Is Mitochondrial Dysfunction a Common Root of Noncommunicable Chronic Diseases?

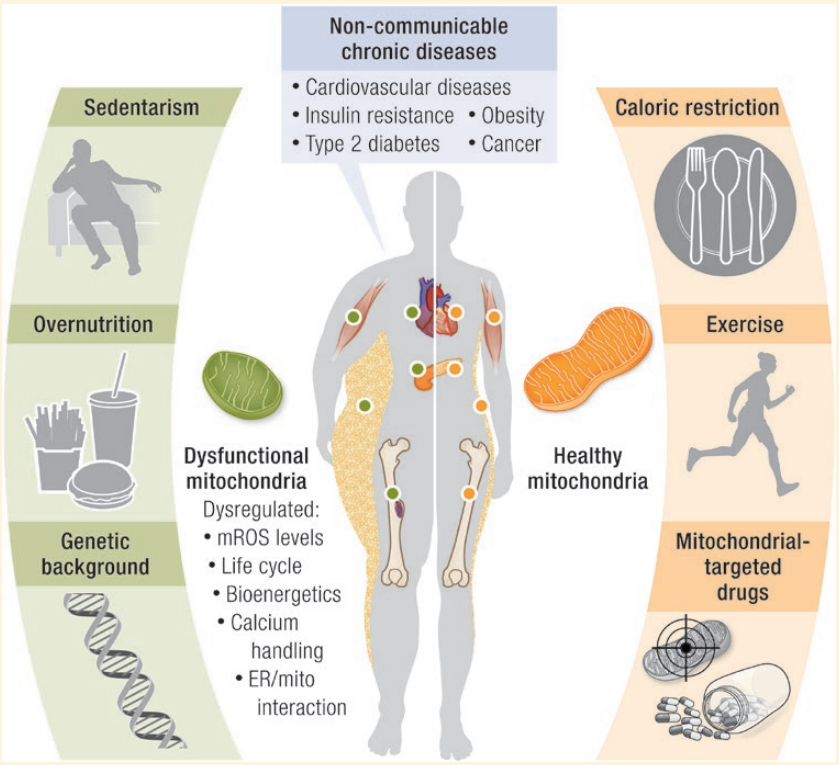
Alexis Diaz-Vegas,[1,2] Pablo Sanchez-Aguilera,[1] James R Krycer,[2] Pablo E Morales,[1] Matías Monsalves-Alvarez,[1,3] Mariana Cifuentes,[1,3,5] Beverly A Rothermel,[4] and Sergio Lavandero[1,4,5]

[1]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad de Medicina, Universidad de Chile, Santiago, Chile; [2]Charles Perkins Centre, School of Life and Environmental Sciences, The University of Sydney, Camperdown, Sydney, NSW, Australia; [3]Institute of Nutrition and Food Technology (INTA), Universidad de Chile, Santiago, Chile; [4]Department of Internal Medicine (Cardiology Division), University of Texas Southwestern Medical Center, Dallas, Texas 75390 ; [5]Center for Studies of Exercise, Metabolism and Cancer (CEMC), Facultad de Medicina, Universidad de Chile, Santiago, Chile.

ORCiD number: 0000-0003-4258-1483 (S. Lavandero).

Abstract Mitochondrial damage is implicated as a major contributing factor for a number of noncommunicable chronic diseases such as cardiovascular diseases, cancer, obesity, and insulin resistance/type 2 diabetes. Here, we discuss the role of mitochondria in maintaining cellular and whole-organism homeostasis, the mechanisms that promote mitochondrial dysfunction, and the role of this phenomenon in noncommunicable chronic diseases. We also review the state of the art regarding the preclinical evidence associated with the regulation of mitochondrial function and the development of current mitochondria-targeted therapeutics to treat noncommunicable chronic diseases. Finally, we give an integrated vision of how mitochondrial damage is implicated in these metabolic diseases.

Graphical Abstract



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Essential Points

- Noncommunicable chronic diseases are complex and multifactorial conditions that collectively pose a huge challenge for public health systems
- Mitochondria are an integrative platform that support indispensable cell functions, including metabolism and cellular signaling
- Several noncommunicable chronic diseases have in common the early development of a marked mitochondrial dysfunction
- Definition of mitochondrial dysfunction and its contribution to cell function is imperative to understand the pathophysiology of noncommunicable chronic diseases
- Understanding the molecular mechanisms involved in the effect of caloric restriction and exercises will pave the way toward new mitochondrial-targeted therapies to combat noncommunicable chronic diseases

Noncommunicable chronic diseases (NCDs) are complex and multifactorial conditions that collectively pose a huge challenge for public health systems in both the industrialized and the developing world. NCDs are, by definition, noninfectious, nontransmissible disorders often associated with low-grade inflammation, among which the most common are obesity, insulin resistance (IR), diabetes, cancer, cardiovascular diseases (CVDs), among others.

According to the World Health Organization, in 2016, 6 of the "top 10" causes of death worldwide were NCDs. These included ischemic heart disease and stroke (representing a combined 15.2 million deaths); chronic obstructive pulmonary disease; Alzheimer's disease and other dementias; trachea, bronchus, and lung cancers, and type 2 diabetes mellitus (T2DM). The latter (T2DM) exemplifies the alarming increase in the prevalence of many of these conditions, killing 1.6 million people in 2016 compared with fewer than 1 million in 2000. It has been estimated that the economic burden of NCDs for low- and middle-income countries will be more than US$7 trillion over the period 2011-2025 (1); thus, the challenge of NCDs is not unique to the developed world.

The urgent need to address this global health epidemic has prompted both scientific and social initiatives to combat NCDs. It is broadly understood that unhealthy eating behaviors and sedentary lifestyles are significant contributors, as being overweight or obese is highly associated with hypertension, IR, T2DM, cancer, nonalcoholic fatty liver disease (NAFLD), and CVD. As the world faces the epidemic of NCDs, basic scientific research has a critical role to play by identifying the cellular and molecular mechanisms that are involved in the pathophysiology and metabolic consequences of NCDs, thereby laying the groundwork for knowledge-based approaches to the prevention and treatment of these conditions.

A combination of genetic and environmental factors contributes to the development of NCDs, including low-grade inflammation and oxidative stress (1). Intriguingly, many of these factors have in common the ability to either damage mitochondria or interfere with mitochondrial repair (1), suggesting that mitochondria play a central role in the pathophysiology of NCDs.

Mitochondria are well placed in this respect: from a genetic standpoint, both mitochondrial and nuclear genomes must cooperate to maintain mitochondrial homeostasis and respond to changes in demand. Thus, mutations in either genomic compartment can have pathological consequences. From an environmental standpoint, mitochondria are central processing hubs for external nutrient input and energy output. In addition to generating adenosine triphosphate (ATP), they synthesize a number of important lipid species and provide metabolic substrates for post-translational modifications that are central to intra- and intercellular signaling (eg, acetylation, fumaration, succination, malonylation). They also act as central mediators of internal and external death signals.

Metabolic aberrations are at the core of many NCDs. Even before the mechanisms linking mitochondrial respiration to ATP generation were elucidated (eg, chemiosmotic theory in the 1960s), altered mitochondrial function was identified as a key feature of certain NCDs. Perhaps the most famous example is Otto Warburg's observation in 1924 (2) that metabolism in cancer cells was very different from that of normal tissues, tending toward glycolysis of glucose to lactate even in the presence of oxygen, thereby linking respiratory defects to cancer. Over the last decade many lines of investigation have converged on mitochondrial dysfunction as a central feature in the development of a variety of NCDs (Table 1). Of particular note is the close association between mitochondrial dysfunction and obesity (40), with mitochondrial dysfunction often proposed as a critical factor

Table 1. Tissue-specific mitochondrial dysfunction observed in noncommunicable diseases (NCDs) and the cellular phenotype change related to mitochondrial dysfunction.

NCDs	Cell type	Mitochondrial dysfunction	Cellular change	References
Obesity	Adipocyte	Mitochondrial fragmentation	Increased proliferation and differentiation	(3,4)
		mtROS overproduction		
	Hepatocyte	Mitochondrial fragmentation	Metabolic shift and cell death	(5,6)
		ETC components reduction		
		Impaired $\Delta\Psi$mt and ATP synthesis		
Insulin resistance	Skeletal muscle cell	Mitochondrial fragmentation	Impaired insulin signaling, reduced glucose uptake and GLUT4 translocation	(7-13)
		Mitochondrial Ca^{2+} mishandling		
		mtROS overproduction		
		MAM's disruption		
	Adipocyte	mtROS overproduction	Impaired GLUT4 trafficking and glucose uptake	(10,12)
	Hepatocyte	MAM's disruption	Impaired insulin signaling and insulin resistance	(14,15)
		Mitochondrial fragmentation		
Type 2 diabetes mellitus	β-cells	Mitochondrial respiratory uncoupling	Reduced insulin secretion	(16-24)
		Impaired ATP synthesis		
		Altered mitophagy		
		Exaggerated fission or fusion		
Cancer	Primary tumor cell	PCG-1α reduction	Adaptative metabolic shift, increased biosynthetic activity, proliferation, tumorigenesis and aggressiveness	(25-29)
		Enhanced mitophagy		
		Excessive glycolytic rate		
		moderate mtDNA mutations		
		Mitochondrial metabolic hyperactivity		
	Circulatory and invasive tumor cell	PCG-1α overactivity	Metabolic shift, cellular migration and invasion	(30-34)
		Enhanced mitochondrial Ca^{2+} uptake		
		mtROS overproduction		
Cardiovascular disease	Smooth muscle cell	mtDNA damage	Increased proliferation	(35)
	Cardiomyocyte	Mitochondrial fragmentation	Hypertrophy, impaired contractility, energetic crisis, calcium overload and cell death	(13,36-39)
		MAM disruption		
		Mitochondrial Ca^{2+} mishandling		
		mtROS overproduction		
		Decreased OCR		

Abbreviations: ATP, adenosine triphosphate; ETC, electron transport chain; mROS, mitochondrial reactive oxygen species; MAM, mitochondrial associated membrane; mtDNA, mitochondrial DNA; OCR, oxygen consumption rate.

mediating the progression of many obesity-related NCDs (Table 1) (41).

The present review addresses the relevance of mitochondrial function to cell homeostasis and adaptation, examining the mechanisms and consequences of mitochondrial dysfunction in the development of NCDs, specifically obesity, IR, T2DM, cancer, and CVD. We focus on these conditions because of their shared characteristics of altered metabolism at the mitochondrial

level and their close association with the obesity pandemic. We want to stress that mitochondrial dysfunction is not simply "broken" or damaged organelles but includes states in which there is a mismatch between mitochondrial activities and cellular demands. Normal human physiology requires continuous adaptation to changes in nutritional input (fasting/feeding, lipolysis/lipogenesis) versus energy expenditure (sleeping/waking, physical activity/sedentary behavior). The highly plastic nature of mitochondria allows them to adapt to these constant changes in order to maintain homeostasis or mediate cell death. The definition of mitochondrial dysfunction is in essence the failure of mitochondria to adapt appropriately or sufficiently and this is what lies at the core of many NCDs. The possible therapeutic opportunities that this knowledge provides will also be discussed.

Mitochondrial Physiology

General information

Mitochondria are bound by a double membrane. The inner mitochondrial membrane (IMM) contains multiprotein complexes of the electron transport chain (ETC) that shuttle electrons from nicotinamide adenine dinucleotide with hydrogen (NADH) and flavine adenine dinucleotide ($FADH_2$) to molecular oxygen, ultimately forming water. In the process, protons are pumped from the internal matrix into the intermembrane space, storing potential energy in the form of an electrochemical gradient that can be used by complex V to generate ATP. The proton motive force (Δp) consists of both a pH gradient (ΔpH) and an electrical gradient (mitochondrial membrane potential, $\Delta \Psi mt$). In addition to oxidative phosphorylation, the IMM and the outer mitochondrial membrane (OMM) contain a diversity of channels and enzymes that along with proteins in the matrix carry out a variety of cellular functions including the synthesis of nucleotides, heme groups, and a subset of essential lipids, among others (42). These organelles are also at the center of cellular life or death decisions. Mitochondria continuously adapt in response to extra- and intracellular stimuli through transient changes in localization or metabolic activity as well as through more pronounced structural modifications, such as changes in morphology or content (43,44). The following section will discuss the basic concepts of structural and functional mitochondrial adaptations.

Mitochondrial dynamics: When the shape illuminates the function

Mitochondria are not static and can exist in a variety of ever-changing shapes, even within the same cell. "Bullet"-, "spaghetti"-, and "donut"-like, and a "mitochondrial reticulum" have all been used to describe mitochondrial architecture (45). These structures are the result of continuous cycles of mitochondrial transport, fragmentation, and fusion events, collectively termed mitochondrial dynamics (Fig. 1) (45). Defects in the fission machinery result in an elongated network. Conversely, reduced activity of profusion proteins leads to a more fragmented state (46,47). The primary functions of mitochondrial dynamics are metabolic adaptation and quality control, with fusion increasing oxidative capacity and fission facilitating degradation of damaged organelles and their replacement (48).

Fusion of the mitochondrial network depends on the activity of large, membrane-localized GTPases. In the early 2000s, evidence pointed to an essential role for Mitofusin (Mfn-1/2) proteins in the tethering and fusion of the OMM (46,49,50). In mammals, overexpression of either Mfn-1 or Mfn-2 results in a more interconnected mitochondrial morphology and increased perinuclear clustering. Conversely, their downregulation increases mitochondrial fragmentation (46,49,50). Mfn molecules localized to the OMM of 1 mitochondrion can interact with Mfn from a different mitochondrion, forming homo- or heterocomplexes to tether mitochondria and facilitate GTP-dependent fusion with the opposing OMM (Fig. 1) (46,51,52). Mice with single deletions of either Mfn-1 or Mfn-2 alone are viable whereas the double deletion is lethal (49), demonstrating functional redundancy. Despite this, complementation experiments suggest that Mfn-1 may play a more dominant role in the fusion process (46,51,53).

Elegant microscopy experiments reveal that the process of IMM fusion is independent and distinct from that of OMM fusion (54,55). Optic atrophy 1 (OPA1), a GTPase on the external surface of the IMM (56) is involved in IMM fusion (57, 58). It also helps shape mitochondrial cristae morphology, promoting tightening of crista junctions (57-59). This, in turn, facilitates ETC super complex formation and increases oxidative phosphorylation (60,61), thereby mediating a mechanistic link between mitochondrial form and function (62,63).

Mitochondrial fragmentation, the opposing process, requires dynamin-related protein 1 (Drp-1), a member of the dynamin family of GTPases (47, 64) that resides in the cytosol. Upon

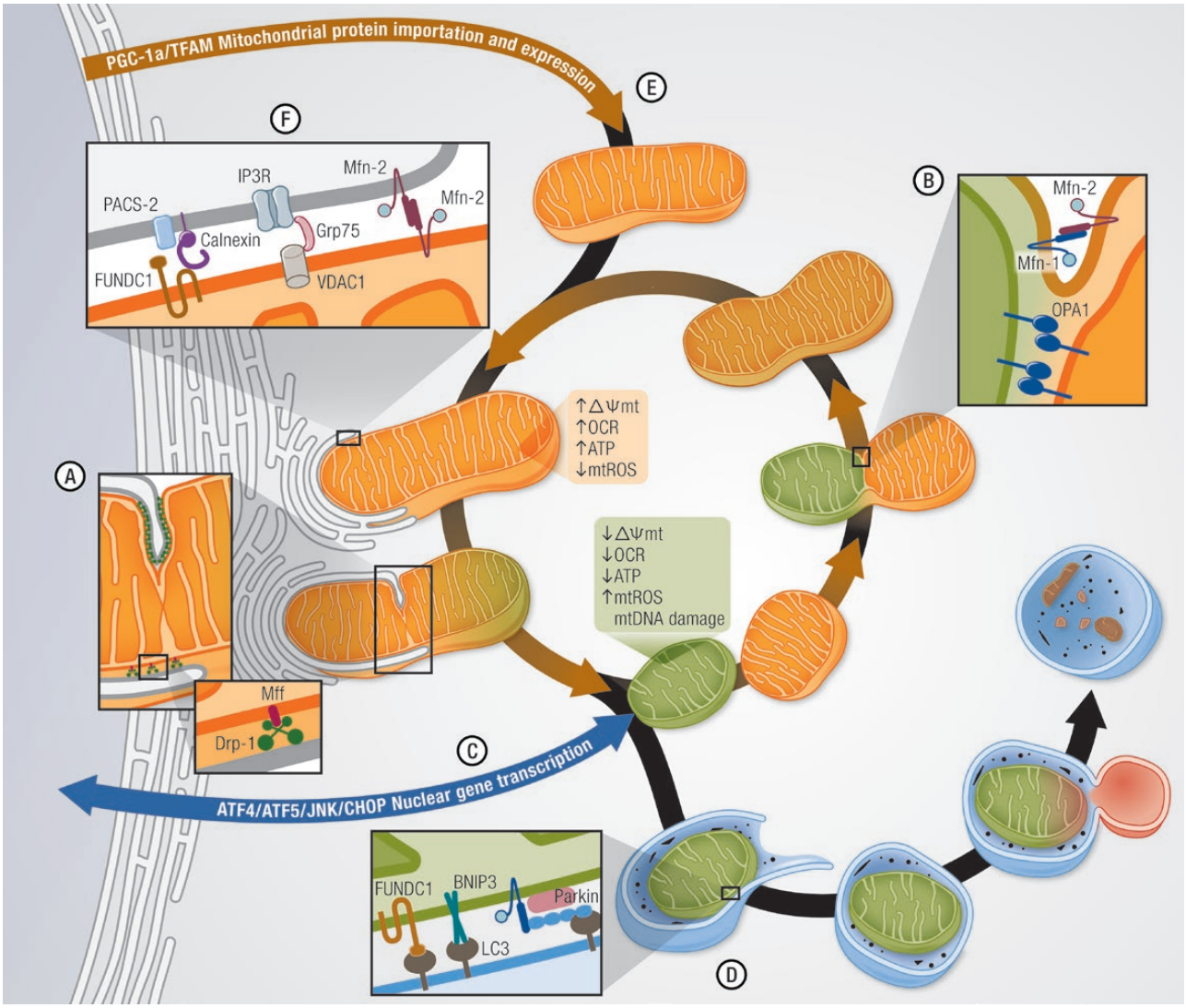

Figure 1. Mitochondrial plasticity regulates the mitochondrial quality control and function. A mitochondrion is a malleable organelle that constantly changes its structure and shape to adapt its function and allow the normal replacement of anomalous mitochondria. (A) Mitochondrial fission is regulated by Drp-1, which binds to outer mitochondrial membrane (OMM) protein adaptor like Mff, which together with the endoplasmic reticulum, (ER) constrict and isolate the impaired mitochondria. On the other hand, mitochondrial fusion (B) is orchestrated by dynamin-related GTPases mitofusin (Mfn) 1/2 and optic atrophy type 1 (OPA1) promoting the OMM and inner mitochondrial membrane (IMM) fusion respectively. This process allows the function of an anomalous mitochondrion to be enhanced and averaged through combination with a healthy mitochondrion. (C) The stressed mitochondria also communicate with the nucleus through the mitochondrial unfolded protein response (UPRmt) and activate ATF4/5 and JNK/CHOP signaling pathways to induce the transcription of nuclear genes associated with mitochondrial survival and quality control. (D) Alternatively, impaired mitochondria may also be eliminated by mitophagy, triggered by the direct association of BNIP3 or FUNDC1 with LC3 or by marking mitochondria through the parkin-mediated polyubiquitination of OMM proteins. (E) Nuclear encoded mitochondrial proteins and mtDNA transcription are induced by PGC-1α that enhance mitochondrial biogenesis and maintain the mitochondrial mass. (F) ER–mitochondria communication through mitochondria-associated membranes (MAMs) allow molecules to be exchanged between these organelles and regulate the mitochondrial metabolism, bioenergetics, and signaling processes. MAMs are composed of ER proteins like PACS-2, Calnexin, and Mfn-2 and by mitochondrial proteins like VDAC, Mfn-2, and FUNDC1 among others.

activation, Drp-1 translocates to the OMM, binds adaptor proteins, and then assembles a collar-like structure to initiate GTP-dependent constriction of the lipid bilayers (Fig. 1) (47,64,65). A second GTPase, dynamin-2, is required for the final step of lipid fusion and organelle division (66). In mammals, several adaptor proteins capable of recruiting Drp-1 to the OMM have been identified, including mitochondrial fission 1 protein (Fis1), mitochondrial fission factor (Mff), and mitochondrial dynamics proteins, 49 kDa and 51 kDa (MiD49 and MiD51) (67-70). Deletion of these proteins often results in elongation and clustering of mitochondria around the nucleus (67,69,70). Recent studies using TAT fusion peptides to selectively disrupt adaptor interactions with Drp-1

suggest that Mff is required for mitochondrial fission under physiological conditions, whereas Fis1 mediates the interaction and subsequent fission under pathological stress (71).

Mitochondrial dynamics facilitate changes in metabolic activity. In general, when the activity of fission proteins is experimentally reduced to generate a more elongated network, it has been observed that cells are more metabolically active than those with smaller, fragmented mitochondria, even when total mitochondrial content is the same. On the contrary, artificially inhibiting fusion increases mitochondrial fragmentation while reducing both the rate of oxygen consumption and $\Delta\Psi mt$ (49, 72). These observations have often led to the impression that a larger, more fused mitochondrion is a "healthier" organelle. Caution should be taken though, as under basal, normally occurring conditions, this statement might be inaccurate. For instance, subsarcolemmal mitochondria on muscle fibers are more spherical and isolated units, while the intermyofibrillar counterparts are organized in a more complex network. Each population is matched to meet the metabolic and biosynthetic demands specific to its subcellular location (73,74). As such, both types of distinctively shaped mitochondria work synergistically to orchestrate cell metabolism. Both fission and fusion are needed to maintain cellular homeostasis. Fission in particular plays an essential role in repair processes. For instance, in adult muscle, damaged mitochondria are electrically and physically separated from the rest of the network, allowing the remaining mitochondria to resume their normal function (75) while the damaged organelle is repaired, replaced, or degraded. Suboptimal or damaged mitochondria are separated through fission events and can be eliminated by mitophagy or undergo fusion with healthy mitochondria to increase their function. The dynamic nature of the mitochondrial life cycle helps maintain a constant mitochondrial mass by establishing an equilibrium between mitochondrial biogenesis and degradation (Fig. 1) (48). These processes will be discussed later in greater detail.

The fission/fusion process participates in many cellular responses and adaptations. For instance, mitochondrial fragmentation can be an essential initiating signal in apoptosis (76). Mitochondrial elongation (fusion) can increase the rate of ATP synthesis and prevent mitochondrial degradation (77). Fusion can occur when cells are exposed to different oxidative substrates to stimulate oxidative phosphorylation (78). However, acute exposure to high glucose causes an immediate and transient increase in mitochondrial fragmentation and mitochondrial reactive oxygen species (mROS) production in cultured hepatocytes and myocytes that is blocked by inhibiting Drp-1 (3). Similarly, increasing OPA1 can protect mitochondria from damage caused by ETC inhibitors (61,79). These findings are physiologically relevant. For example, insulin action promotes mitochondrial fusion in cardiomyocytes in vivo, increasing mitochondrial metabolism via an OPA1-dependent mechanism (80). Taken together, there is a preponderance of evidence that mitochondrial morphology and function are intimately associated and mutually regulated.

Mitochondrial ROS: It depends on context and fuel

A hyperpolarized Δp increases the potential for generating ROS, which can damage cellular content including DNA. Thus, since a more fused mitochondrial network is associated with higher metabolic activity, fusion also tends toward an increase in the capacity for ROS generation. However, not all ROS generation is pathological, and it is now widely recognized that ROS can function as an important cellular signaling molecule. Because increased mROS is often evoked as a primary mechanism through which dysfunctional mitochondria contribute to the pathology of NCDs, it is relevant to understand what regulates mitochondrial ROS production (81). Transfer of a single electron to molecular oxygen (O_2) produces a highly reactive superoxide ($O_2^{\bullet-}$) molecule. At physiological pH, $O_2^{\bullet-}$ is rapidly converted to peroxide (H_2O_2) with the help of superoxide dismutase in the cytoplasm and mitochondrial matrix (SOD1 and SOD2, respectively). Even though H_2O_2 is not actually a reactive radical, it is considered a ROS because in the presence of a transition metal (ie, Fe^{2+}) it is converted to a highly reactive hydroxyl radical ($HO^{\bullet}$) via the Fenton reaction. At least 11 mitochondrial sites are known to produce $O_2^{\bullet-}$ and/or H_2O_2 ($O_2^{\bullet-}/H_2O_2$) (82). Here we will only discuss ROS production at complex I, as it is thought to be the primary site in vivo, and particularly important with respect to NCDs. Furthermore, it provides an excellent example of how various factors that predispose individuals toward developing NCDs can influence mitochondrial ROS production.

Generation of $O_2^{\bullet-}$ is directly dependent on (1) Δp (40), the local O_2 concentration, and (41) the ratios of the redox donor–acceptor pairs $NADH/NAD^+$ and $CoQH_2/CoQ$ (the coenzyme Q pool) (83). Under conditions where cellular demand for ATP keeps pace with its production,

mitochondrial Δp is dissipated by complex V and thus remains relatively depolarized. The NADH/NAD^+ ratio is also reduced because of the flow of reducing equivalents from NADH into the ETC. Finally, respiration consumes O_2, lowering its local concentration. This combination of conditions minimizes ROS generation from complex I. In contrast, there are 2 main modes of ROS generation at complex I. The first mode, forward electron transfer (FET), requires that the flavin mononucleotide (FMN) in complex I is in an almost fully reduced state. This only occurs when the NADH/NAD^+ ratio is high. Any one of a variety of cellular perturbations that inhibit specific component of the ETC will raise the NADH/NAD^+ ratio and increase ROS generation via this mechanism (84,85), including damage to or mutation of the complex, ischemia, loss of cytochrome *c*, or a low cellular demand for ATP. Conversely, conditions that lower the NADH/NAD^+ ratio tend to suppress ROS production. In fact, the addition of complex I substrates augments NADH levels and increases H_2O_2 production in isolated mitochondria (86). Subsequent administration of ADP (to model an increase in demand for energy, eg, physical exercise) results in oxidation of NADH to NAD^+ and reduced H_2O_2 levels (87). Thus, a careful balance between energy demand and supply is necessary to control mROS generation. Although responsive to the NADH/NAD^+ ratio, the FET mode of ROS generation is relatively independent of Δp, occurring at both hyper- and hypopolarized membrane potentials (83).

The second mode of ROS generation involves reverse electron transfer (RET) from $CoQH_2$ through complex I, and reducing NAD^+ to NADH (87,88). This mode of ROS generation can occur during oxidation of succinate or fatty acids (88). For instance, succinate accumulation and RET-dependent mROS generation has been observed during heart ischemia (89). Inhibition of succinate dehydrogenase attenuates mROS production via RET, thereby protecting the heart from mROS-induced damage (89). In contrast to ROS generation via FET, a hyperpolarized Δp is essential for RET and very small depolarization in Δp can abolish it, so as long as ATP demand remains high, a shift to fatty acid oxidation does not necessarily increase ROS production. It is reasonable to speculate the increase in mitochondrial fission that is often noted in metabolic disease could in part be a compensatory measure to reduce RET-mediated ROS.

It is important to note that mitochondrial fission often generates daughter organelles with an unequal distribution of Δp, ETC component, mtDNA, etc. (90). Furthermore, deletion of Mfn-1/2 to prevent fusion evokes stochastic depolarization of a subset of mitochondria (49,72). Subsequently, daughter mitochondria with depolarized Δp can be preferentially removed by mitophagy, whereas, the daughter mitochondria capable of maintaining or restoring Δp may merge again with the mitochondrial network (91).

The mitochondrial uncoupling proteins (UCPs) provide an additional compensatory mechanism for reducing RET-mediated mROS generation. UCPs are sensitive to glutathionylation (92) and they have been implicated in many pathological conditions. For instance, overexpression of UPC2 in cancer cells confers a drug-resistant phenotype (93). Downregulation of UCP3 increases oxidative damage, impairs fatty acid oxidation, and compromises cardiac function (94), whereas overexpression of UCP3 in skeletal muscle protects against high-fat diet-induced IR (95). An acute increase in mROS production can provoke UCP2/3-dependent proton leak across the IMM (96,97) depolarizing mitochondria and thereby providing negative feedback to reduce RET-dependent mROS production (96,97) and maintaining mitochondrial–redox homeostasis.

Another important mode of mitochondrial ROS generation relevant to NCDs is ROS-induced ROS release (RIRR). This occurs when an initial burst of ROS production triggers a much larger feed-forward release from both the originating and neighboring mitochondria. The mechanisms of RIRR are reviewed in detail elsewhere (98). This is the mode of ROS release though to occur in response to ischemia reperfusion. The mitochondrial translocator protein is an integral OMM protein involved in gating RIRR and is involved in the propagation of cardiac arrhythmias following a heart attack and those associated with T2DM (99). Whether RIRR plays a role in NCDs requires further investigation.

Mitochondrial unfolded protein response: The whisper between nucleus and mitochondria

The human mitochondrial proteome is composed of nearly 1500 different proteins, only 13 of which are encoded by the maternally inherited mitochondrial genome (mtDNA) (100). The large number of nuclear-encoded mitochondrial proteins imposes a tremendous workload on the mitochondrial protein import system (101). It is therefore not surprising that mechanisms have evolved for mitochondrial–nuclear communication. For instance, mitochondrial stress can lead to remodeling of

nuclear chromatin and global gene silencing (102). An important aspect of the mito-nuclear communication system is the mitochondrial unfolded protein response (UPRmt). The name "UPRmt" was originally coined based on its role to re-establish mitochondrial proteostasis. However, the UPRmt is now thought of as an integrated response to a variety of mitochondrial stressors, including ETC damage, and accumulation of misfolded proteins and mROS. Activation of the UPRmt can trigger compensatory adaptations (eg, changes in expression of nuclear encoded glycolytic enzymes) to sustain proper cell function (103).

Work primarily in yeast and worms has provided important insights into mechanisms mediating mito-nuclear communication (104,105). Although mammalian cells share some features, the specifics of the mammalian system are yet to be fully elucidated. The mammalian transcription factor ATF5 has been proposed to act in a fashion similar to that of ATFS-1 in *Caenorhabditis elegans* (105,106). Under basal conditions, ATF5 translocates to and accumulates in mitochondria. However, upon inhibition of ETC complex I or V, ATF5 moves to the nucleus, inducing the expression of hallmark UPRmt response genes (Fig. 1) (106). Other stresses, such as dissipation of $\Delta\Psi mt$ or inhibition of mitochondrial protein translation, results in the induction of a similar, but not identical, gene/protein signature committed to the re-establishment of mitochondrial homeostasis, via an eIF2a/ATF4-dependent pathway (107). Similar to the accumulation of unfolded proteins in the ER, the accumulation of misfolded proteins in mitochondria increases the expression of the stress-induced transcription factor C/EBP homologous protein (CHOP) (108). Accordingly, several genes induced by the UPRmt have CHOP binding sites in their promoters (108, 109). Both mROS and JNK2 activation are also involved in the response as their inhibition decreases expression of UPRmt response genes (108).

Although the mammalian UPRmt is not fully defined, there is evidence of its relevance in obesity and NCDs, with mROS generation acting as a mechanism for mediating mito-nuclear communication. In response to diet-induced obesity, ROS levels have been shown to increase in skeletal muscle, triggering the activation of AMP-activated protein kinase (AMPK) and PPAR gamma coactivator-1 alpha (PGC-1α) signaling pathways, leading to mitochondrial biogenesis and rewiring of systemic metabolism (110,111). Similar systemic changes in whole-body metabolism have been observed when mitochondrial translation was impaired by cardiomyocyte-specific deletion of mitochondrial aspartyl-tRNA synthetase (DARS2). Remarkably, expression of UPRmt-responsive genes, such as the mitokine FGF21, occurred even before evidence of respiratory chain deficiency, resulting in reduced body fat mass and glycemia (112). In contrast, deletion of DARS2 in skeletal muscle caused respiratory chain deficiency but did not activate the UPRmt or mediate system changes in metabolism, suggesting tissue specificity in both activation of the UPRmt and its downstream consequences. Taken together, the evidence indicates that upon mitochondrial oxidative or proteostatic stress, mitochondria engage a nuclear response, with consequences not only to the organelle and the cell in which it resides but that can have systemic effects on the whole organism.

Endoplasmic reticulum–mitochondria communication: A matter of distance

Mitochondria often interact directly with other organelles including the endoplasmic reticulum (ER). This was first described 60 years ago (113), and has subsequently received substantial attention because of its implications at the cellular and systemic level (114,115). Some of the first studies aimed at understanding the role of the ER–mitochondria interaction involved phospholipid metabolism (116). As reviewed elsewhere, synthesis of phosphatidylserine, phosphatidylethanolamine, and other phospholipids relies on lipid translocation between endoplasmic reticulum and mitochondria (117,118). After subcellular fractionation, crude mitochondrial extracts were observed to still retain enzymatic activities associated with ER lipid biosynthetic pathways, suggesting that some ER membrane was still attached to mitochondria (117,118). Those tightly bound membranes are currently known as mitochondria-associated membranes (MAMs). MAMs are involved in several important cellular processes including phospholipid synthesis, autophagosome formation (119,120), mitochondrial fragmentation (121), replication and distribution of mtDNA (122), and cell signaling (123,124), to name a few. ER–mitochondria communication is also intimately involved in mitochondrial activity and metabolism. Early studies from Denton et al. and McCormack et al. showed that several dehydrogenase enzymes involved in the Krebs cycle are activated by an increase in intramitochondrial Ca^{2+} (125,126). It is now known that release of Ca^{2+} from ER stores through IP_3R Ca^{2+} channels generates Ca^{2+} "hotspots," promoting Ca^{2+} entry into the mitochondrial matrix, thereby increasing the production

of reducing equivalents (NADH and $FADH_2$) and ATP synthesis (126-130). Therefore, the close apposition of mitochondria to the ER is essential for the transfer of Ca^{2+} and metabolic regulation.

From a physical point of view, ER–mitochondria contact sites must be tightly regulated. The gap between ER and mitochondria is in the 10 to 30 nm range (Fig. 1) (131,132). This distance allows the accommodation and proper distribution of the protein entities that tether mitochondria to the ER. For efficient transfer of Ca^{2+}, IP_3R in the ER side and VDAC1 on the mitochondrial side are connected by the chaperone Grp75 (133,134). Physical binding of the organelles is maintained by additional proteins, such as Mfn-2, located on both the OMM and the ER (135), calnexin and PACS-2 on ER (136,137), and the recently described OMM protein FUNDC1, that interacts with calnexin (Fig. 1) (138).

Given the involvement of ER–mitochondria communication in mitochondrial metabolic activity, it is not surprising that it can also impact systemic metabolism. Genetic deletion of MAM constituent proteins interferes with proper liver and muscle insulin signaling in vivo. Conversely, increasing MAM formation has the opposite effect, protecting against palmitate-induced IR (7,14). Reduction of MAM formation in these tissues results in poor plasma glucose handling, highlighting the profound metabolic impact of MAM stability (7,8,14). In the opposite direction, mitochondria can regulate ER functions. For instance, studies in L6 myotubes show that palmitate-dependent mtDNA damage promotes ER stress and that protecting mtDNA from damage reduces markers of ER stress (139,140).

Regulation of mitochondrial content: Biogenesis and mitophagy

The processes discussed thus far are mechanisms associated with the response of the existing mitochondrial pool to different internal or external cues in order to sustain mitochondrial health and respond to energy demands. However, under certain circumstances total mitochondrial mass needs to change to meet cellular demands. This is achieved through 2 opposing mechanisms, namely mitochondrial biogenesis and autophagic, lysosomal degradation of mitochondria, also known as mitophagy (44,141).

Mitochondrial biogenesis involves the synthesis of new mitochondria from pre-existing mitochondria. It is a complex process that involves mtDNA replication, synthesis of mtDNA and nuclear DNA-encoded proteins, and the formation of new lipid membranes (142). Although a number of transcription factors are known to be involved, such as NRF-1/2, TFAM, and ERRα (142), the ultimate coordinator of mitochondrial biogenesis is PGC-1α. First described as a thermogenic factor in fat tissue (143), PGC-1α was soon after shown to induce the expression of ETC proteins and increase mitochondrial activity (144,145).

Degradation of mitochondria involves the coordinated action of both the general autophagy machinery and the specific signaling from mitochondria. Mitophagy is a "selective" form of autophagy, in the sense that mitochondria are specifically marked for processing through the autophagic machinery (146). The most well-studied mechanism for mitophagy involves the PINK1/Parkin pathway. When mitochondrial membrane potential is lost, the PTEN induced kinase 1 (PINK1) is stabilized at the OMM where it recruits and phosphorylates the E3 ligase protein Parkin, which, in turn, polyubiquitinates proteins in the OMM ((147-149). Adaptor proteins recognize these ubiquitinated tags and direct the mitochondria for degradation via autophagosomes (150). In addition, proteins, such as BNIP3, Nix/BNIP3L, and FUNDC1, can elicit autophagy-mediated degradation of mitochondria independent of Parkin or ubiquitination (Fig. 1) (151-153).

Mitochondrial generation and degradation are usually intimately associated and occur simultaneously. For instance, myoblast differentiation is accompanied by an increase in both PGC-1α and in markers of mitophagy (154,155). In vivo, mitochondrial biogenesis in skeletal muscle in response to exercise occurs in parallel with an increased expression of autophagy-related genes (156,157). Both genetic and pharmacological reduction of either PGC-1α or autophagic activity results in the inhibition of the opposing process (156,158). Mechanistically, protein kinase AMPK (159) or the protein PARIS (160,161) have been shown to coordinately modulate mitochondrial biogenesis and mitophagy.

Retrograde mitochondrial signaling: Beyond ATP

Mitochondria are well known for their central role in generating ATP, but as previously mentioned, these organelles also have biosynthetic and intracellular signaling functions (Fig. 2) (162). These mitochondrial processes act together with the rest of the cell to maintain homeostasis or adapt to environmental changes. For instance, the Krebs cycle in the mitochondrial matrix generates metabolic intermediates (eg, acetyl-coenzyme A) that can be used to generate new cellular molecules

(biosynthetic activity), as well as $FADH_2$ and NADH to provide reducing equivalents to feed the ETC for ATP synthesis (bioenergetic activity). ETC activity, in turn, is coupled to the generation of mROS, which can activate redox-sensitive proteins in the cytoplasm (signaling activity) modulating function of the whole cell (48).

A current challenge in the field is to identify specific mitochondrial processes driving specific pathological conditions. Currently, most interventions targeting mitochondria affect every other organelle function. For example, inhibition of the ETC with specific drugs (eg, Antimycin A) reduces oxygen consumption rate (OCR) but also reduces ATP synthesis (bioenergetic), damps tricarboxylic acid (TCA) activity (biosynthetic intermediate), and increases mROS production (signaling). Therefore, interpretation of experimental results from these approaches can be difficult and need to be taken in the context of the whole system.

There is overwhelming evidence that mitochondrial activity impacts intracellular signaling pathways (163,164). It is also clear that multiple mechanisms are involved (Fig 2). Certainly, as previously discussed, production of mROS and the subsequent redox modification of cytoplasmic proteins are the mechanisms most frequently considered relative to NCDs (98). As an example, mROS has been shown to impact cell proliferation and differentiation via activation of redox-sensitive proteins such as HSP-70 (165). Mitochondria are also key players in a metabolic–genomic regulatory axis because they regulate levels of key metabolites (NAD^+, acetyl-CoA, alpha-ketoglutarate, ATP, among others) used in epigenetic chromatin modification

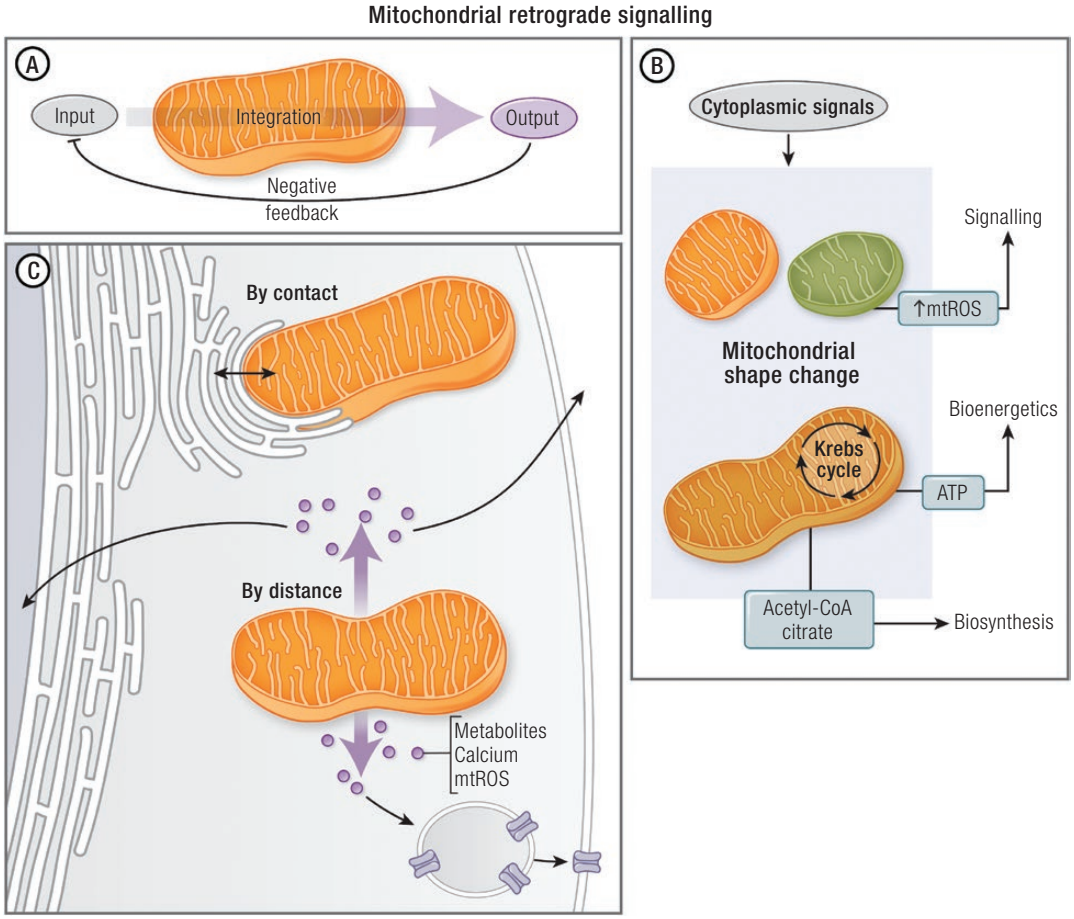

Figure 2. Mitochondrial retrograde signaling. (A) Mitochondria can be considered as an integration node. This organelle receives signals from the cytoplasm (Input), integrates the information, and generates responses (output). This response may regulate several cellular functions, generating a negative feedback on the input. (B) Cytoplasmic signals induce morphological and functional changes in mitochondria that regulate several intracellular functions such as signaling (eg, mROS generation), bioenergetics (ATP synthesis), and biosynthetic (eg, acetyl-CoA) roles. These roles are necessary for the whole cell function. (C) There are 2 mechanisms by which mitochondria regulate whole cell function: (1) by contact (eg, ER–mitochondria interaction), and (2) by distance (eg, mROS activate transcription factors to modulate nuclear gene expression). Communication by distance would include several molecules, such as small metabolites, peptides, and ions. These molecules would regulate nuclear gene expression, vesicle trafficking, and cell death, among others.

(Fig. 2) (166,167). Another emerging example are mitochondria-derived peptides (168). The most recently described is the MOTS-c peptide that is derived from an open reading frame in the mitochondrial 12S rRNA-c gene (169). MOTS-c activates AMPK and regulates nuclear gene expression in response to metabolic stress (170). These mechanisms by which mitochondria control the cell functions beyond the ATP production are collectively known as retrograde signaling (Fig. 2) (164).

Although the retrograde signal was initially described in yeast this signaling pathway is now receiving greater attention in mammalian cells. Crucial questions remain. How do mitochondria communicate stress signals to another cellular compartment? Which transduction mechanisms are involved? What cytoplasm factors respond to and/or regulate mitochondrial signaling? Current omics technology, high-resolution microscopy, and base-machine learning technology create a formidable stage to study the retrograde signaling of mitochondria in mammals and its role in the pathology, prevention, and treatment of NCDs.

Regulation of mitochondrial enzymatic activity

Mitochondrial activity exhibits several enzymatic regulation nodes. Some of these nodes are controlled by metabolic intermediates such as ADP/ATP, NAD^+/NADH, acetyl-CoA, among others (171). The levels of these metabolic intermediates are in turn influenced by the energetic state of the cell, providing a tight relationship between energy demand and mitochondrial metabolism.

ADP is thought to be one of the primary molecules controlling the rate of oxidative phosphorylation (172). The addition of adenosine diphosphate (ADP) increases the OCR of isolated mitochondria and permeabilized cells. Furthermore, declines in ADP levels decreases OCR even in presence of excess ETC substrates (172). Thus, ADP produced by ATP hydrolysis during cellular metabolism (energy demand) can signal an increase in OCR (energy supply).

Another point of control is the mitochondrial Ca^{2+}-sensitive dehydrogenases that supply NADH and $FADH_2$ to the ETC (173). Activation of these dehydrogenases increases ATP synthesis. Among these is pyruvate dehydrogenase (PDH), the rate-limiting enzyme of glucose oxidation that is inhibited when phosphorylated by pyruvate dehydrogenase kinase (PDK), or in the presence of acetyl-CoA or NADH (171). An increase in mitochondrial Ca^{2+} activates pyruvate dehydrogenase phosphatase 1 (PDP1c), the phosphatase responsible for dephosphorylating PDH and restoring its activity, and increasing glucose oxidation (173). Inhibiting mitochondrial Ca^{2+} uptake leads to hyperphosphorylation of PDH, and reduction of both glucose oxidation and OCR (174, 175). There are many additional posttranslational modifications known to regulate mitochondrial metabolism that have been reviewed in detail elsewhere (176,177).

Mitochondrial dysfunction: What is the gold standard to define it?

Classically, mitochondrial dysfunction has been defined as the inability of mitochondria to sustain ATP synthesis sufficient to satisfy cellular demands (178). However, as discussed above, mitochondrial dysfunction exhibits many faces, causes, and consequences. For this reason, diagnosis of mitochondrial dysfunction needs to integrate measures of the organelle's function and structure with the availability of substrates and reducing equivalents. Ultimately, these parameters need to be placed in the context of human physiology. Despite this complexity, functional readouts from isolated mitochondria can still yield important insights and provide a first step toward integration with other cellular parameters.

OCR and ATP production are fundamental functions of mitochondria. Brand and Nichols propose that OCR is the parameter that can provide the most information (179). OCR can yield insights into the processes of substrate transport, substrate metabolism, proton pumping, electron delivery through the ETC, as well as others. Measuring $\Delta\Psi mt$ can provide indirect information regarding proton motive force, although in many ways it can be less sensitive than OCR (179). For instance, deletion of complex I subunit NDUFAF3 reduces OCR and ETC activity without changing the mitochondrial membrane potential (180). This is because $\Delta\Psi mt$ can be generated and maintained via the reverse activity of the F_0F_1 ATP synthase even in the absence of oxidative phosphorylation and ETC activity (181). Thus, $\Delta\Psi mt$ cannot always be taken as an indication of mitochondrial metabolic activity. Measures of mROS generation, ATP production, mitochondrial mass, morphology, and distribution are likewise not exempt from false-positive or negative errors and need to be interpreted with appropriate caution (179).

Recently, Fisher-Wellman et al. designed and validated an elegant multiplexed assay platform that provides a comprehensive phenotyping of mitochondrial functions under conditions that model in vivo changes in energy supply and demand

(182). They provide detailed rationales for each of their assays, offering an excellent resource for anyone undertaking similar analyses. This important approach, however, uses isolated mitochondria and is therefore devoid of information regarding the contribution of the intact mitochondrial network or its interaction with other organelles. An additional issue is that mitochondrial isolation from whole organs yields mitochondria from a variety of cell types which could mask important changes in a specific population. Despite these limitations, when the technique was applied to mitochondria isolated from the hearts of mice on a high-fat diet, there was a generalized decrease in oxygen flux, regardless of substrate, and an increase in proton leak as well as increased H_2O_2 production (183). Although these changes were small, they demonstrate the power of the approach and validate the presence of fundamental changes in cardiac mitochondria in the setting of obesity. Although the approach proposed by Fisher-Wellman et al. does not incorporate measures of mitochondrial Ca^{2+} retention or the mitochondrial permeability transition pore activity (mPTP), it represents a major advancement in the assessment of mitochondrial function, complementing other comprehensive approaches (184-186). Future studies should focus on developing the same kind of workflow to define cell type-specific mitochondrial adaptation in intact living cells and tissues.

Other methodological approaches have been developed that avoid the invasiveness, the lack of biological context and tissue destruction. Near infrared spectroscopy gives information regarding OCR and its reduction by cytochrome C oxidase in human and murine skeletal muscles (187). Magnetic resonance spectroscopy can also be used to measure the OCR and ATP synthesis in vivo, providing measures of mitochondrial coupling in skeletal muscle (188). These 2 techniques avoid artefacts associated with tissue extraction but so far have only been applied to skeletal muscle.

Mitochondrial (dys)function in NCDs

Remarkably, a common feature of many NCDs is a bioenergetic crisis characterized by a reduction in mitochondrial function. In this section, we will discuss the role of mitochondrial dysfunction in NCD pathophysiology.

Mitochondrial dysfunction in obesity

The mitochondrial network is an important node that integrates nutrient supply with the energy requirements of the cell. In obesity, nutrient oversupply generates mitochondrial alterations leading to a dysfunctional organelle. While mitochondrial fusion is associated with higher oxidative capacity and ATP production, mitochondrial fission is associated with an opposite response. Changes in mitochondrial morphology have been documented in a variety of organs in the setting of metabolic disease (189). Importantly, fission/fusion events depend partly on the balance between nutrient supply and demand. Starvation and higher metabolic demand (eg, during exercise) are associated with increased network fusion (190,191). The higher $\Delta\Psi mt$ favored by fusion increases the potential for ROS generation, yet fasting or exercise would tend to decrease the $NADH/NAD^+$ ratio, lowering the potential for mROS generation (83). In turn, nutrient overload (eg, high glucose) promotes mitochondria fragmentation that occurs in conjunction with only a transient increase in mROS generation (3); however, this coupled with a sedentary lifestyle that lowers ATP demand, raising the $NADH/NAD^+$ ratio could lead to increase in mROS generation. It may be that increased mitochondrial fragmentation in metabolic disease is a compensatory response to limit this by reducing OCR. Compelling evidence indicates that oxidative stress plays a causal role in the development of obesity-related diseases, but the exact ROS source is still under investigation. Due to the strong association between mitochondrial function and nutrient availability, mROS may play a central role in adipogenesis. Recently, Tormos et al. demonstrated in primary human mesenchymal stem cells undergoing differentiation into adipocytes that complex III-derived ROS production promotes adipocyte differentiation via mTORC1 (4).

NAFLD is a liver disease strongly associated with being either overweight or obese. The disease can progress to inflammatory nonalcoholic steatohepatitis, liver fibrosis, and cirrhosis, driven finally to liver failure (192). Importantly, no drugs/therapies are approved for NAFLD treatment. It has been suggested that a metabolic shift, characterized by a reduction of mitochondrial lipid oxidation, is responsible for NAFLD development. Although there is elevated mitochondrial function in response to the higher lipid availability in NAFLD, this adaptation is insufficient to prevent lipotoxicity in the long term. Teodoro et al. showed in a choline-deficient NAFLD model that mitochondrial function exhibits a biphasic response (5). They observed an increment in the ETC components in the short term; however, the long term was characterized by a reduction of these components, alongside a drop in $\Delta\Psi mt$ and ATP synthesis (5). Moreover, mitochondrial network

remodeling appears to be crucial for NAFLD development. For instance, in primary mouse hepatocytes, mitochondrial fission plays a vital role in the progression of NAFLD (6). An elegant work of Hammerschmidt et al. recently showed that prevention of mitochondrial fission by deletion of Mff rescues NAFLD in mice fed a high-fat diet (15). In spite of the strong relationship between mitochondrial fission and NAFLD, the mechanism that connects these events remains poorly understood.

Are mitochondrial perturbations upstream of insulin resistance?

Current evidence indicates that in addition to increasing glucose uptake, insulin also stimulates mitochondrial activity in adipocytes (193), and skeletal and cardiac muscle (80,194). Conversely, mitochondrial damage can lead to impaired insulin signaling (9,10). Canonical insulin signaling that promotes glucose uptake involves sequential activation of a phosphorylation cascade. This signaling initiates when insulin binds to its receptor, leading to the activation of several proteins, including IRS1, PI3K, and Akt (proximal components of insulin signaling) (195,196). Deficiencies in this pathway have been used as molecular markers of IR. However, the removal of plasma membrane cholesterol restores insulin-stimulated glucose uptake in adult skeletal muscle without recovery of Akt phosphorylation in obese mice (197). Also, the reduction of Akt phosphorylation is not consistently observed across different models of IR (198). These results suggest that a mechanism independent of Akt could be responsible for the development of IR (199).

As mentioned, optimal mitochondrial function is ensured by a quality control system which is tightly associated with fusion–fission events. There are studies that point to a role for mitochondrial dynamics in IR development. For instance, Sebastián et al. showed that Mfn2 deficiency increases H_2O_2 production leading to IR in skeletal muscle and liver (8). In addition, del Campo et al. showed in L6 myotubes that deletion of Mfn2 and OPA1 (to promote mitochondrial fission) reduced insulin-dependent GLUT-4 translocation and impaired mitochondrial Ca^{2+} uptake. Interestingly, inhibition of mitochondrial Ca^{2+} uptake recapitulated IR without affecting mitochondrial dynamics (9). These results suggest that mitochondrial Ca^{2+} mishandling is downstream of mitochondrial fragmentation and provides signals that promote IR.

On the other hand, Taddeo et al. described that opening of mPTP is required to develop IR in skeletal muscle. In vitro, inhibition of mPTP with cyclosporin A prevented insulin resistance evoked by C2-ceramide or palmitate in L6 myotubes. Moreover, mice lacking cyclophilin D were protected against diet-induced IR in skeletal muscle. Interestingly, although no changes were observed in the mitochondrial morphology, the ability of mitochondria to take up Ca^{2+} was reduced in the different models of insulin resistance (200).

We and others showed that mROS promotes IR in skeletal muscle cells and adipocytes (11,12). We recently demonstrated that mROS is sufficient to produce IR without affecting either the proximal component of insulin signaling or mitochondrial respiration (10). Of interest, mROS can activate mitochondrial fragmentation (201). Some authors have proposed mROS generation as a physiological signal to prevent nutrient overload (11). This signal would be crucial at limiting further substrate uptake when fuel supply exceeds metabolic demand (eg, overnutrition and sedentarism). In fact, antioxidant treatment of IR has shown contradictory results (202,203). It is worth noting that the subcellular ROS source may determine the effect of these molecules. For instance, cytoplasmic NADPH oxidase 2-dependent ROS production is necessary to promote GLUT4 translocation in response to insulin (204) and physical exercise (205) in muscle cells. Thus, the development of mitochondria-targeted antioxidants to prevent mROS-mediated damage could have important therapeutic implications.

Hammerschmidt et al. showed that the sphingolipid ceramide 16:0 physically interacts with the adaptor protein Mff to promote mitochondrial fission and hepatic IR (15). Interestingly, downregulation of Mff prevented ceramide-dependent IR. Of note, mitochondrial fission is often associated with a $\Delta\Psi mt$ depolarization which can reduce the ability of mitochondria to take up and buffer Ca^{2+} (43). However, the authors did not evaluate the mechanism by which mitochondrial fragmentation generates IR. This will be an important question to focus on in the future.

The ER–mitochondria interaction has also been suggested as critical for maintaining insulin sensitivity. Disruption of the ER–mitochondria interaction dampens insulin action in neonatal cardiomyocytes and in skeletal muscle cells (7,13). Tubbs et al. elegantly showed that disruption of ER–mitochondria interactions is a common characteristic in various mouse models of obesity and T2DM (7). Of interest, an experimental increase in ER–mitochondria interactions prevented palmitate-induced IR in human myotubes. However, no correlation was observed

between insulin sensitivity and ER–mitochondria interactions in myotubes from lean, obese subjects and subjects with T2DM (R^2 = 0.11, P = .04). However, it is important to note that this author used insulin-dependent Akt phosphorylation as a molecular marker of insulin signaling, instead of a more specific readout such as GLUT-4 translocation or glucose uptake. Although conflicting findings have been published regarding the role of ER–mitochondria interactions in insulin sensitivity in the liver (14,206), these are united regarding the concept that a proper interaction between these 2 organelles is crucial to maintain insulin sensitivity in hepatocytes.

Much of the evidence connecting mitochondrial dysfunction with IR has been obtained in the setting of established IR, making it difficult to determine whether mitochondrial dysfunction is cause or consequence. Recently, Fazakerley et al. (10) reported that 2 hours of mitochondria-targeted paraquat (mPQ) treatment, which promotes mROS production by redox cycling at the flavin site complex I of ETC (207), leads to IR in 3T3-L1 adipocytes, L6-myotubes, and adult skeletal muscle (10). Of interest, mPQ treatment did not affect mitochondrial respiration or insulin-dependent phosphorylation of either Akt or TBC1D4 (10). This suggests that acute mROS generation is sufficient to promote IR independent of AKT/TBC1D4 signaling. However, whether mPQ elicits effects other than increased mROS generation (eg, mitochondria fission) to prevent insulin-dependent GLUT4 translocation requires further investigation.

A pattern of mitochondrial alterations (eg, mROS, mitochondrial fission, impaired ER–mitochondria interaction, mitochondrial Ca^{2+} mishandling) appears to be conserved in the insulin target tissues, such as hepatocytes, adipocytes, skeletal myocytes, and cardiomyocytes under the IR state. However, the important question that remains is whether the features of the mitochondrial dysfunction are connected or converge on a common signal that promotes IR. Further efforts are necessary to dissect the molecular mechanisms by which mitochondrial damage generates IR across different cellular and animal models of IR.

Impact of mitochondria function on insulin release

Impaired or overtly absent insulin secretion is a common feature of type 1 DM and long-lasting T2DM (208-210). Mitochondrial function is vital for proper insulin release from pancreatic β-cells. A simplistic overview of this connection is as follows: after its uptake through GLUT2, glucose is rapidly metabolized by glycolysis to generate pyruvate, which is later incorporated to the TCA. Increased Krebs cycle activity leads to a rise in mitochondrial NADH levels, mitochondrial membrane hyperpolarization, and, ultimately, an increase in ATP synthesis. Once it is translocated to the cytosol, ATP binds to and closes nucleotide-sensitive K_{ATP}/SUR1 channel complexes, leading to β-cell membrane depolarization and generation of cytosolic Ca^{2+} elevations. Finally, an increase in cytosolic Ca^{2+} leads to the fusion of insulin-containing granules to the plasma membrane, releasing their content outside the cell (211,212).

This linear flux of events has been challenged and complemented over time. Contributing roles for insulin secretion have been associated with the NADH cytosolic–mitochondrial shuttle (213), mitochondrial Ca^{2+} signaling (214,215), mitochondria-originated ROS (216,217), TCA metabolic intermediates (218), and so on. As noted, most of these signals are related in some way to mitochondrial function, emphasizing the role of this organelle in β-cell function.

The idea that mitochondria are essential for insulin release is supported by the identification of *UCP2* as a quantitative trait loci for obesity and human insulin-dependent T2DM (219). In vitro studies later demonstrated that overexpression of this uncoupling protein leads to $\Delta\Psi mt$ hyperpolarization in response to a glucose challenge in rat islets. In turn, this decreased mitochondrial response resulted in impaired ATP synthesis, and, therefore, diminished insulin secretion (16,17,18). Accordingly, islet levels of UCP2 were increased in obese mice and biopsies from T2DM patients, alongside reduced ATP levels (17,19). The presence of an A/G polymorphism at -866 position on *UCP2* promoter has been associated with reduced glucose-induced insulin secretion in human islets, in a manner dependent on A-allele dosage (220). Mechanistically, –866A/A is preferentially bound by the β-cell transcription factor PAX6, which promotes its expression over their G-allele counterparts (221). Therefore, it seems possible that upregulation of UCP2 in β-cells could be responsible, at least in part, for the reduced insulin secretion in genetically susceptible subjects carrying the polymorphism. Additional studies identify this polymorphism as having a population-specific role relative to predisposition for obesity and T2DM (221-223).

A Korean population study revealed a similar effect of *PARK2* gene variant (encoding Parkin protein) on insulin release (20). The presence of

the G allele on rs10455889 and rs9365294 SNPs was associated with increased fasting glucose and reduced circulating insulin in males (20). In vitro studies demonstrated that reduced Parkin levels in INS-1 β-cells results in reduced glucose-induced ATP synthesis, reverberating on insulin secretion (20).

Mitochondrial morphology impacts insulin secretion from pancreatic β-cells. For instance, Drp-1 deletion results in impaired glucose-stimulated insulin secretion, despite generating a more connected and fused mitochondrial network (21-23). Altered metabolite delivery into mitochondria resulting in reduced ATP synthesis, or mishandling of Ca^{2+} mitochondrial signaling have been proposed as possible mechanisms for the reduced insulin release (22, 23). Notably, β-cell–specific deletion of *OPA1* recapitulates the aforementioned results (24). Mitochondrial dynamics in tissues other than the pancreas also influences insulin secretion from β-cells. Deletion of the profusion protein Mfn1 in POMC neurons results in reduced glucose-induced insulin secretion from pancreatic islets (224). Remarkably, deletion of OPA1 or Mfn2 in the same neurons did not alter insulin secretion (224). Together, these observations point to a crucial role for mitochondrial dynamics in insulin secretion. The question is, however, which morphological state of the mitochondrial network is better suited for glucose sensing and the subsequent insulin release in diabetic patients. As both exaggerated fragmentation and elongation result in altered insulin secretion, it seems that an equilibrium between these opposing processes is the most plausible answer.

The (dys)function of mitochondria in cancer

Since the seminal work published by Warburg (1956) (225), it is well known that cancer cells in culture catabolize glucose by lactic fermentation even in presence of oxygen. This suggests a potential role for mitochondrial metabolism in cancer. In fact, mutation of key mitochondrial enzymes is associated with cancer progression in humans (226). Thus, mitochondrial metabolism is frequently considered an important player in the development and progression of cancer.

PGC1-α, which promotes mitochondrial biogenesis has been proposed as a tumor suppressor (227). In fact, the hypoxia inducible factor-1 alpha (HIF-1α) has been shown to reduce PGC1-α levels leading to a higher glycolytic rate in tumorigenic cells (25). Importantly, mitochondria still maintain their biosynthetic activity in cancer cells, thus providing metabolic intermediates for cell proliferation and tumorigenesis (228).

PGC-1α expression may also play a key role in malignancy and survival of some types of cancer. In hepatocellular carcinoma, PGC-1α promotes cellular migration and invasion (30). The metabolic switch induced by PGC-1α is sufficient to enhance mitochondrial function and cell motility needed to develop an invasive phenotype in circulating cancer cells (31). Conversely, when circulating cancer cells achieve their target tissue, they undergo a metabolic switch to aerobic glycolysis that ensures the cell survival (229).

Recently, Tosatto et al. showed a role for mitochondrial Ca^{2+} uptake in breast cancer progression. In triple-negative breast cancer—the most aggressive breast tumor subtype—mitochondrial Ca^{2+} uptake and mROS are required for tumor growth and metastasis in vivo. Mitochondrial Ca^{2+} accumulation could promote mROS production which is necessary for HIF-1α activation (32). In line with these results, Cárdenas et al. reported, in several tumorigenic cancer cell lines and in vivo tumors, that inhibition of Ca^{2+} transfer from ER to mitochondria reduces mitochondrial Ca^{2+} levels, generating a bioenergetic crisis and reducing cell viability (33). In the mitochondrial matrix there are several Ca^{2+} sensitive enzymes—such as isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase—in the TCA cycle. It is possible that constitutive mitochondrial Ca^{2+} accumulation is required to maintain sufficiently high TCA cycle activity. In fact, TCA are the main source of biosynthetic intermediates that are required to sustain the high rate of cancer cell proliferation (34). Interestingly, Cárdenas et al. provided evidence that cancer cell death induced by the reduction of mitochondrial Ca^{2+} uptake was prevented by exogenous nucleoside supply (33).

It has been described that mtDNA also plays a key role in cancer development. An extensive description of mtDNA copy number was performed in several types of human cancer biopsies. As a common feature, and despite the large variability among tissues, several cancers displayed a reduction in mtDNA (230) and mtRNA copy numbers (231). Of interest, mtRNA expression correlated positively with the overall survival of the patients (231). Despite these results, the mechanisms that link mtDNA copy with cancer phenotype requires further investigation.

mtDNA mutations have also been strongly associated with oncogenesis and metastasis. The characteristic way of how mtDNA is replicated favors heteroplasmic point mutations in both strands of

mtDNA (232). In fact, a large proportion of cancers have ~60% of these somatic mtDNA mutations (26). The role of mtDNA mutation in carcinogenesis has been supported by clinical studies. In patient-derived prostate cancer biopsies, a correlation between cancer aggressiveness and mtDNA mutations was observed (27). Moreover, mtDNA replacement of nonmetastatic cancer cells with mtDNA from a highly metastatic cancer cell increased their malignancy and metastatic potential (28). Based on these data and recent findings in cells with a tunable grade of mtDNA point mutations, it is possible that mitochondrial dysfunction derived by mtDNA mutation stimulates oncogenesis and metastatic potential (232). Nevertheless, an excessive grade of mtDNA mutation and mitochondrial dysfunction deeply alter cell viability and reduce the aggressiveness of cancer cells (232).

Based on single cell RNAseq, Xiao et al. showed that malignant cells are enriched for the expression of genes that confer metabolic plasticity (29). The authors observed that both glycolysis and Krebs cycle were enhanced in cancer cells. These data could explain why cancer cells can adapt to their environment so rapidly and change their phenotype favoring growth and invasiveness.

Altogether, these results suggest that a variety of mitochondrial functions can contribute from different angles to the development of a pathological state. Therefore, it will be important to fully understand the relative contribution of each to tumor development and progression.

Mitochondrial dysfunction in cardiovascular diseases

CVDs are the main cause of death worldwide. Initial observations showed that mitochondrial alterations are characteristic of dilated cardiomyopathy in humans and mice (233,234). Also, mitochondrial damage plays a central role in vascular pathologies. For instance, mitochondrial DNA damage promotes atherosclerosis by increasing smooth muscle cell proliferation (35). To date, it is widely believed that mitochondrial damage is involved in the pathophysiology of CVD (114)

Heart muscle exhibits a high metabolic demand, and this may explain the central role mitochondrial metabolism plays in its pathophysiology. Pennanen et al. showed that norepinephrine promoted mitochondrial fragmentation which correlated with cardiomyocyte hypertrophy. Importantly, while a dominant-negative Drp-1 prevented the norepinephrine-dependent hypertrophy, the downregulation of Mfn2 was sufficient to promote cardiomyocyte hypertrophy (36). Norepinephrine also disrupted ER–mitochondria interactions, impaired mitochondrial Ca^{2+} handling, increased mROS, and reduced mitochondrial efficiency, thereby driving cardiomyocytes to an energetic crisis (13). Interestingly, Mfn1/2 deletion in adult heart reduces fractional shortening, increases left ventricular end-diastolic volume and promotes dilated cardiomyopathy (37). Deletion of Drp-1 in adult heart likewise leads to heart failure, highlighting the need for both fission and fusion processes. Finally, $Opa1^{+/-}$ mice are more sensitive to pathological cardiac remodeling induced by aortic constriction and to ischemia–reperfusion (I/R) damage (38).

Recently, Parra et al. showed both in vitro and in vivo that reduction of the regulator of calcineurin 1 (RCAN1) promotes mitochondrial fission by a calcineurin–Drp-1 dependent mechanism. In RCAN-1 depleted cardiomyocytes, mitochondria exhibited reduced capacity for Ca^{2+} uptake, and decreased OCR. In addition, RCAN1 knockout mice were more susceptible to ischemia/reperfusion damage. Interestingly, pharmacological inhibition of Drp-1 restored mitochondrial function and conferred protection against ischemia/reperfusion damage (39). These results highlight the role of mitochondrial metabolism in the development of heart diseases and suggest Drp-1 as a therapeutic target.

Key Experiments and Unaddressed Questions

Important questions require further investigation.

- Is mitochondrial dysfunction a cause or consequence of NCDs? This is a contentious point because most studies have assessed mitochondrial parameters in the diseased state rather than across its progression.
- What specific mitochondrial properties promote NCDs development? Mitochondrial damage has many faces including impairment of mitochondrial dynamics, ER–mitochondria interactions, mROS production, mitochondrial Ca^{2+} handling, mitochondria respiration, among others. Thus, a big challenge is to determine which alterations are involved in NCDs, which are cause or consequence, and how they interact under pathological conditions.
- How does mitochondrial dysfunction lead to NCDs? Do all mitochondrial changes associated with NCDs share a common, downstream pathological mechanism?

Emerging omics technology and machine learning approaches generate an outstanding opportunity to explore the effect of specific mitochondrial alterations in whole cell/organism physiology to help address these questions.

Mitochondrial Dysfunction: Can it Be Reduced or Reverted?

Caloric restriction modulates mitochondrial function

Reduction of mitochondrial respiratory capacity is reported as a common feature of several NCDs. For instance, short-term high-fat diet promotes adipose tissue mitochondrial uncoupling. This uncoupled respiration generates local hypoxia and IR of the adipose tissue (235). Furthermore, obese, IR and diabetic subjects present reduction of their respiratory capacity on different tissues such as skeletal muscle (236-238), liver (237), and adipose tissue (239). The decrease in the OCR has also been observed in some CVDs such as atherosclerosis (240) and hypertension (241-243). Similarly, cancer has been proposed as a metabolic disease (244,245). However, because of the high diversity of cancer cell types in a tumor and the number of different tumor types, it is not possible to conclude that mitochondrial dysfunction is a common feature of all cancers (246). Given the common feature of mitochondrial dysfunction in NCDs, can similar approaches be taken to improve mitochondrial function across this diversity of pathologies? In the following paragraph we discuss mechanisms by which caloric restriction (CR) may improve mitochondrial function and its application to NCDs.

As previously mentioned, mitochondria are important nodes that integrate environmental clues associated with nutrient availability. CR is well known as a potent and reliable strategy to reduce the incidence of NCDs among different species including mice and nonhuman primates (247-249). In *Rhesus* monkeys, for instance, CR increases lifespan and also reduces the prevalence of obesity, CVD, cancer, and T2DM (247,248,250).

It has been proposed that CR improves mitochondrial function by modulating molecular targets related to energy sensing (Fig. 3). One of the most important energy sensors is the AMPK protein, which is upregulated in different experimental models of CR (251-253). AMPK is an important promoter of mitochondrial biogenesis through direct phosphorylation of PGC-1α at Thr177/Ser538 inducing the expression of several nuclear genes involved in mitochondrial biogenesis and adaptations (254). AMPK mRNA levels are decreased in both obese and IR humans alongside a reduction of the NAD^+-dependent deacetylase SIRT1 (255).

In addition to the previously discussed role of the NADH/NAD^+ ratio in mROS generation, NADH and NAD^+ are important regulators of many enzymes and transcription factors. CR-dependent increases in NAD^+ are sufficient to promote sirtuin activation of PGC-1α (Fig. 3) (256,257). CR also induces mitochondrial biogenesis and improves mitochondrial function by a PGC-1α-dependent mechanism (258-260). However, skeletal muscle-specific overexpression of PGC-1α did not promote adaptations typical of CR and instead increased the susceptibility to high-fat diet-induced IR (261). These results suggest that under CR, PGC-1α may play a cell- or organ-specific role.

In vitro experiments have shown that CR modulates not just mitochondrial content but also its efficiency. López-Lluch et al. showed in vitro and in vivo that despite finding no change in ATP levels, CR elevated mitochondrial mass and reduced both mitochondrial respiration and mROS production (262). This maintenance of ATP levels implies a more efficient or "coupled" ETC under caloric restriction. Remarkably, these adaptations have been consistently observed in human and murine models under CR (263-265). Importantly, caloric restriction has been associated with a more connected mitochondrial network and increased mitophagy, both of which are necessary to maintain a healthy mitochondrial network (77).

Like any intervention, CR has limitations. For instance, in the long run, CR increases the risk of developing some pathologies such as osteoporosis, slow wound healing, and sensitivity to cold environments, among others (266).

Physical exercise and mitochondrial function

Physical inactivity is associated with the prevalence of NCDs (Fig. 3). Like CR, physical exercise generates a negative energy balance that improves mitochondrial function (Fig. 3). These 2 strategies trigger similar intracellular molecular pathways. Pioneering work by John Holloszy, more than 50 years ago, showed that sustained, strenuous physical exercise doubled mitochondrial content and activity in skeletal muscle (Fig. 3) (267). It also increased mitochondrial coupling, suggesting important changes in efficiency and function. Since then, experimental evidence has repeatedly shown the profound effect of physical exercise on mitochondrial function/structure, although the

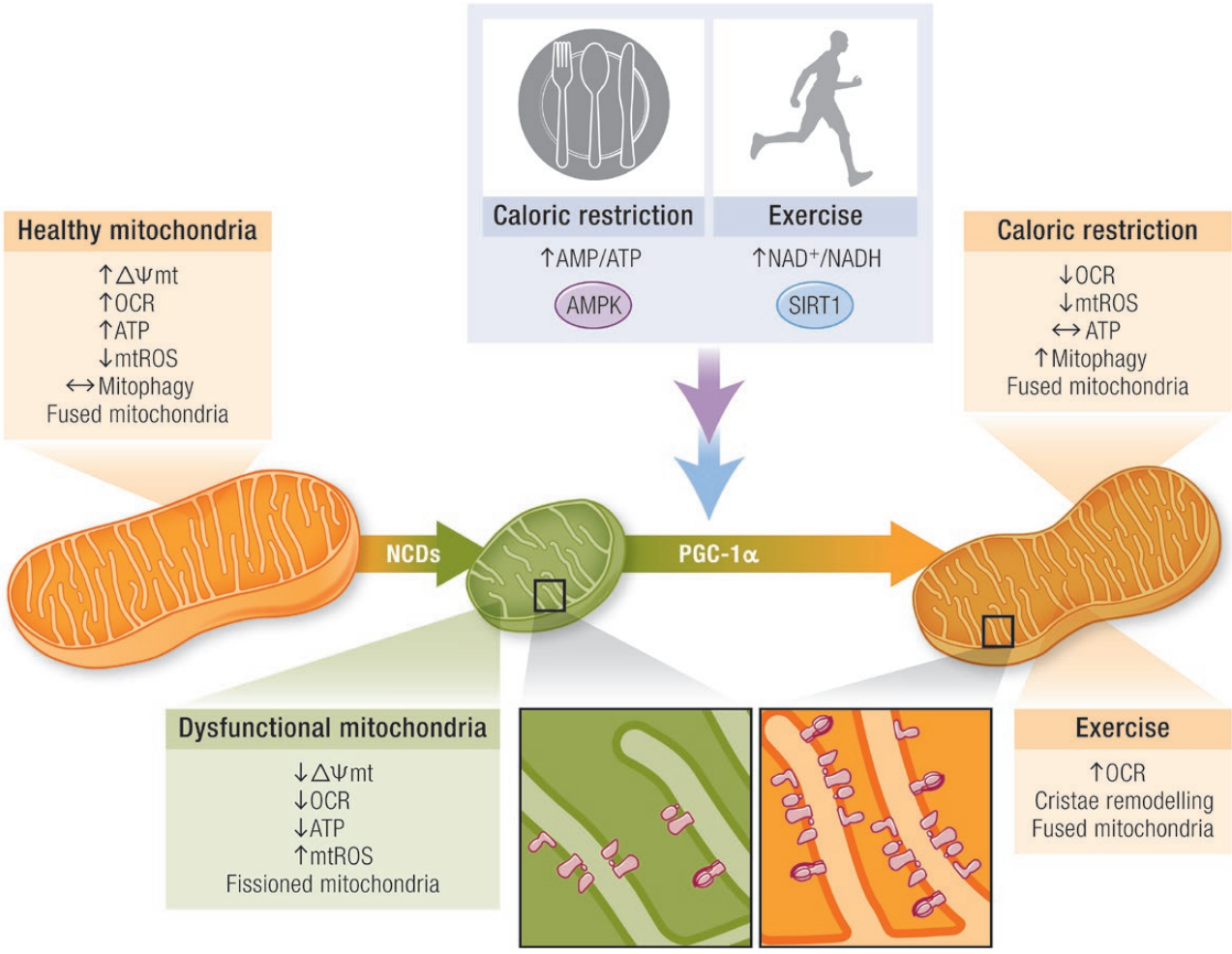

Figure 3. Caloric restriction and exercise restore mitochondrial dysfunction. NCDs induce an alteration of functional and structural features of mitochondria. The energetic stress challenge induced by caloric restriction and exercise create a negative balance by altering the AMP/ATP and NAD+/NADH ratios. These changes are sensed by 2 main proteins, AMPK and SIRT1, that are able to phosphorylate and deacetylate correspondingly the mitochondrial biogenesis regulator PGC-1α. Alteration of energy homeostasis increases mitophagy, changes mitochondrial morphology (more elongated) allowing mROS production to be reduced, antioxidant capacity to be increased, and, on a differential manner, oxygen consumption rate (OCR) to be altered to ensure more efficient ATP to be maintained during caloric restriction or ATP synthesis for muscular work. These ATP-driven differences are allowed by more coupled electron transport chain assembly induced by changes in mitochondrial mass and a fused pattern encouraged by initial energetic sensors.

mechanisms involved are not completely understood (268).

Mitochondrial responses to physical exercise can be divided in 2 types: acute and chronic adaptations. Acute adaptations involve changes in mitochondrial function during a bout of exercise. Of interest, the change of mitochondrial function during acute exercise are extremely hard to determine. As we previously mentioned, noninvasive methods such as ^{31}P magnetic resonance spectroscopy and near-infrared spectroscopy have been used to estimate mitochondrial function (187, 269). Nevertheless, these techniques are constrained by the enzymatic equilibrium and the subcellular distribution of the molecules.

Currently, most of the evidence available has been obtained from in vitro models of physical exercise, for example, skeletal muscle fibers contraction or permeabilized cells. In vitro muscle fiber activation increases mitochondrial OCR (127) and promotes mitochondria fusion (270), without changing mitochondrial content. This is a relevant model because (1) it avoids exposing the cells to supraphysiological concentrations of mitochondrial substrates; (2) it prevents the artefacts associated with mitochondrial isolation protocols and maintains mitochondrial environment intact (ie, mitochondria–ER interaction). On the other hand, permeabilized muscle allows exposing mitochondria to different metabolites whose

concentration changes during exercise. This is an interesting approach, especially because it can be applied to human muscle biopsies. For instance, initial work from Walsh et al. (271). reported that sequential addition of ADP, creatine (Cr), and phosphocreatine (PCr) modulates mitochondrial function. The authors showed for the first time that PCr reduces ADP-stimulated respiration whereas Cr has the opposite effect. Of interest during transition from rest to high-intensity exercise, decreases of PCr/Cr ratio increase ADP-dependent mitochondrial respiration (271). Indeed, the addition of creatine or phosphocreatine in assay media completely changes the interpretation of how chronic exercise training affects mitochondrial function (272). Despite these experimental observations, mitochondrial response during acute exercise will also depend on (1) vasculature, (2) O_2/nutrient availability, (3) cardiac output, (4) mitochondrial mass, and (5) total effective surface of capillaries (273). In consequence, conclusions from these in vitro experiments need to be interpreted in the appropriate physiological context.

Chronic adaptations occur in response to regular physical exercise training. These adaptations include increases in mitochondrial mass (274), higher oxidative phosphorylation (275), cristae remodeling in the IMM (276). The specifics of mitochondrial adaptation can depend on the duration, intensity, modality, and frequency of physical exercise. Remarkably, ROS, generated in the cytoplasm, is emerging as an essential signal in mitochondrial adaptation to exercise in heart and skeletal muscle (268,277,278).

Similar to CR, physical exercise promotes the activation of the molecular energy sensor AMPK (254). Acetylation of PGC-1α by GCN5 suppresses its activity, whereas deacetylation by SIRT1 increases its activity. PGC-1α is deacetylated in response to either fasting or endurance exercise. During fasting, deacetylation by SIRT1 controls PGC-1α activity (279), whereas the exact mechanism of exercise-induced deacetylation of PGC-1α remains controversial and may also rely on suppression of GCN5-mediated acetylation (280). Interestingly, supplementation with nicotinamide mononucleotide—a NAD+ precursor that promotes SIRT1 activation—improves mitochondrial function in obese mice (281). In humans, physical exercise increases expression of nicotinamide phosphoribosyltransferase, the rate-limiting enzyme of the NAD+ salvage pathway (282). In line with the idea that this mechanism contributes to mitochondrial biogenesis, nicotinamide phosphoribosyltransferase transgenic mice show higher mitochondrial respiration in response to voluntary wheel training (283). To better understand the molecular mechanisms triggered in response to physical exercise Hoffman et al. undertook a global phosphoproteomic analysis of physical exercise in human skeletal muscle. Remarkably, the majority of the exercise-regulated phosphoinositides and kinases identified had not been previously associated with physical exercise (284). These remarkable findings raise many important questions.

- How these global changes are integrated with ROS signals?
- How different are the global responses to different types of exercise?
- How do different tissues respond to physical exercise?
- Are responses attenuated/altered in NCDs?
- How does the genetic background impact the global response to exercise?
- Which molecular mechanisms are key to the beneficial effects of exercise?
- Do different stimuli, such as caloric restriction and physical exercise converge on common pathways? Address these questions will pave the way toward new mitochondria-targeted therapies to combat NCDs.

Current Challenges and Futures Perspectives: From the Bench to Clinical Trials

As discussed, mitochondria play a leading role in many NCDs. Substantial efforts over the last 3 decades to have been directed toward the development of specific mitochondria-targeted therapies. Several mitochondria-related molecules have been identified as possible interventional tools. However, there are many obstacles to advancing mitochondria-targeted drugs toward clinical trials. These concerns include drug pharmacokinetics, biodistribution, and off-target effects, among others (285). The following section will touch on some of the current therapies that are being tested in clinical trials with positive outcomes (286).

The synthetic tetrapeptide SS-31 (also called elamipretide or Bedavia) represents a novel generation of drugs. SS-31 targets cardiolipin, a mitochondria-specific lipid that resides in the IMM (287). SS-31 protects mitochondria form insults by preventing cardiolipin peroxidation, promoting cytochrome C retention and the appropriate supercomplex maintenance at the IMM

(287). This peptide has been successfully used in animal models of IR (11) or CVD (288). Notably, a recent study showed that elamipretide also has an acute and cardiolipin-independent effect over mitochondria in cardiac ventricular samples from healthy and heart failure patients. This drug increases complex I-dependent oxygen flux and promotes supercomplex formation in the IMM (289). Randomized clinical trials to treat human pathologies where mitochondria play a critical role have been carried out. In patients with heart failure, elamipretide significantly improved ventricular function without adverse effects in high doses (0.25 mg/kg/hour), placing this molecule as a good candidate for heart failure treatment (290).

Mitoquinone (MitoQ) is another molecule that has been extensively studied. MitoQ is a lipophilic cation consisting of a triphenylphosphonium moiety covalently attached to a ubiquinone (antioxidant CoQ10). It accumulates on mitochondria up to 1000-fold driven by mitochondrial membrane potential and has been shown to reduce mROS levels (291,292). This feature makes it a perfect candidate in those pathologies with an excess generation of mROS. For instance, MitoQ treatment improves mitochondrial function and lipid metabolism in skeletal muscle of obese rats (293). In the same line, as other mitochondrial antioxidants (11), MitoQ rescued insulin sensitivity in different models of IR (294). MitoQ has also shown positive effects in the cardiovascular system. For example, 4 weeks of oral MitoQ treatment prevented the development of arterial stiffness in aged rats (295). In a transverse aortic constriction-induced heart failure model, oral treatment with MitoQ improved mitochondrial function, restored Ψmt, oxygen consumption and β-oxidation (296). A randomized controlled-trial showed that long-term treatment with MitoQ in chronic heart failure patients improved the symptoms and significantly reduced cardiac events, decreasing cardiovascular deaths by 43% (297). Despite the fact that MitoQ has been used in in preclinical cancer models (298,299), to our knowledge, there are not current trials on MitoQ to cancer treatment in humans.

Mitochondria-targeted therapies under current study for their potential role on cancer progression are centered on some metabolic modulators of the TCA cycle. These include isocitrate dehydrogenase (IDH1/2), PDH, and PDK1 inhibitors. Treatment with AG-221 (enasidenib), a selective IDH2 inhibitor in IDH2 mutant patients, results in suppression of the onco-metabolites (R)-2-hidroxyglutarate production in tumor xenograph models (300). In contrast to chemotherapy, AG-221 seems to increase myeloid differentiation and the population of functional and mature neutrophils (300). Early-phase trial AG-221-treated patients have a response rate of 40% (301). AG-221 is currently under clinical trial phase I/II on subjects with advanced hematologic malignancies with IDH2 mutations (Clinical Trial identifier: NCT01915498). Modulation of glucose-related metabolism by dichloroacetate (DCA) or CPI-613 has also shown interesting and promising results. DCA acts as a PDK1 inhibitor, and it has been demonstrated that it modulates proliferation in colorectal cancer cells by increasing proteasomal degradation, without changes in apoptosis (302). A recent DCA human trial in patients with solid tumors showed a mild decrease in glucose uptake, and reported toxicity issues on some of the patients (303). On the other hand, CPI-613 inhibits both PDH and alpha ketoglutarate dehydrogenase: the former through activation of PDK, while the latter is mediated by a rapid change on redox signal in mitochondrial tumor (304). In vitro CPI-613 treatment on cancer stem cells showed a reduction on sphere-forming capacity and tumorigenicity, with an important reduction in CD133+ and DC117 cell frequency, suggesting a relevant role in improving treatment for ovarian cancer (305). CPI-613 has also been tested on patients suffering metastatic pancreatic adenocarcinoma within a phase I trial, where the combination with folfirinox chemotherapy was well tolerated in most of the patients and showed favorable outcomes (306).

Another promising area of mitochondrial therapeutics is directly centered on ETC. In this regard, metformin, a drug originally used for treating T2DM, is one of the most widely reported ETC-targeting drugs tested in the context of cancer due to its safety and apparent harmlessness. Metformin works by directly blocking complex I (NADH dehydrogenase), reducing oxygen consumption and ATP production, while increasing the AMP/ATP ratio that leads to AMPK activation and reduction of mTOR signaling pathways (a more compressive review can be found in reference (307). Metformin has been tested on different types of cancer, such as endometrial cancer (308), metastatic pancreatic cancer (309), colorectal cancer (310), and breast cancer (311), among others. Overall, metformin treatment is well tolerated and shows mixed results regarding survival and clinical benefit, depending on the patient's characteristics and pharmacological interactions.

Undoubtedly, mitochondrial therapies are promising and represent a fresh perspective for

treatment of long-lasting diseases. However, they are still at early stages of development.

Conclusion

NCDs are the most important cause of death worldwide and they generate extremely high economic cost (1). Importantly, the major part of these diseases is generated by an environmental–genetic interaction; therefore, they are partly preventable (1). In this review we discussed the concept of mitochondrial dysfunction and its emerging role as a common root for NCDs. Because of the many faces of mitochondrial dysfunction, it is challenging to definitively identify how these various mitochondrial properties interact to promote NCDs. To date, most evidence suggests a common pattern of mitochondrial alterations although the contribution of each to disease progression may vary. In consequence, the therapeutic control of specific mitochondrial alterations is a crucial step in mitochondrial physiology and its application may depend on the pathological context. New tools for analysis and therapeutic application are being aggressively pursued.

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Additional Information

***Correspondence and Reprint Requests*:** Sergio Lavandero, PhD, Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Olivos 1007, Santiago 8380492, Chile. E-mail: slavander@uchile.cl.

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Abbreviations:ADP, adenosine diphosphate; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; COQ, coenzyme Q; CR, caloric restriction; CVD, cardiovascular disease; DCA, dichloroacetate; Drp-1, dynamin-related protein 1; ER, endoplasmic reticulum; ETC, electron transport chain; FADH2, flavine adenine dinucleotide; FET, forward electron transfer; FMN, flavin mononucleotide; IDH, isocitrate dehydrogenase; IMM, inner mitochondrial membrane; IR, insulin resistance; MAM, mitochondria-associated membrane; Mfn, Mitofusin; MitoQ, Mitoquinone; mPQ, mitochondria-targeted paraquat; NADH, nicotinamide adenine dinucleotide with hydrogen; NAFLD, nonalcoholic fatty liver disease; NCD, noncommunicable chronic disease; OCR, oxygen consumption rate; OMM, outer mitochondrial membrane; OPA1, Optic atrophy 1; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; RCAN1, regulator of calcineurin 1; RET, reverse electron transfer; RIRR, ROS-induced ROS release; ROS, reactive oxygen species; SOD, superoxide dismutase; T2DM, type 2 diabetes; TCA, tricarboxylic acid; UCP, uncoupling protein; UPRmt, mitochondrial unfolded protein response