated their useful effects (Table I). In one study, mitochondria-targeted antioxidants were employed in β-cells such as RINm5F and HIT-T15 under conditions of glucolipotoxic and glucotoxic stress typical of type 2 diabetes.[161] Results in β-cells under oxidative stress conditions showed an increase in the levels of mitochondrial antioxidant enzymes (such as MnSOD), the expression of mitochondrial ETC complex subunits and lipogenic enzymes (such as ATP-binding cassette transporter A1 [ABCA1]), FA synthase (FAS), and acetyl-CoA carboxylase (ACC), as well as induction of apoptosis, intracellular lipid droplet accumulation, presence of oxidative stress and ER stress, mitochondrial membrane depolarization, expression of sterol regulatory element binding protein 1c (SREBP1c), and NF-κB, together with a decrease in citrate synthase activity, ATP concentration, and insulin release. These changes were related with mitochondrial oxidative stress and were prevented by the mitochondria-targeted antioxidants MitoQ or Mito Tempol, which protected β-cells, thereby improving insulin secretion and the survival of said cells. The protective effects of complications associated with diabetes, such as diabetic nephropathy and retinopathy. Li et al. described a beneficial effect of peptide SS31, a mitochondria-targeted antioxidant, on hyperglycemia-induced damage in human retinal endothelial cells (HRECs).[162] They reported that exposure to SS31 decreased mitochondrial ROS production, diminished the release of cytochrome c from the mitochondrion to the cytosol, stabilized $\Delta\Psi_m$, decreased the expression of caspase-3, and enhanced the expression of Trx-2 in HRECs. Mitochondria-targeted antioxidants have demonstrated their potential therapeutic effect in models of tolerance to nitroglycerine (GTN) in cardiometabolic diseases. In different studies of nitrate tolerance, the effects of GTN on mitochondrial O_2 consumption and ALDH-2 activity were shown to be prevented by MitoQ.[163,164]

There is also *in vivo* evidence for the beneficial effects of MitoQ in cardiometabolic pathologies. Chacko et al. demonstrated that, when administered orally over a 12-week period, MitoQ improved glomerular and tubular function in an animal model of diabetes type 1, $Ins2^{(+/-)}$ (AkitaJ) mice.[165] MitoQ did not significantly change creatinine levels, but reduced urinary albumin to levels similar to those exhibited by nondiabetic animals. In addition, MitoQ prevented the increased nuclear accumulation of the pro-fibrotic transcription factors phospho-Smad2/3 and β-catenin.

The beneficial effects of MitoQ have been reported in animal models of metabolic syndrome and atherosclerosis (fat-fed $ApoE^{-/-}$ and $ATM^{+/-}/ApoE^{-/-}$ mice, which are haploinsufficient for ataxia telangiectasia mutated protein kinase, ATM). This antioxidant prevented

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Table I. Mitochondria-Targeted Antioxidants which Have Shown Protective Effects in In Vivo (Animal) and/or In Vitro (Cell) Models of Cardiometabolic Pathologies

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Compound	Cardiometabolic condition	References[a]
MitoQ	Cardiac I/R injury	[1,2]
	Endothelial nitroglycerin tolerance	[3]
	Hypertension with cardiac hypertrophy	[4]
	Kidney damage in type 1 diabetes	[5]
	Oxidative damage of beta cells	[6]
	Atherosclerosis and the metabolic syndrome	[7]
	Obesity	[8]
	Coronary artery disease with diabetes	[9]
SS31	Cardiac I/R injury	[10]
	Hypertensive cardiomyopathy	[11]
	Insulin resistance	[12]
	Diabetic retinopathy	[13]
MitoTEMPO	Hypertension	[14]
	Oxidative damage of beta cells	[6]
SkQ1	Cardiac I/R injury	[15]

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Table I. Continued

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Another recently published study has demonstrated a beneficial role of mitochondria-targeted antioxidants in obesity-related comorbidities. Zucker obese fatty (ZOF) rats have high levels of ROS in smooth muscle cells and the aortic endothelium and display increased UCP2 and antioxidant enzyme activity in comparison with Zucker control rats. MitoQ significantly reduced lipid peroxides in ZOF rats to levels similar to those seen in lean rats and improved the metabolic profiles. This beneficial effect restored coronary collateral growth in response to repetitive ischemia to the level of the control animals.[167]

Liposomal carriers are constituted by phosphatidylcholine, phosphatidylglycerol, and cholesterol, which can inclose small molecular weight antioxidants, antioxidants enzymes, or a combination of various agents with antioxidant activities.[168] It appears that antioxidants, such as the liposomally encapsulated *N*-acetylcysteine[169], quercetin, and Siliphos (a complex formed by silybin and phospholipids), have therapeutic effects (Fig. 4). Siliphos has been shown to be hepatoprotective in a rat model of steatosis[170] and to ameliorate liver enzyme levels in NAFLD patients[171] by enhancing mitochondrial function and through an insulin-sensitizing action.

MITO-Porter is a liposome-based nanocarrier that delivers cargo to mitochondria through a membrane fusion mechanism (Fig. 4) based on the multifunctional envelope-type nanodevice (MEND), which consists of a condensed plasmidic DNA core and a lipid envelope that mim-ics envelope-type viruses.[172] This mechanism seems to be capable of transporting functional nucleic acids, proteins, and small bioactive molecules.[173] These delivery systems are useful as they can transport encapsulated molecules of varying physicochemical characteristics or size. Mitochondrial delivery using MITO-Porter takes place in three steps: (i) delivery of the carrier from the extracellular space to the cytosol; (ii) intracellular trafficking of the carrier, includ-ing mitochondrial targeting; and (iii) mitochondrial delivery via membrane fusion. Also, a conjugate nanocarrier that targets mitochondria and contains a mitochondria-targeting signal peptide (MTS) and MITO-Porter has been developed very recently.[174]

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Szeto-Schiller (SS)-peptides[175] and mitochondria-penetrating peptides (MPPs)[176] are peptide-based targeting ways of delivering antioxidants to mitochondria. SS-peptides are antioxidant compounds with three positive charges in homeostatic pH conditions. In vitro cell studies have shown their rapid uptake through the cellular membrane in a concentration-dependent way by which they accumulate 1000-fold in mitochondria where they bind to the IMM through a mechanism that is not completely described.[177,178] It is important to mention that the particular mitochondrial uptake of these compounds does not take place in response to $\Delta\Psi_m$;[177,179] this is advantageous, as mitochondrial membrane polarization is not disturbed and that the process is not self-limiting. SS-peptides have been shown to exert a protective effect against oxidative stress in cellular models of disease, including insulin resistance, and in isolated mitochondria (Table I), with SS-31 demonstrating to be the most beneficial.[175,180,181] In terms of the increase of MPPs in the mitochondrial matrix, hydrophobicity and electric charge seem to be of great relevance,[182] although the mechanisms by which MPPs are transported through the phospholipid bilayer and the role of $\Delta\Psi_m$ are unclear.

Novel mitochondria-targeted molecules and their therapeutic potential have recently been described. XJB-5–131 is an electron and ROS scavenger containing the Leu-D-Phe-Pro-Val-Orn fragment of gramicidin S, a membrane-active cyclopeptide antibiotic.[183] (Fig. 4). Due to the high affinity of this type of antibiotics for bacterial membranes, which resemble mitochondrial membranes, XJB-5–131 can be targeted successfully to the mitochondrion. This compound has been shown to be beneficial in acute tissue ischemia, such as that produced in rat enterocytes exposed to lethal hemorrhagic shock.[184]

Another approach to targeting bioactive molecules to the mitochondrial matrix is platform technology using biodegradable polymers.[185] This method involves a rationally designed mitochondria-targeted polymeric nanoparticle (NP) system and the combination of a targeted poly(D,L-lactic-co-glycolic acid)-block (PLGA-b)-poly(ethylene glycol) (PEG)- TPP polymer (PLGA-b-PEG-TPP) with either nontargeted PLGA-b-PEG-OH or PLGA-COOH. In particular, the construct PLGA-b-PEG-TPP NP shows great promise as a component of therapy for mitochondrial dysfunction-related metabolic diseases, such as obesity.

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A. Summary and Perspectives

Oxidative stress is clearly related to the pathogenesis of cardiometabolic diseases, while the pathophysiological importance of different molecules requires further study. In general, the data obtained with antioxidant strategies employed to limit the pathophysiological effects of insulin resistance are scarce and inconsistent. Mitochondria play a critical role in cardiometabolic diseases, such as metabolic syndrome and diabetes. The level of mitochondrial ATP is crucial in regulating insulin release; mitochondrial ROS, which otherwise exerts a vital role as secondary messengers, impair this process. Mitochondrial function is a key factor in insulin sensitivity in tissues, such as muscle, liver, and adipose tissue. In this review, we have focused on the mechanisms of the mitochondrial dysfunction related with the pathophysiology of insulin resistance and type 2 diabetes in different tissues, and have considered the process of cardiometabolic diseases from a mitochondrial perspective. We have discussed the potential beneficial effects of mitochondria-targeted antioxidants as a tool for modulating mitochondrial function in cardiometabolic diseases, and particularly in diabetes. The future of mitochondrial pharmacology will depend on the development of mitochondria-targeted antioxidants and new and accurate methods of assessing all aspects of mitochondrial function in patients. To conclude, we believe that mitochondrial pharmacology has great potential as an emerging therapeutic element to be used in many aspects of medicine.

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Milagros Rocha *obtained his undergraduate degree at Madrid's Complutense University in 1995 and obtained her Ph.D. in 2003, focusing on the control of sexual steroids on segregated products by adipose tissues and the hypothalamic neuropeptides implicated in food intake. As postdoctoral researcher, Dr. M Rocha was at University of Valencia and University Hospital Doctor Peset from 2004 to 2010. She is now a Miguel Servet researcher of ISCIII at University Hospital Doctor Peset. She has spent time working in different international and national research centers, including the University of Liverpool (Dr. G Williams), and the Barcelona Biomedical Research Group (PRBB) (Dr. R. Herance). Currently, Dr. Rocha's work focuses on:*

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2. Influence of functional foods in cardiovascular risk, inflammation, and oxidation.
3. Hypolipemic pharmacological agents and endothelial dysfunction.

Dr. Rocha has been the main investigator of several regional funded competitive research projects and has published more than 40 articles in different international journals, including Circulation Research, Hepatology, JCEM, Fertility and Sterility, and Journal of Nutritional Biochemistry.

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and is an author of 19 original peer-reviewed papers, eight review articles and one editorial as guest editor. She has had more than 40 communications in national and international meetings and has supervised several doctoral and master theses. Nadezda has won several awards including the award of the best graduated student at the Faculty of Mathematics and Natural Sciences at "St. Cyril and Methodius" University for the year 2001 or the EPHAR Young Investigator Award in 2011. Her research over the last years has been focused on the participation of mitochondria in cellular pathophysiology regarding parameters, such as oxidative stress, survival mechanisms, and induction of cell-death programs. Recently, her investigation has had a special emphasis on deciphering the bases of several pharmacological mitotoxicites in cellular models.

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Victor M. Victor *obtained his degree at Madrid's Complutense University in 1995. He subsequently trained as a postgraduate student in the laboratory of Dr. Monica de la Fuente at the Department of Physiology of the same university, where he obtained his Ph.D. in 2001 in the role of antioxidants in immune function in murine models of endotoxic shock. After a 6-year period at CNIC (National Centre of Cardiovascular Disease Research) from 2002 to 2008, he is now a researcher at University Hospital Doctor Peset and an associate professor at the Faculty of Medicine, University of Valencia. Over the years, Dr. Victor has also spent time in different laboratories in Cambridge, London, Dublin, and New York analyzing different aspects of mitochondrial bioenergetics and related diseases. Nowadays Dr. Victor's work focuses on mitochondrial and endothelial dysfunction and related diseases, such as type 2 diabetes and metabolic syndrome. Additionally, he is involved in the following lines of research: (i) the role of mitochondria-targeted*

antioxidants in different models of oxidative stress. (ii) The potential role of nanoparticles in antioxidant function. (iii) The effect of different drugs in lipid profile. Dr. Victor has been the principal investigator of several nationally funded research projects and has published more than 70 articles in different international journals including Diabetes Care, Circulation Research, Immunity, PNAS, Hepatology, and JCEM. He is an international reviewer and member of the editorial board of several journals.

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