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Review

Intracellular to Interorgan Mitochondrial Communication in Striated Muscle in Health and Disease

Neoma T. Boardman,[1] Giulia Trani,[2,3] Marco Scalabrin,[2,3] Vanina Romanello,[2,3] and Rob C. I. Wüst[4]

[1]Cardiovascular Research Group, Department of Medical Biology, Faculty of Health Sciences, UiT-The Arctic University of Norway, 9019 Tromsø, Norway
[2]Veneto Institute of Molecular Medicine, 35129 Padova, Italy
[3]Department of Biomedical Sciences, University of Padova, 35100 Padova, Italy
[4]Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Amsterdam Movement Sciences, Vrije Universiteit Amsterdam, 1081 BT Amsterdam, The Netherlands

Correspondence: Neoma Tove Boardman, PhD, Cardiovascular Research Group, Department of Medical Biology, Faculty of Health Sciences, MH2, 9019, Tromsø, UiT-The Arctic University of Norway. Email: neoma.boardman@uit.no; or Vanina Romanello, PhD, Veneto Institute of Molecular Medicine, via Orus 2, 35129 Padova, Italy. Email: vanina.romanello@unipd.it; or Rob Wüst, PhD, Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Amsterdam Movement Sciences, Vrije Universiteit Amsterdam, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands. Email: r.wust@vu.nl.

Abstract

Mitochondria sense both biochemical and energetic input in addition to communicating signals regarding the energetic state of the cell. Increasingly, these signaling organelles are recognized as key for regulating different cell functions. This review summarizes recent advances in mitochondrial communication in striated muscle, with specific focus on the processes by which mitochondria communicate with each other, other organelles, and across distant organ systems. Intermitochondrial communication in striated muscle is mediated via conduction of the mitochondrial membrane potential to adjacent mitochondria, physical interactions, mitochondrial fusion or fission, and via nanotunnels, allowing for the exchange of proteins, mitochondrial DNA, nucleotides, and peptides. Within striated muscle cells, mitochondria-organelle communication can modulate overall cell function. The various mechanisms by which mitochondria communicate mitochondrial fitness to the rest of the body suggest that extracellular mitochondrial signaling is key during health and disease. Whereas mitochondria-derived vesicles might excrete mitochondria-derived endocrine compounds, stimulation of mitochondrial stress can lead to the release of fibroblast growth factor 21 (FGF21) and growth differentiation factor 15 (GDF15) into the circulation to modulate whole-body physiology. Circulating mitochondrial DNA are well-known alarmins that trigger the immune system and may help to explain low-grade inflammation in various chronic diseases. Impaired mitochondrial function and communication are central in common heart and skeletal muscle pathologies, including cardiomyopathies, insulin resistance, and sarcopenia. Lastly, important new advances in research in mitochondrial endocrinology, communication, medical horizons, and translational aspects are discussed.

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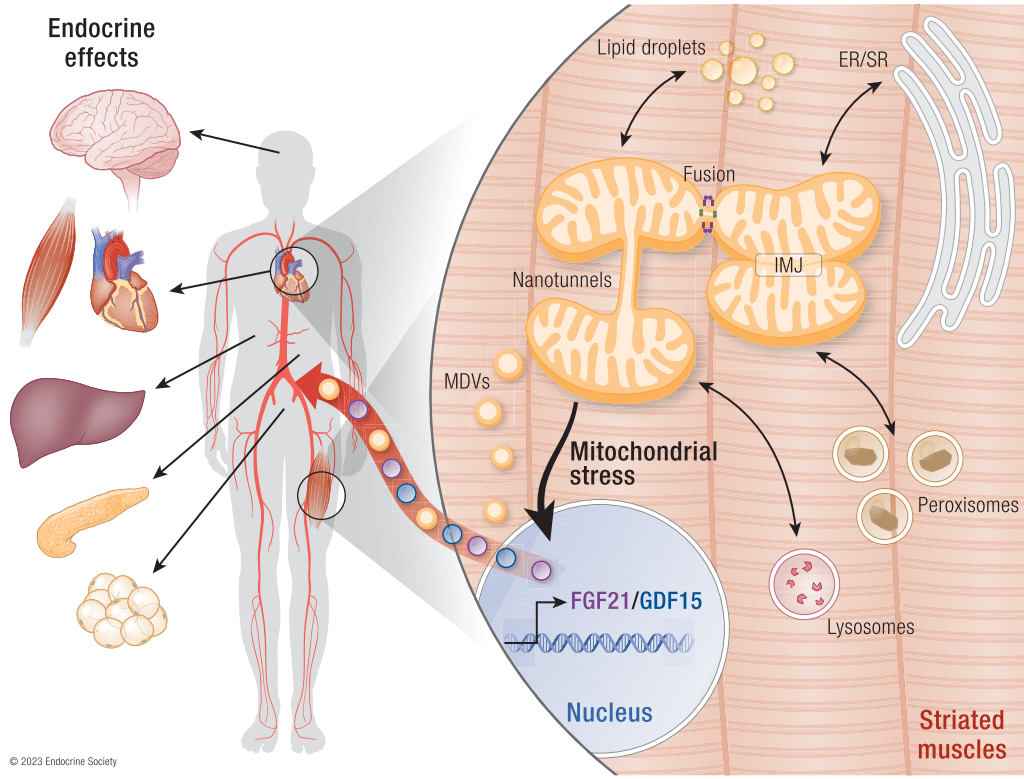
Graphical Abstract



Key Words: mitochondrial dynamics, mitochondrial cristae, respiratory supercomplexes, mitochondria-organelle interactions, myokines, FGF21, GDF15

Abbreviations: AMPK, adenosine monophosphate–activated protein kinase; ATF5, activating transcription factor 5; ATP, adenosine triphosphate; CHOP, C/EBP homologous protein; Drp1, Dynamin-related protein 1; ER, endoplasmic reticulum; FGF21, fibroblast growth factor 21; GDF15, growth and differentiation factor 15; MCU, mitochondrial calcium uniporter; MFN, mitofusin; MICOS, mitochondrial contact site and cristae organizing system (includes Mic60 and Mic10 components); miR, microRNA; mtDNA, mitochondrial DNA; mtUPR, mitochondrial unfolded protein response; NAD, nicotinamide adenine dinucleotide; OPA1, Optic atrophy type 1; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; Plin2/5, perilipin 2/5; ROS, reactive oxygen species; SR, sarcoplasmic reticulum; TFAM, transcription factor A mitochondrial; TGF-β, transforming growth factor β; TOM20, translocase of outer mitochondrial membrane 20.

ESSENTIAL POINTS

- Mitochondria in striated muscle provide energy for contraction but they are also signaling organelles and modulate different cell functions
- Intermitochondrial communication between adjacent mitochondria contributes to optimal energy production during high energy demand and the synchronization of the mitochondrial reticulum can signal cellular stress
- Mitochondrial communication with other organelles highlights the role of mitochondria in optimizing overall cell function
- Myomitokines, such as FGF21, GDF15, and mtDNA, can alter whole-body physiology and systemic responses

Skeletal and cardiac muscles depend on vast amounts of energy for optimal excitation-contraction coupling and require efficient energy supply and demand pathways, such that glucose and fat can be taken up, stored, and utilized by the mitochondria (1-4). Mitochondria harbor key processes of cellular energy metabolism, such as oxidative phosphorylation for the synthesis of adenosine triphosphate (ATP), and a large number of anabolic and catabolic pathways. In the last decade, it has become clear that mitochondria are not solely on the receiving end of substrate oxidation, but that mitochondria actively communicate with other mitochondria as well as other organelles, such as the nucleus, lipid droplets, peroxisomes, and endo-/sarcoplasmic reticulum (5). Cardiac and skeletal muscle mitochondria even communicate with other cells or organs by compounds we now consider as *myokines*, *mitokines*, or *myomitokines* (6).

In this review, we provide an overview of the state-of-the-art in the field of mitochondrial communication with the focus on striated muscle. Here, we define mitochondrial communication as the processes by which mitochondria share content and bioenergetic potential with neighboring mitochondria in the reticulum, as well as the physical interactions and exchange of molecules and metabolites between other mitochondria and organelles. Mitochondrial endocrine communication is discussed, as this represents the molecular information transferred between mitochondria and distant organ systems. We specifically focus on skeletal and cardiac muscle mitochondria, due to their role under conditions of high energy

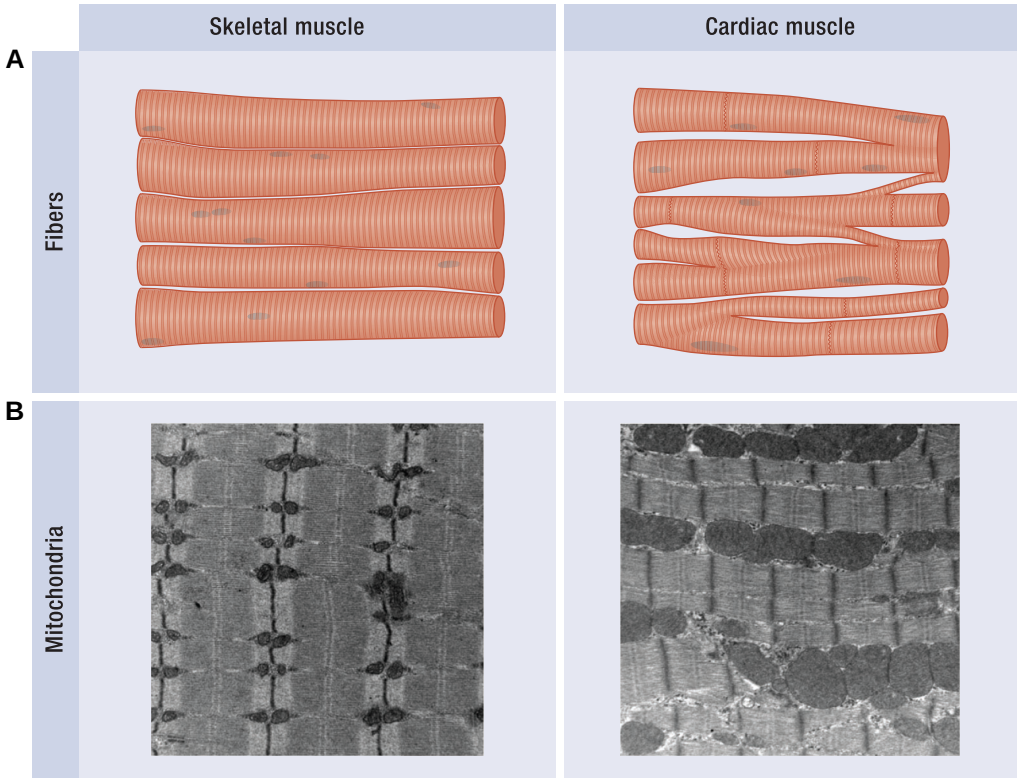


Figure 1. Mitochondrial distribution in skeletal and cardiac muscle. Due to physical constraints within the muscle fibers, intrafibrillar mitochondria of striated muscle undergo a lower rate of dynamics and can communicate through direct intermitochondrial interaction. A) Schematic view of striated muscle fiber organization. Skeletal muscle fibers are polynucleated and juxtaposed, while cardiomyocytes are mononucleated and branched. B) Electron microscopy images showing the "grid-like" mitochondrial distribution (in dark) in striated muscle fibers.

demands; the mitochondrial reticulum is a clear example of intermitochondrial communication and skeletal muscle is the largest organ in the body that produces myokines. Striated muscle fibers are postmitotic cells, and hence they cannot divide. As a result, their mitochondria predominantly rely on intrinsic fitness for optimal function. While striated muscle mitochondria were traditionally only seen as energy-producing organelles, their roles of sensing, integrating, and communicating with other organelles are emerging as multifaceted, and are discussed here in more detail.

Communication Between Neighboring Mitochondria in the Reticulum

The molecular machinery that regulates mitochondrial morphology and ultrastructure is coordinated with mitochondrial pathways that respond to the metabolic demand, cellular stress, and damage (7-9). The coordination of mitochondrial morphology and structure can therefore provide greater insight into different types of stressors and how the cells in striated muscle respond to them. The next section describes the role of mitochondrial morphology and structure as determinants of mitochondrial integrity and how these may be altered under physiological and pathological conditions (Fig. 1).

Intermitochondrial Communication via the Electrical Grid

Due to the physical constraints, and high energy requirement in striated muscle, mitochondria develop in a network that facilitates energy distribution (10-13). This mitochondrial reticulum was first described in rat diaphragm in the 1970s (10). Already in 1988, scientists observed that contact points between adjacent mitochondria, called *intermitochondrial junctions*, are permeable for H^+ ions, allowing for the mitochondrial proton motive force to be transmitted between mitochondria (11).

Mitochondrial subpopulations within striated muscle may have different roles, as subsarcolemmal mitochondria preferentially contain more mitochondrial complex IV while intramyofibrillar mitochondria have more ATP synthase (1, 14). The communication between these different subpopulations through specific contact sites (15) underscores the importance of shared power conduction through the networks for optimal energy production during conditions of high energy demands.

The oscillatory behavior of mitochondrial network membrane potentials was demonstrated through computational model analyses, where individual mitochondria were shown to affect the behavior of their neighboring mitochondria in a coordinated network (16, 17). Moreover, the patterns of oscillations differed under normal vs abnormal conditions. Membrane potential oscillates within a wide frequency range in normal conditions, whereas cellular stress and subsequent accumulation of reactive oxygen species (ROS) to a critical threshold caused a synchronization of membrane potential to act as a cellular alarm signal of a stressed mitochondrial network (17, 18). Further evidence has supported the highly coordinated function of the mitochondrial network, similar to that of an electrical power grid, via conduction of the mitochondrial membrane potential to adjacent mitochondria in

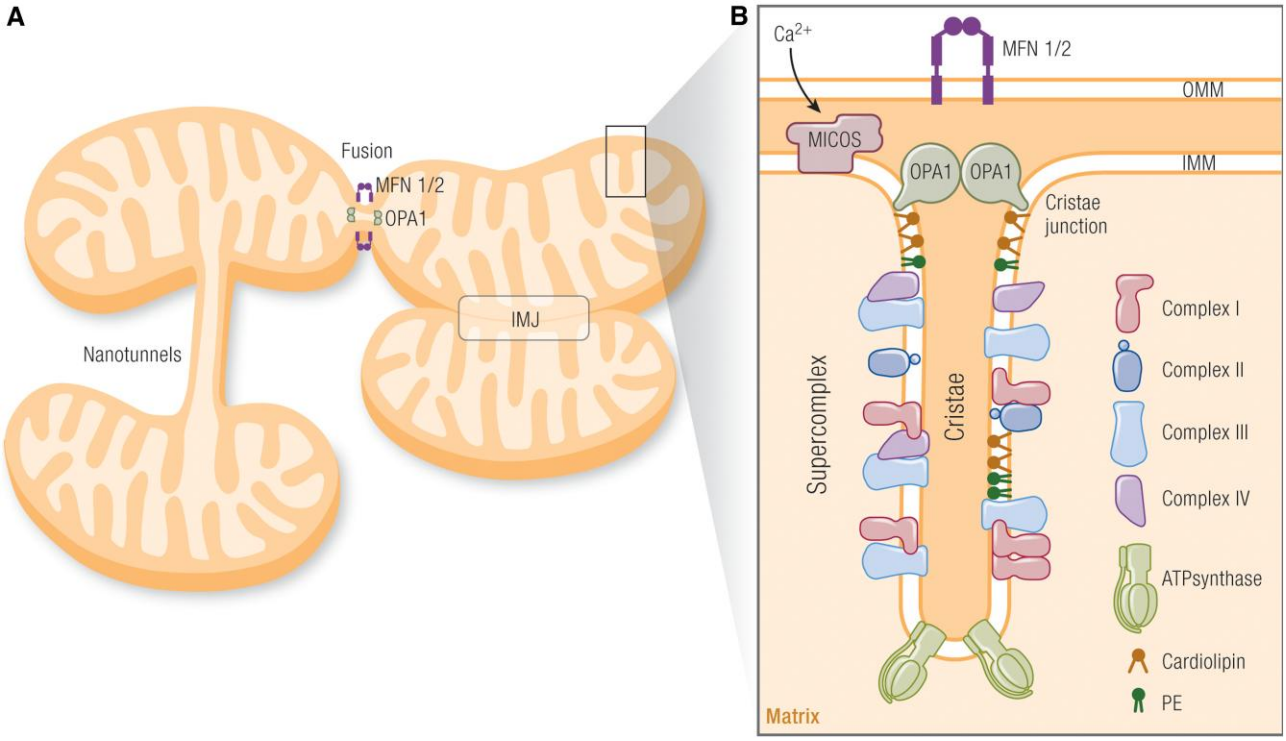


Figure 2. Schematic representation of intermitochondrial communication. A) Mitochondrial fusion, nanotunnels, and intermitochondrial junctions (IMJ) promote intermitochondrial communication and exchange of proton-motive force, mtDNA, and mitochondrial proteins. Optic atrophy 1 (OPA1) and Mitofusin 1/2 (MFN1/2) are the main proteins involved in the mitochondrial fusion process. B) Magnification of mitochondrial cristae organization. The mitochondrial contact site and cristae organizing system (MICOS), ATP synthase, and OPA1 determine the cristae shape. The mitochondrial respiratory complexes, localized in the inner mitochondrial membrane (IMM), may also communicate through formation of supercomplexes that dynamically adapt to the metabolic requirements within the cell.

both skeletal muscle (1) and cardiomyocytes (12). As such, regional uncoupling of the skeletal muscle mitochondria network by dissipation of the membrane potential could be rapidly restored through the intermitochondrial conduction of the electrical gradient (19) at intermitochondrial junctions (1, 19) (Fig. 2). Following stress, the synchronization of the mitochondrial membrane potentials was dependent on the strength of the coupling between adjacent mitochondria as well as the size of the oscillating cluster in cardiomyocytes (20), resulting in altered network behavior, also shown to be substrate-dependent (12). The significance of the coordination of the network is underscored by the rapid quality control mechanisms in place to electrically separate depolarized mitochondria from the mitochondrial reticulum (21), limiting the spread of dysfunction within the cardiomyocyte (19).

Interestingly, cristae are both more abundant and aligned at the intermitochondrial junction (22) between adjacent mitochondria. The formation of intermitochondrial junctions between mitochondria may be modulated by their energetic demand (12), that is, can be increased in correlation with increasing oxidative phosphorylation capacity (23-25) in addition to the electrochemical coupling discussed above (16). These intermitochondrial junctions are dynamic (22) and respond to changes in cellular metabolic demand (26). This might further enhance electrochemical communication between neighboring mitochondria (26).

Under stress conditions or mitochondrial damage, the synchronization of the network oscillations in membrane potential toward a stable depolarized state (18) led to broken intermitochondrial junctions where damaged mitochondria were physically separated from the network, allowing fragmented mitochondria to be cleaned up by mitophagy (19). Mitochondrial fragmentation has been linked to both increased fission and impaired fusion in heart failure (27), induction of apoptosis (28, 29), and may also indicate activation of mitochondrial quality control processes in the heart (28, 30). Moreover, this has been demonstrated under many pathological conditions in striated muscle (6, 31-33). Overall, these findings demonstrate the mechanisms in which altered coordination of the mitochondrial network plays a key role and possibly a key cellular signal to adapt to a new energetic state and prevent further damage.

Nanotunnels

Adjacent mitochondria may participate in a sudden intermitochondrial content transfer, known as *kissing*, both in the heart (34, 35) and skeletal muscle (36). Although shared contact sites between adjacent mitochondria, including the intermitochondrial junctions (22), are frequently observed, the actual exchange of contents happens less often (34, 35) and the steps occurring prior to content exchange are not fully known. Mitochondrial nanotunnels provide means for communication between distant mitochondria within the network. Previously called string mitochondria (37), these rapidly extending and retracting tubules can be formed between mitochondria that may be nearby but can even occur across long distances (35, 38, 39). The tunnels are characterized by thin, double membrane, tubular extensions with a diameter of no more than 200 nm (40) that reach out from the original

mitochondria. Nanotunnels have an inner- and outer mitochondrial membrane and contain matrix components as well as extensions of cristae (34, 35, 40).

Mitochondrial nanotunnels are more common in cardiac muscle than in skeletal muscle (13) and are not observed in mitochondria from other organs (13, 19). Thus, without changing mitochondrial position, nanotunnels likely contribute to slower, albeit efficient, content exchange between mitochondria in the heart (34, 35, 41) and would be advantageous in striated tissue where the mitochondria are constrained within the muscle fibers. In particular, cardiac nanotunnels have been observed in pools of mitochondria residing close to capillaries (also called paravascular mitochondria (1)), connecting to form an intermitochondrial junction with one or more adjacent mitochondria (19). In skeletal muscle, mitochondrial DNA (mtDNA) mutations and highly fragmented mitochondrial networks were associated with increased nanotunnel formations (8, 42). This suggests that under conditions where many small, fragmented mitochondria and a disrupted mitochondrial network are observed, increased nanotunnel formation could indicate mitochondrial stress and/or deficiency in oxidative phosphorylation. Impairments in calcium handling and homeostasis, through cardiac ryanodine receptor 2 (RyR2) mutations, also led to higher frequency of long-distance nanotunnels, and specifically between smaller mitochondria (35). Despite disruption of calcium homeostasis, and subsequent mitochondrial stress, intermitochondrial communication was ensured via nanotunnels. In this case, the induction of nanotunneling was not associated with increased fragmentation (35, 43) but rather linked to the proximity of microtubules which followed the path of the nanotunnels.

In vitro studies have elucidated that dynamic mitochondrial tubulation, a process like nanotunneling, is required for the maintenance and growth of the mitochondrial network (44). Driven by the microtubule kinesin KIF5B, thin tubules extend from the mitochondria and are formed into lattices that generate a series of interconnected lattices within the mitochondrial network (40). These tubules have been shown to follow in parallel with nanotunnels and thus may also contribute to dynamic changes in tunneling (35), potentially generated by an active pull on the mitochondria (35).

Although deficiencies in oxidative phosphorylation capacity, mitochondrial stress and/or altered calcium homeostasis are involved in the stimulation of nanotunnels (8, 35, 42, 45), the physiological and pathophysiological conditions that drive nanotunnel formation are still largely unknown. In accordance, the extent to which nanotunnel formation may improve bioenergetic status of dysfunctional mitochondria, prior to separation from the network, remains unknown. The physical interaction and exchanged cargo between the nanotunnel and the receiving mitochondria must be explored further (41) under conditions of normal physiology and disease.

Fusion and Fission—Mitochondrial Dynamics

Mitochondria fuse and divide such that their structure and morphology meet the energetics needs of the muscle fiber and maintain the distribution and maintenance of mtDNA (7) and also to optimize mitochondrial quality control systems (46-48). As such, morphological changes alter the physical interactions between neighboring mitochondria and contribute to altered coupling of membrane potential, exchange of contents, or other signaling factors (49). Despite occurring at a slower rate in striated muscle as compared to other organs, mitochondrial fusion and fission remain essential for the health of both cardiac and skeletal muscle mitochondria (28, 33, 50-56). Mitochondrial dynamics are controlled by a set of dynamin-related GTPase proteins, including fission proteins (Dynamin-related protein 1 [Drp1] and its outer membrane receptors Fis1, Mitochondrial Fission Factor [Mff], Mitochondrial Dynamics protein 49 and 51 [MID49] and [MID51]), as well as fusion proteins (Optic atrophy type 1 [OPA1], and Mitofusins 1 and 2 [MFN1/2]).

OPA1 is an important modulator of inner mitochondrial membrane fusion (57, 58), located on the inner mitochondrial membrane or in the intermembrane space where it participates in mitochondrial fusion with the outer mitochondrial GTPases, MFN1 and MFN2 (57, 59). In addition, OPA1 has an independent role in maintaining cristae structure (55). In cardiac muscle, fusion was dependent on intracellular calcium oscillations occurring during excitation-contraction coupling (60), as well as maintained mitochondrial DNA stability and metabolic state (61).

OPA1 mutations are associated with a wide range of pathologies in which mitochondrial dysfunction occurs, including neuromuscular degenerative and cardiac diseases (62). Impaired respiration is a hallmark of the obese/diabetic heart (63-65) and is associated with the downregulation of OPA1 (66). Increased OPA1 expression levels (through targeted pharmaceutical treatment) were reported to promote mitochondrial fusion and inhibition of apoptosis through increased expression levels of 2 components of ATP synthase (66, 67), rescuing cardiac function (66). The mild systemic overexpression of Opa1 protects from muscle atrophy as well as heart and brain ischemia (68). The general consensus is that fusion promotes oxidative phosphorylation (69). Therapies designed to stimulate mitochondrial fusion may do so through targeting the processing and oligomerization status of OPA1 (55) although future translational studies are warranted to study the full potential for the cardiac mitochondrial reticulum and cell function.

In skeletal muscle, the pro-fission molecule DRP1 plays an important role in development of contractile strength as well as during muscle wasting (50, 70-73). The critical role of fission was demonstrated by skeletal muscle–specific inhibition of DRP1 that led to a disruption of the mitochondrial–sarcoplasmic reticulum (SR) tethering by MFN2. Subsequently, mitochondrial calcium uptake was increased, reducing calcium availability for muscle contraction (50). Conversely to fusion, mitochondrial fragmentation may be a response to lowered ATP-availability mediated by the adenosine monophosphate (AMP)–activated protein kinase (AMPK) (50, 72). Furthermore, this was associated with activation of a retrograde response to the nucleus to drive a FoxO-dependent atrophy program modulated by AMPK activation (72).

When this balance in mitochondrial dynamics is disrupted, this can lead to pathological consequences such as heart failure, diabetes, and sarcopenia (28, 33, 50-56, 74). Similarly, in the heart of animal models of overload-induced cardiac hypertrophy and failure (27) and metabolic disturbances (64, 75), the balance between mitochondrial fission, fusion, and fragmentation is disturbed. Dysregulation of either mitochondrial fusion or fission machinery is associated with a decreased mitochondrial respiration (28, 50, 66, 76),

supercomplex formation (50, 69), and membrane potential (31, 56) that suggests that the mitochondrial shape and/or network organization are crucial for overall function. It is, however, difficult to dissociate the role of mitochondrial dynamics proteins in regulating mitochondrial structure from their importance in mitochondrial quality control. Fusion and fission can trigger different signaling pathways and biological processes, and as such are not direct opposites of the same spectrum. This phenomenon has recently been highlighted in a study where both knockdown and overexpression of DRP1 late in life caused skeletal muscle atrophy and impaired mitochondrial quality (77). In accordance, mitochondrial fission in cardiac muscle is an early step in the induction of PINK1/Parkin-mediated mitophagy (78, 79), and similarly, deficiency of the fusion protein MFN2 is also associated with dysregulated PINK1/Parkin-mediated mitophagy (28) in the failing myocardium. These findings are supported by other studies that demonstrate the importance of mitochondrial dynamics for maintenance of mitochondrial integrity and regulation of key mitochondrial functions in striated muscle (32). Although the physiological cues driving mitochondrial shape changes in striated muscle during disease remain to be elucidated, regulating the balance between fusion and fission may be an important therapeutic strategy.

Endocrine effects on mitochondrial dynamics are an exciting new perspective that may shed light on the physiological cues underlying shape and network organization (covered in the section "Extracellular Communication of Mitochondria to Maintain Function and Homeostasis"). Whether future therapeutic targets to block fission (and stimulate fusion) or to block fusion (and stimulate fission) will provide translational perspective for patients suffering from metabolic disease affecting heart or skeletal muscle, remains to be answered.

Mitochondrial Cristae Ultrastructure and Function

Mitochondrial cristae are tubular or lamellar invaginations of the inner mitochondrial membrane into the mitochondrial matrix. In line with the notion of a mitochondrial reticulum in striated muscle, the cristae of neighboring mitochondria are also coordinated at intermitochondrial junctions (22, 80) allowing for communication between neighboring mitochondria in the network, and the formation of a mitochondrial microcompartment optimized for ATP production (81). Important determinants of cristae shape are the mitochondrial contact site and cristae organizing system (MICOS), and the F_1F_0-ATP synthase (82, 83). MICOS is located at the cristae junctions and contains several identified subunits that are linked to regulation of cristae membrane shape and oxidative phosphorylation capacity (82, 84) as well as formation of the cristae junctions (Mic60) and laminar cristae (Mic10) (82). MICOS subunits interact with a variety of different proteins that are essential for basal mitochondrial function, such as mitochondrial calcium transport (85), autophagy/mitophagy (86-88), and the lipid composition of the mitochondrial membrane (86, 89).

Following loss of membrane potential, apoptotic signaling passes through a mitochondrial network in waves (90), through OPA1-mediated cristae aligned intermitochondrial junctions between neighboring mitochondria (80). In this case, the inversion of the curvature of the cristae is necessary for cytochrome c to be completely released (55, 91) in addition to promoting interaction between the fusion protein OPA1 and MICOS (specifically Mic60) (86, 92).

In addition to its role in apoptotic signaling, OPA1 determines both the cristae width as well as the width of the cristae junction (92) supporting ATP-linked respiration (80). There is general consensus that cristae ultrastructure is a determinant of mitochondrial respiration (93-95) in part through maintenance of the assembly and stability of mitochondrial respiratory complexes and supercomplexes (9, 69). Depending on the cell type, energy requirements, and tissue location, the cristae membrane morphology can vary greatly. Cristae density is highest in metabolically active tissues, such as cardiac and skeletal muscle, with a cristae surface packed with respiratory (super)complexes and ATP synthase (58, 96). Recent work has indicated that, in cultured cells, cristae can be regarded as independent operating individual bioenergetic units within the mitochondria (97). Whereas lower intra-cristal space was associated with low respiration levels, higher respiration rates were associated with higher intra-cristal space (58, 86, 98, 99). As such, mitochondrial cristae density increased in line with increased leg muscle power in endurance-trained athletes after training (93). Because of the large amount of cellular and molecular adaptations associated with mitochondrial dysfunction, it remains unclear whether changes in cristae architecture are a symptom or a cause of altered mitochondrial function. Likely however, cristae morphology represents a marker of overall mitochondrial fitness in striated muscle.

Altered cristae shape following deletion of mitochondrial fusion protein OPA1 in skeletal muscle contributes to the destabilization of the respiratory complexes and supercomplexes (33, 57, 58, 69). Barth's syndrome is a cardiolipin-associated disease with a clinical manifestation of cardiomyopathy and loss of skeletal muscle strength (100), as well as severely impacted cristae ultrastructure and altered MICOS complex assembly (86). Similarly, in type 2 diabetes, the MICOS protein Mic19 was among other mitochondrial proteins shown to be downregulated in skeletal muscle (101). As cristae generally respond to alterations in metabolic factors including glucose and ADP levels or hypoxia (102), cristae restructuring may provide insight to many pathological conditions in striated muscle.

Communication Between Mitochondria and Other Organelles

Not only do mitochondria communicate with each other within the reticulum, mitochondria extensively communicate with other organelles in cardiac and skeletal muscle fibers. Traditionally, this communication has been linked to either the intracellular concentration of ATP or ATP production rates, as energy demand is high in striated muscle. Here we particularly highlight some of the more novel ways that mitochondria use to communicate with other organelles. First, we describe the concept of mitochondrial-derived vesicles and subsequently discuss the structural and functional link between mitochondria and nucleus, glycogen, lipid droplets, peroxisomes, and endoplasmic/sarcoplasmic reticulum (Fig. 3).

Mitochondrial-Derived Vesicles

Mitochondrial-derived vesicles were originally characterized based on 3 criteria: they contained mitochondrial cargo but

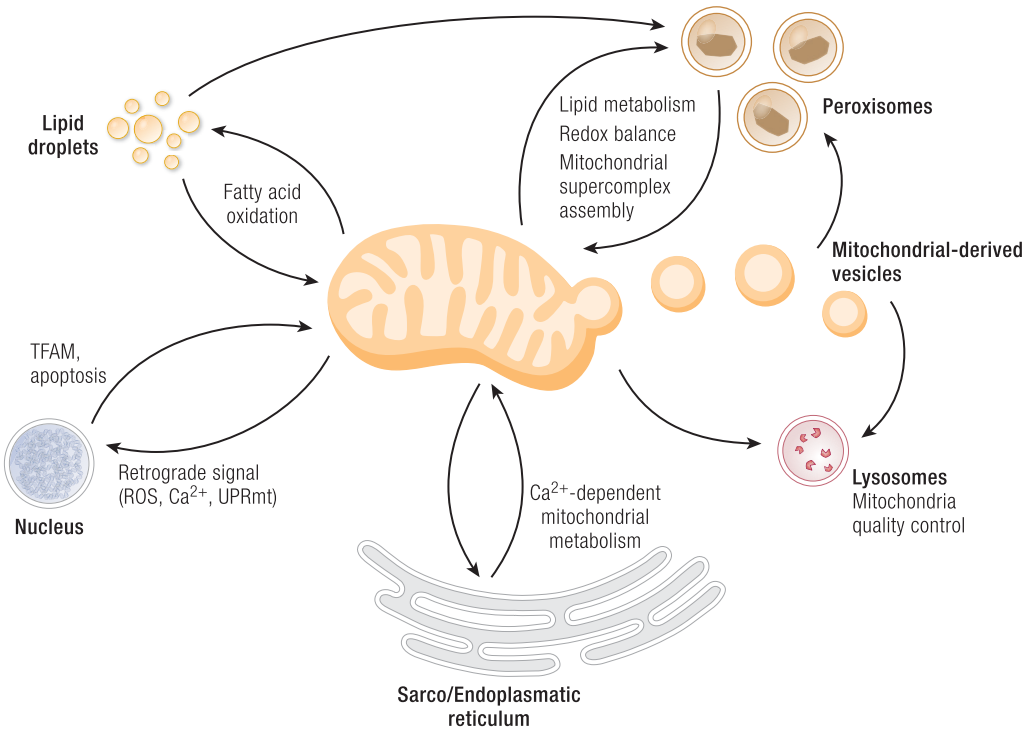


Figure 3. The physiological relevance of mitochondrial-organelle communication. Intracellular mitochondria-organelle communication is mediated by direct membrane contact sites, the exchange of metabolites, redox state, as well as through mitochondrial-derived vesicles. Whereas mitochondria-sarcoplasmic reticulum interaction is required for calcium-dependent energy metabolism and mitochondria-peroxisome communication regulates lipid metabolism and peroxisomal biogenesis, these mitochondria-organelle interactions also contribute to redox balance and respiratory supercomplex assembly. In turn, fatty acid oxidation is modulated by the metabolic interplay between mitochondria and lipid droplets. Mitochondrial biogenesis and apoptosis can dictate cell fate and are regulated by mitochondrial-nucleus crosstalk. Reactive oxygen species (ROS) play important signaling roles, in addition to altered ATP levels, calcium accumulation, triggering mitochondrial unfolded protein response (mtUPR), the integrated stress response and mitonuclear retrograde signaling. Various stressors, including ROS, stimulate the release of mitochondrial-derived vesicles, and the delivery of mitochondrial cargo to the lysosomes for degradation.

not cristae; they were highly uniform single or double membrane-bound circular structures with a diameter from 60 to 150 nm; and they were formed independently from Drp1-induced mitochondrial fragmentation (103-106).

To date, mitochondrial-derived vesicles have been shown to transport cargo to the peroxisomes (105-107), lysosomes (104), and endosomes (106). More recent evidence has demonstrated that mitochondrial-derived vesicles may also deliver their cargo to extracellular vesicles (108-110) and, as such, circulate from source tissues with potential to functionally impact distant target organ systems (49). The fate of the vesicles is not always clear but likely depends on the incorporated cargo. Mitochondrial-derived vesicles are important as a first line of defense against mitochondrial stress (104, 108, 111) in addition to mitochondrial housekeeping (104). Although the protein cargo is mostly undefined, proteins from oxidative phosphorylation complexes, Krebs' cycle enzymes, and other major enzymes related to metabolic and redox signaling, have been identified as destined for lysosomal degradation after (oxidative) stress-induced damage (104, 108). Other studies have linked the exposure to stress to a several-fold higher concentration of these proteins and enzymes, reenforcing the role for mitochondrial-derived vesicles as a response to cellular stress and their contribution in the removal of damaged mitochondria or oxidized mitochondrial components (112, 113). Thereby, mitochondrial-derived vesicles act as a mechanism of mitochondrial quality control (39, 106), and allow for the quick removal of damaged mitochondrial proteins prior to detectable mitochondrial dysfunction or mitophagy (104). In the heart, mitochondrial-derived vesicles were shown to contain transporters of the outer membrane 20 (TOM20) and matrix-derived pyruvate dehydrogenase (45), and vesicle formation was observed under basal conditions as an active quality control condition, whereas increased formation of TOM20-containing mitochondrial-derived vesicles was seen following oxidative stress where mitochondria became fragmented and dysfunctional (45, 111).

Emerging evidence indicates that cells package and release some of their mitochondrial content to circulating extracellular vesicles (108, 110). This was demonstrated either by detection of cell-free mitochondria within the extracellular vesicle (114, 115) or through the detection of mitochondrial components, such as TOM20 (116). Thus, it is possible that, in line with what has been shown in mesenchymal stem cells (115), mitochondrial-derived vesicles use extracellular vesicles to outsource mitophagy to other cells (110, 115). On the other hand, the transfer of mitochondrial content may also be a mechanism to rescue metabolic activity, through mtDNA transfer (117), or potentially also as a biomarker or passing on information to the rest of the body regarding mitochondrial stress. In fact, the nature of mitochondrial stress, and/or extent of damaged proteins within the mitochondrial content, was shown to play a determining role in whether mitochondrial-derived vesicles were sent to extracellular vesicles or to lysosomes for degradation (116). In this case, the PINK/Parkin pathway was shown to inhibit incorporation

of oxidized mitochondrial components in extracellular vesicles, thereby possibly preventing a deleterious inflammation and immune response (116) elicited by mitochondrial damage–associated molecular patterns (mtDAMPs; see section "Mitochondrial DNA"). PINK/Parkin regulates the transport of mitochondrial-derived vesicles induced by stress to the lysosomes (118), thus avoiding immune activation unless the stress is excessive and above their transport capacity (110, 116). The role of mitochondrial-derived vesicles in antigen presentation during infection has been elegantly demonstrated in the etiology of Parkinson disease (119, 120). Thus, determination of a wider physiological role of mitochondrial-derived vesicles clearly deserves further attention in cardiac and skeletal muscle, under both pathological and physiological conditions, particularly during the progression of metabolic diseases.

Communication Between Mitochondria and Nucleus

While most cardiomyocytes have a single nucleus, skeletal muscle cells have multiple nuclei due to their much larger fiber size. The DNA of almost all mitochondrial proteins (including the subunits of the mitochondrial complexes) is in the nucleus. Mitochondria still retain their own circular DNA (mtDNA), encoding the mitochondrial 16S and 12S rRNA, 22 tRNAs, and 13 core subunits of the oxidative phosphorylation system, including 7 crucial subunits of mitochondrial complex I (45, 121, 122). Mitochondria therefore require their own transcription and translation machineries. The stoichiometric balance between both genomes is tightly regulated via mitochondria-nuclear (*mitonuclear*) communication, and is crucial for increasing mitochondrial volume density, for example, in the heart (24). Hence, intricate mitonuclear communication facilitates both coordination and synchronization of transcription and translation, and a dedicated import of mitochondrial proteins.

There are various forms of mitonuclear communication, one of which occurs via transcription factor A, mitochondrial (TFAM). The gene for TFAM is located in the nucleus and is transcribed and translated upon activation by peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and nuclear respiratory factors (NRF1 in particular). Together with mitochondrial transcription factor B1 and 2 (TFB1M; TFB2M), TFAM initiates mitochondrial transcription by specifically binding to transcriptional start sites in the mtDNA (21).

The pathways that are involved in the activation of PGC-1α in skeletal muscle are relatively well studied (123). AMP-dependent protein kinase (AMPK) becomes activated after acute endurance exercise due to higher AMP concentrations and lower glycogen concentrations. This cellular stress sensor is the starting point of gene transcription and translation at the nucleus that ultimately leads to a higher PGC-1α, TFAM, NRF1 and other proteins required for increasing mitochondrial biosynthesis (124), and subsequent mitochondrial transcription and translation. The importance of TFAM for regulating mitochondrial volume density is highlighted by the observation that mice with a skeletal muscle–specific disruption of TFAM appear healthy until ~13 weeks, after which the mice suffer from ragged-red muscle fibers, accumulation of abnormally appearing mitochondria, excessive mitochondrial calcium uptake, progressively deteriorating oxidative phosphorylation capacity, and reduced muscle force production (125, 126).

While most mitochondria are located between the myofilaments in longitudinal chains (intramyofibrillar mitochondria), there is another population of mitochondria located around nuclei: the perinuclear (sometimes called paranuclear) mitochondria (15, 19, 127). These perinuclear mitochondria are much less studied but are exclusively found in cardiac muscle (15, 19, 127). Recent work has highlighted that the majority of translation occurs in the perinuclear region of the mitochondrial network (127, 128). Perinuclear mitochondria are also more mobile and appear to participate readily in fission/fusion dynamics (127). As such, the nuclear mitochondrial subpopulation may be more critical during mitochondrial turnover and for the regulation of nuclear function and protein import and export.

Mitochondria also communicate with the nucleus via retrograde signaling, which modifies the transcriptome to overcome cellular stress. This consists of various signaling pathways that involve perinuclear mitochondria (127), the production of reactive oxygen species, local ATP concentrations, and the mitochondrial unfolded protein response (mtUPR) (129). The mtUPR system is activated upon mitochondrial (proteotoxic) stress, and breakdown products (from proteostasis) communicate to the nucleus via retrograde signaling to inhibit nuclear protein translation, breaking down misfolded proteins, induce chaperones that help with protein folding, or even initiate apoptosis via the release of cytochrome c into the cytosol. Typical markers for the mtUPR are ATP-dependent zinc metalloprotease (Yme1L1), heat shock protein 60 (Hsp60), LON protease 1 (Lonp1), and caseinolytic peptidase *P* (CLpP) (129).

In the heart, initiating activating transcription factor 5 (ATF5)-mediated mtUPR signaling may be cardioprotective following ischemia-reperfusion (130) and in chronic pressure-overload (131, 132). Thus, stimulation of an endoplasmic reticulum (ER)-stress response via mtUPR activation is linked to preserved mitochondrial function and cell viability. Similarly, mitochondrial dysfunction in skeletal muscle during insulin resistance also induced ATF5-mediated mtUPR (133). Yet, this stress response has a critical, physiological role in skeletal muscle demonstrated by the exercise-induced elevation in mtUPR markers in muscle from aged mice that corresponded with enhanced mitochondrial function (134). These effects are associated with higher nicotinamide adenine dinucleotide (NAD^+) levels in both heart (131) and skeletal muscle (135, 136).

Various cell culture and invertebrate studies have observed a crosstalk between mitochondrial stress and the activity of the proteasome. However, little is known about the regulation of these in skeletal and cardiac muscle during more physiological relevant (environmental) stressors such as physical (in)activity.

In mammals, the mtUPR may not be the primary response to mitochondrial dysfunction, but it is embedded within the integrated stress response helping the cell adapt to a variable environment (137). Both the mtUPR and the integrated stress response reduce cytosolic translation, protein folding capacity, ubiquitination, and proteasome degradation and autophagy (129), partly via the phosphorylation of eukaryotic translation initiation factor 2a (eIF2a) and the activation of ATF4-dependent adaptive stress cellular response. The integrated stress response can lead to either beneficial or

detrimental effects depending on the duration of the type of initial stress. Recent work on cardiac cells identified a regulatory mechanism mediated by C/EBP homologous protein (CHOP) that fine-tunes integrated stress response activation upon mitochondrial dysfunction (137). CHOP competes with ATF4 to bind to CCAAT/enhancer-binding protein b (C/EBPb), reducing the transcriptional levels of ATF4 and the downstream targets of the integrated stress response. The effects of CHOP are independent of mtUPR and rely on the attenuation of the integrated stress response overactivation by reducing the excessive ATF4-mediated stress response activation that results in cardiotoxic effects. Accordingly, CHOP deficiency exacerbates mitochondrial dysfunction and switches the integrated stress response from acute to chronic activation, which is detrimental for overall cell function (137). Therefore, the mitochondrial stress response depends on the interplay between CHOP, C/EBPb, and ATF4 to regulate an adaptive transcriptional response, which overlaps with the integrated stress response. Upon activation of the integrated stress response, CHOP and ATF4 regulate the transcription of fibroblast growth factor 21 (FGF21) and growth and differentiation factor 15 (GDF15), two metabolic hormones with autocrine, paracrine, and endocrine actions. Muscle-derived FGF21 and GDF15 are systemic mediators of the integrated stress response (138, 139), and mediate the communication between cardiac and skeletal muscle with distant organs upon mitochondrial stress (see section "Extracellular Communication of Mitochondria to Maintain Function and Homeostasis").

Communication Between Mitochondria and Glycogen

Metabolic flexibility is the ability of striated muscle to adapt cellular metabolism by substrate sensing, transport, storage, and utilization, depending on availability and requirement (3). Glycogen and lipid droplets are local energy stores that mitochondria interact with to produce the required energy for muscle contractions. As such, mitochondria are metabolic sensors that are sensitive to nutritional overload (140).

Glycogen is a major source of energy in skeletal muscle cells, particularly during high-intensity exercise (3). Granules of these branched glucose polymers are visible by transmission electron microscopy. In skeletal muscle, these granules have a distinctive distribution depending on species, fiber type, and the history of activity (141). About 65% to 80% of the glycogen is located in the intermyofibrillar space, in the close vicinity of mitochondria (141, 142), with other glycogen stores located near the sarcoplasmic reticulum (SR) or adjacent to the sarcolemma (142). Only with detailed and labor-intensive electron microscopy imaging is it possible to provide physiological insights into the spatial-temporal behavior of glycogen deposition and utilization in skeletal muscle during exercise (141, 142). The utilization rates of the various subcellular muscle glycogen storages are higher in glycolytic type 2 fibers and depend on its subcellular localization and exercise duration, albeit with a large single-fiber heterogeneity (141). Chronic aerobic exercise increased resting skeletal muscle glycogen content, but only in the subsarcolemmal region (143). In skeletal muscle of patients with type 2 diabetes mellitus, skeletal muscle glycogen content was not significantly different from healthy controls (143), likely due to insulin resistance and subsequent reduced glucose uptake. Currently, it is unknown how exactly the glycogen pool communicates with the mitochondria. It is conceivable that the activity and subcellular location of glycogen phosphorylase (rate-limiting step in glycogen breakdown) and other enzymes play a key role here.

Studies with such spatio-temporal resolution in subcellular glycogen stores are currently lacking in the heart. While the overlap in substrate utilization and metabolic pathways between skeletal and cardiac muscle might suggest a similar glycogen accumulation, the constant energy requirement of the heart might result in different glycogen uptake and utilization rates. Glycogen accumulation was higher in cardiomyocytes, particularly around mitochondria, in a porcine model of experimental type 2 diabetes mellitus (75).

It is currently unknown whether glycogen accumulation causes advanced glycation end products (AGEs) which can negatively affect mitochondrial function in conditions of nutritional oversupply in the heart. Shunting of glucose into alternative pathways, including glycogen storage, has been linked to cardiac hypertrophy and dysfunction but not with mitochondrial structural changes (144). Nonetheless, that excessive glycogen accumulation causes cardiac dysfunction and myopathy is evident from glycogen storage diseases, which are heterogenous inherited inborn errors of carbohydrate metabolism. Hypertrophic cardiomyopathy, skeletal muscle myopathy, and exercise-induced rhabdomyolysis are common symptoms in various glycogen storage diseases, such as McArdle disease (145). A clear contributing role for a defect glycogen to mitochondrial communication in these genetic metabolic diseases requires further studies. The involvement of insulin resistance and altered mitochondrial metabolites suggests that glycogen-induced mitochondrial overload clearly has physiological and pathological consequences in these diseases (146).

Communication Between Mitochondria and Lipid Species

Not only do mitochondria communicate with glycogen, but also with intracellular lipid droplets. These lipid droplets can vary dramatically in size and number and have a phospholipid monolayer coated with proteins involved in lipid droplet synthesis and degradation. In contrast to insulin-mediated glucose uptake, lipid import into striated muscle mitochondria is a less-strictly regulated process and occurs via the transmembrane glycoprotein cluster of differentiation 36 (CD36) (147). Intramuscular lipid storage and oxidation in skeletal muscle is highly muscle-fiber-type-specific, and depend critically on the abundance of key proteins, such as adipose triglyceride lipase, hormone-sensitive lipase, and the lipid droplet–coating proteins perilipin 2 (Plin2) and perilipin 5 (Plin5) (148).

Intramyocellular lipid storage in skeletal and cardiac muscle has often been negatively associated with insulin resistance (64, 149). Paradoxically, also endurance-trained athletes have similar levels of lipid droplets, but do not suffer from insulin resistance. This athlete's paradox suggests that lipid droplets are not solely responsible for the compromised insulin sensitivity (150). In endurance-trained athletes, lipid droplets are smaller and more abundant in the intramyofibrillar region of type I fibers, whereas the lipid droplets are larger and in the subsarcolemmal region of type II fibers in individuals with type 2 diabetes mellitus (150). In addition, the make-up of the lipids probably plays a role, as the lipotoxicity of

long-chain acylcarnitines, but also diacylglycerols and ceramides, contributes to the development of intracellular insulin resistance (64).

An excessive lipid accumulation in the diaphragm muscle has been suggested to underlie the alterations in mitochondrial and metabolic alterations after mechanical ventilation (140). This nutritional overload and subsequent responses of cellular metabolic sensors (AMP-activated protein kinase and sirtuins) induced diaphragmatic oxidative stress, which caused mitochondrial (DNA) damage, with functional consequences for overall muscle function (140). In addition, disorders of mitochondrial long-chain fatty acid oxidation and the carnitine shuttle are associated with a compromised energy homeostasis and accumulation of long-chain acylcarnitines (151). Similar to lipotoxicity in the diabetic heart, an excessive accumulation of lipid species reduces cardiac function, leading to the induction of ventricular fibrillation in patients and animal models of long-chain fatty acid oxidation disorders (151, 152). Also exercise-induced rhabdomyolysis has been observed in these patients (151). In a mouse model of very long-chain acyl-coenzyme A dehydrogenase deficiency, mitochondria in heart and soleus muscle appear more heterogeneous in size, increased in number, and are disorganized in the subsarcolemmal region (152).

The lipid droplet–mitochondria interaction provides a rapid transfer of fatty acids from lipid droplets to mitochondria for breakdown and ATP production. In addition, fatty acids released by the autophagic degradation of membranous organelles can shuttle back to lipid droplets, acting as a lipid buffering system that prevents lipotoxic dysregulation of mitochondria (153). Peridroplet mitochondria in brown adipose cells have distinct characteristics, such as a unique proteome due to reduced fusion and fission dynamics and enhanced ATP production that promotes triacylglycerides synthesis rather than fatty acid breakdown to support lipid droplet expansion (154). It is currently unknown if a similar subpopulation exists in striated muscle. The directionality between lipid synthesis and lipid breakdown might be regulated by the Plin protein family. Plin5 regulates lipid storage and utilization by establishing close contacts between lipid droplets and mitochondria (155). Plin5 inhibition causes skeletal muscle insulin resistance associated with ceramide accumulation (156), while its overexpression, specifically in skeletal muscle, causes protective metabolic effects in mice fed a high-fat diet, and the beneficial effects are mediated by the myokine FGF21 (157). Conversely, Plin5 overexpression in the heart leads to myocardial steatosis and dysfunction (158). Plin2 regulates lipid accumulation, and its levels mirror the lipid content (159). Accordingly, Plin2 levels are increased in several metabolic disorders, such as type 2 diabetes, insulin resistance, atherosclerosis, and cardiovascular diseases (160). In age-related sarcopenia, increased Plin2 levels correlate with the decline in muscle mass and force (161). Plin2 inhibition in skeletal muscle increases myofiber size and intramuscular lipidome modulation, while its overexpression does not affect muscle mass (162). Mitochondria-lipid droplet interaction is impaired in type 2 diabetes. Mitochondria are fragmented, lipid droplets are higher in size but reduced in number, and Plin2 levels are higher than Plin5 compared with healthy individuals, suggesting that the Plin composition might regulate lipid droplet dynamics (163).

As substrate storage and utilization is important for optimal mitochondrial function, the interplay between glycogen and lipid droplets is still relatively poorly understood. Studying this communication in striated muscle requires a combination of imaging (electron microscopy) and metabolite and molecular analyses, preferably combined with functional assays of contraction and/or fatigue resistance. Particularly the role of peroxisomes, important for very long-chain fatty acid oxidation, in skeletal and cardiac muscle is not studied in detail.

Communication Between Mitochondria and Peroxisomes

Peroxisomes are ubiquitous organelles that participate in several pathways, such as overall cellular lipid and reactive oxygen species metabolism. A critical role of peroxisomes in skeletal muscle is thought to be the maintenance of lipid metabolism by breaking down very long-chain fatty acids before they are further processed in the mitochondria via the β-oxidation (151). Mitochondria and peroxisomes are physically and functionally connected (164), and this close interaction is a prerequisite for efficient transfer of metabolic intermediates. These 2 organelles work together to achieve key cellular processes, such as lipid metabolism, redox balance, antiviral signaling, and peroxisomal proliferation.

In mammals, β-oxidation requires both mitochondria and peroxisomes, and there is a need for bidirectional peroxisome-mitochondria metabolite transfer. Since peroxisomes in mammals can only metabolize very long-chain fatty acids, intermediates must be shuttled to mitochondria in the form of acylcarnitine ester or free acids to ensure complete oxidation. These coordinated metabolic processes also produce reactive oxygen species (ROS) and alterations in peroxisomal ROS metabolism impact the mitochondrial redox balance (165). Moreover, to maintain peroxisomal β-oxidation, NADH generated in peroxisomes must be routed to the mitochondria for energy-efficient reoxidation of NADH back to NAD^+ (166). Peroxisomal membrane protein 34 (PMP34) is encoded by the SLC25A17 gene, and it is so far the only transporter identified in human peroxisomes that shows substrate specificity toward NAD^+ (167).

Another example of the metabolic link between peroxisomes and mitochondria is that peroxisomal-derived ether lipids drive the mitochondrial respiratory supercomplexes assembly under pyrimidine deficiency (168). Peroxisomes primarily proliferate by growth and asymmetric division (fission) of pre-existing organelles. The peroxisomal fission machinery comprises Pex11b, Fis1, MFF, and DRP1, which except for Pex11b, are shared with mitochondria, suggesting coordinated division under certain conditions (169, 170). Peroxisomal fission in yeasts occurs at peroxisomal-mitochondrial contact sites, and it is required to eliminate superfluous or dysfunctional organelles through selective autophagy, known as pexophagy (171). How this process is regulated in skeletal and cardiac muscle is currently unknown. Another route for peroxisomal biogenesis in mammalian cells is the de novo formation of peroxisomes which requires the contribution of mitochondria and the endoplasmic reticulum (ER) (107). Under certain experimental conditions, mitochondria selectively release mitochondrial-derived vesicles containing Pex3 and Pex14, which fuse with ER-derived vesicles carrying Pex16 to form pre-peroxisomes (107).

Although the mechanisms of communication between the 2 organelles are not clear, the close interaction between them might be mediated by membrane contact sites (172) and

mitochondrial-derived vesicles (173). To date, most of this work has been performed in yeast cells. The peroxisomal Pex34 protein physically interacts with the mitochondrial protein Fzo1, the yeast homolog of the mammalian mitofusin 2 (174). This interaction is responsible for transfer of β-oxidation products from peroxisomes to mitochondria (174).

In mammalian cells, peroxisome-mitochondria contact sites have been observed (175), but the tethering components are still elusive. The dual-localized peroxisomal and mitochondrial acyl-CoA binding protein ACBD2, has been proposed as a molecular mechanism to drive peroxisomes and mitochondria into proximity promoting steroid biosynthesis in Leydig cells (176).

The importance of peroxisomes for optimal mitochondrial function in striated muscle is exemplified by the observation that peroxisomal dysfunction is known to cause mitochondrial abnormalities (177). Muscle biopsies from patients with peroxisomal biogenesis disorders display a secondary mitochondrial myopathy with enlarged mitochondria, reduced mitochondrial respiration, and lipid accumulation (178, 179). Whether the metabolic alterations seen in patients with disorders of mitochondrial long-chain fatty acid oxidation are due to an abnormal peroxisome-mitochondria communication is currently unknown and requires further study. The complete elucidation of the communication mechanisms of the 2 organelles is crucial to understanding how organelle cooperation contributes to human health and disease.

Mitochondria and the Sarcoplasmic/Endoplasmic Reticulum

Mitochondria and the sarco/endoplasmic reticulum (SR/ER) in striated muscle are connected at multiple sites through what are known as mitochondria-associated ER membranes (MAMs) (180, 181). These membrane associations regulate important basal functions such as altering intracellular calcium concentrations upon contraction (4, 182), as well as lipid metabolism (183, 184), autophagy (28), oxidative stress (9, 185, 186), and handling of unfolded proteins (180, 187). In striated muscle, mitochondria are located near the SR, in so-called calcium hotspots, where mitochondria form junctions with sarcolemmal T-tubules and the SR terminal cisternae (188-192). The structural organization differs slightly between heart and skeletal muscle; in the heart, one junctional SR and one t-tubule form a dyad whereas 2 junctional SR and one t-tubule form triads in skeletal muscle (181, 193). This allows calcium to accumulate inside the mitochondrial matrix on a contraction-to-contraction basis (194) and allows for bidirectional signaling between these 2 organelles (192). Mitochondrial calcium uptake is tightly regulated both by the electrochemical gradient across the mitochondrial membrane and via the mitochondrial calcium uniporter (MCU) complex on the inner mitochondrial membrane. The complex is comprised of various pore-forming proteins (195), and the calcium-sensitive "gatekeepers" MICU-1 and 2 (196) that in combination, regulate the threshold for calcium import and coordinate channel opening (197, 198). As such, the MCU complexes are strategically located at calcium hotspots (188-190).

Elevated mitochondrial calcium in turn activates major matrix dehydrogenases (including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase complex, and isocitrate dehydrogenase) which contribute to NADH generation (199). Furthermore, mitochondrial calcium uptake activates the aspartate/glutamate shuttle, the ATP-Mg/P_i carrier and ATP synthase (199-202) thereby linking respiration to ATP synthesis (182, 199, 202, 203) In the heart, this contraction-energetic coupling is considered the key mechanism during dynamic changes in cardiac workload (199, 204) and is necessary for mitochondrial fusion to occur (60). In turn, mitochondrial calcium accumulation in skeletal muscle is key for muscle hypertrophy through the regulation of IGF-1/AKT/protein kinase B (PKB) and PGC-1α4 pathways (205) as well as playing a role in muscle strength (206) and regeneration (207). Despite its central role in driving mitochondrial metabolism, calcium is also a powerful trigger of mitochondrial permeability transition pore (mPTP) opening and subsequently release of cytochrome c (normally between complex III and IV) into the cytosol, where it interacts with the intrinsic apoptotic pathways (208, 209). The intimate interaction between the SR/ER-mitochondria is therefore important for the sensitization of injured cells to calcium overload (210, 211) and, as such, the determination of overall cell fate.

The relevance of SR/ER-mitochondria communication in striated muscle physiology is again demonstrated by the physical tethering of mitochondria and SR via the outer mitochondrial membrane fusion protein MFN2 (210, 212, 213), considered to be central for the maintenance of local calcium microdomains or calcium "hotspots" (189, 214). As such, the knockdown of MFN2 increased SR-mitochondria distance (215, 216) and resulted in a hampered activation of matrix dehydrogenases in the heart (211). Similarly, low MFN2 abundance in human skeletal muscle, also seen during type 2 diabetes, was associated with increased SR-mitochondria distance and altered calcium homeostasis. In addition, lower cristae density, reduced muscle strength, lower maximal oxygen uptake, and altered blood lipid profile (217) were observed. Emerging evidence has elegantly linked altered integrity of SR-mitochondria interaction, detectable prior to mitochondrial dysfunction, to muscle insulin sensitivity (218, 219), possibly underlying the development of insulin resistance during obesity. Although it remains unclear whether the SR-mitochondria membrane associations are dispersed (218) or increased (220), both occur during insulin resistance, where pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4) was shown to play a role in modulating integrity of the SR-mitochondria membrane association (220).

The communication between SR/ER-mitochondria ensures the formation of autophagosomes (221) and depletion of MFN2 compromises their formation (222). In cardiac muscle, SR-mitochondria tethering by MFN2 also regulates mitochondrial quality control processes (28) through binding of Parkin to the outer mitochondrial membrane initiating outer membrane protein ubiquitination and the amplification of PINK1/Parkin-mediated mitophagy (78, 79). Mitochondrial shaping proteins such as DRP1 have also been shown to reversibly impact calcium homeostasis and alter SR-mitochondria tethering in skeletal muscle (50). DRP1 deletion resulted in upregulated levels of mitochondrial calcium uniporter and mitochondrial swelling (65), in parallel with unfolded protein response pathway activation and ER stress, all associated with lower SR/ER-mitochondria association (9, 219, 223). Importantly, lipid biosynthesis and transfer also occur at the SR/ER-mitochondria membrane association

(221). This includes the enrichment of the mitochondrial membrane with phosphatidylethanolamine (PE) and other lipids essential for mitochondrial morphology (224) and cristae biogenesis (225). During unfolded protein response (see section "Communication Between Mitochondria and Nucleus"), the ER increases its folding capacity to counteract aggregation of misfolded proteins (226) and ER-mitochondria membrane association is increased (227).

The general consensus is that disruption of skeletal muscle calcium homeostasis and subsequent altered mitochondrial calcium uptake (204) resulted in oxidative stress, mitochondrial damage, and muscle atrophy (50, 203, 228). Importantly, these are at the core of common pathological conditions such as age-related sarcopenia (229) and insulin resistance (218, 219), as described above. In the heart, mitochondrial calcium accumulation is important for increased cardiac workload in health (194) and is higher in type 2 diabetes mellitus (230). Increasing mitochondrial calcium uptake through MCU overexpression in the diabetic myocardium resulted in ameliorated cardiac energetics and substrate metabolism (231). Similarly, the sodium-glucose inhibitor empagliflozin, a highly promising therapy for heart failure, was not only associated with acutely increased mitochondrial calcium accumulation and improved cardiac contractility (232), but also improved ER stress (233), resulting in restored unfolded protein response and reduced inflammation and oxidative stress (234). Combined with the empagliflozin-induced increase in MFN2 (235), these findings suggest that SR/ER-mitochondrial communication is important for cardiac health. Re-establishing physiological calcium dynamics and SR/ER-mitochondrial membrane association are therefore potential novel therapeutic targets for insulin resistance and overall mitochondrial fitness.

A complicating factor in the understanding of SR-mitochondrial communication is the interacting contribution of the peroxisomes. Lipid droplets and peroxisomes are structurally located close to mitochondria and their contact sites increase with increased fatty acid oxidation (236). Excessive cardiac lipid droplet accumulation was recently observed upon a disrupted SR-mitochondrial contact sites and calcium handling (237). Furthermore, subtle changes in local calcium concentrations modulate contact sites between lipid droplets, mitochondria and SR (184). Thus, calcium may play a yet-to-discover role in the regulation of peroxisomal fatty acid oxidation in the heart. In skeletal muscle, the percentage of intermyofibrillar SR-mitochondria interactions positively related to whole-body lipid oxidation and smaller lipid droplet size (183). Although a direct cause-and-effect relationship has not been demonstrated yet, communication between peroxisomes, sarco/endoplasmic reticulum, and mitochondria via calcium signaling seems crucial for optimal striated muscle function.

Extracellular Communication of Mitochondria to Maintain Function and Homeostasis

Striated muscle fibers communicate with each other through direct interaction via axonal signaling, by secreting soluble factors such as enzymes, cytokines, hormones, and growth factors (238), and/or extracellular vesicles (exosomes) containing peptides, DNA (such as mtDNA), mRNA, microRNA (miRNA), and other RNA species (238, 239).

Also, heat is an important nonmolecular signal transduction function of mitochondria. A stable body temperature in endotherms is primarily derived from mitochondrial activity. Using a temperature-sensitive fluorescent probe, mitochondria were ~10 °C warmer at maximal respiration at a constant external temperature of 38 °C (240, 241). This process in skeletal and cardiac muscle is likely mediated by thyroid hormones (242) and uncoupling proteins (241). This temperature gradient between mitochondria and other intracellular and extracellular compartments is required to keep body temperature around 37 °C.

In this review, we particularly focus on molecular factors that can exert autocrine, paracrine, or endocrine effects on other skeletal or cardiac muscle fibers, but their communication also includes distant organs, such as the liver or brain. Myokines are small muscle-derived substances including cytokines, peptides, and metabolites (239). Mitochondria also contribute to the release of these substances into the bloodstream as mitochondrial dysfunction induces stress signals, which are known as myomitokines (6).

Mitochondrial stress activates the mitochondrial unfolded protein response and the integrated stress response (see section "Communication Between Mitochondria and Nucleus"). FGF21 and GDF15 respond to mitochondrial stress by inducing a mitohormetic dual-dose response via autocrine, paracrine, and endocrine signaling (138). FGF21 and GDF15 have been reported to have a role in both health promotion as well as disease progression (6, 243) (Fig. 4). A low level of stress promotes health and lifespan by activating local adaptive responses to increase stress resistance and systemic effects, improving whole-body metabolism. In contrast, higher stress stimuli can lead to adverse outcomes (138), suggesting a failure in the capacity of myomitokines to exert homeostatic compensatory mechanisms that protect against the insult. As such, a functional mitochondrial network is critical for preserving cardiac and skeletal muscle homeostasis (244), and impaired mitochondrial function results in the activation of catabolic pathways leading to muscle loss and weakness (2, 53). In this section, we describe the latest research on how FGF21, GDF15, and mtDNA in striated muscle is produced and what endocrine effects these compounds have outside striated muscle tissue.

Fibroblast Growth Factor 21

FGF21 is an atypical member of the FGF family because it lacks the heparin-binding domain and therefore can be released into the bloodstream to regulate lipid and glucose metabolism. The expression of FGF21 in striated muscle is almost undetectable under healthy conditions, and the circulating FGF21 is predominantly produced and released by the liver (245, 246). However, FGF21 and the expression of its co-receptors β-klotho, FGFR1, and FGFR4 are highly increased in the heart under stress and in catabolic conditions. Muscle-derived release of FGF21 occurs under physiological and pathological conditions, particularly the integrated stress response. Examples include physical exercise, starvation, ER stress, mitochondrial dysfunction, obesity, cardiac stress, mitochondrial myopathies, and during aging (247, 248). FGF21 exerts local autocrine/paracrine signaling effects, as well as endocrine interorgan crosstalk, aimed at restoring tissue homeostasis (33, 245, 246, 248-251). However, FGF21 displays a double-edged sword behavior,

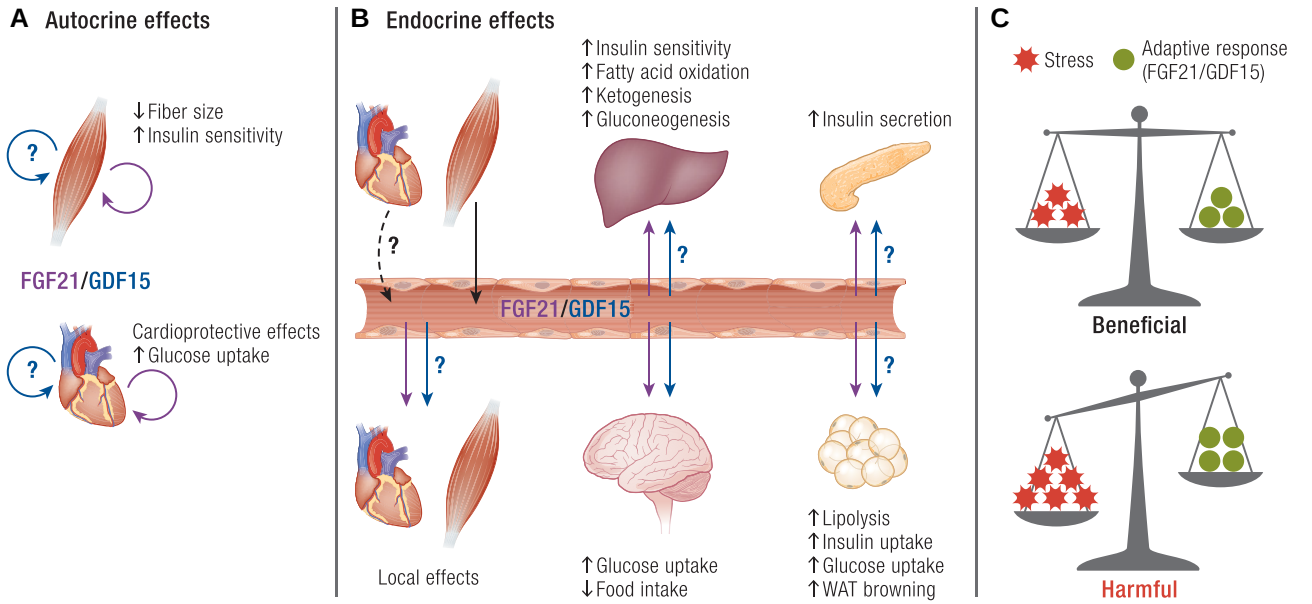


Figure 4. Endocrine communication of mitochondrial stress–derived FGF21 and GDF15 to maintain whole-body homeostasis and metabolism. A) Autocrine/paracrine effects of FGF21 and GDF15 include muscle fiber size reduction and increased insulin sensitivity in skeletal muscle, while in the heart, FGF21 and GDF15 induce glucose uptake and are associated with cardioprotective effects. B) When muscle-derived FGF21 and GDF15 are systemically released, they induce several effects in distant organs, such as white adipose tissue (WAT) browning. C) FGF21 and GDF15 respond to mitochondrial stress by inducing a mitohormetic dual-dose response. A low dose of stress stimuli promotes health and lifespan by activating local adaptive responses to increase stress resistance and systemic effects, improving whole-body metabolism. Conversely, higher stress stimuli can lead to adverse outcomes, suggesting a failure in the myomitokines capacity to exert homeostatic compensatory mechanisms that protect against the insult. Question marks indicate that it is still unknown how the peripheral GDF15 effects are mediated since the only GDF15 receptor identified so far is the GFRAL receptor localized in the hindbrain. The cardiac contribution of FGF21 and GDF15 to the general circulation has not been demonstrated to date.

establishing a fine line between its associated positive and negative outcomes. As such, both cardiac and skeletal muscles are a source but also a target for FGF21 (33, 246, 249).

Muscle-derived FGF21 finds its origin in mitochondrial stress. A dysbalance in skeletal muscle mitochondrial fusion and/or fission increased circulating FGF21 with negative dose-, time-, and context-dependent effects on survival (6). For example, deletion of the muscle-specific mitochondrial fusion protein OPA1 results in higher muscle and serum FGF21, systemic inflammation, accelerated aging and premature death (33). Conversely, muscle-specific mitochondrial fission protein DRP1 null mice have lower FGF21 levels than OPA1 knockout mice, no inflammatory cytokine production, and a normal lifespan (50). Rebalancing mitochondrial dynamics by the simultaneous ablation of OPA1 and DRP1 in muscle leads to a transient increase in circulating FGF21 and GDF15, and the mitigation of age-associated features that rescues the lethal phenotype of OPA1 (51).

Recent work from Lehtonen and colleagues, in Suomalainen's group, has dissected how FGF21 in skeletal muscle coordinates a stage-specific integrated stress response and metabolic remodeling progression from early to chronic response in mice and humans with a genetic mitochondrial disease (252). This integrated stress response is controlled by the anabolic mTOR pathway in skeletal muscle and can lead to the occurrence of ragged-red fibers (139).

Increased muscle-derived FGF21 has beneficial effects in diet-induced obesity and insulin resistance (76, 253), and overexpression of FGF21 in mice increases longevity (254). In skeletal muscle FGF21 is sufficient to activate muscle atrophy by activating the removal of damaged mitochondria through Bnip3-dependent mitophagy (245). Similarly, muscle-specific FGF21 knockout mice are protected from fasting-induced muscle loss by lower mitophagy and maintained protein synthesis rate (245). FGF21 in muscle enhances basal and insulin-stimulated glucose uptake in mouse and human myotubes via a currently unknown autocrine/paracrine mechanism (249, 255, 256).

A mild increase in FGF21 in the heart is required for physiological cardiac remodeling during pregnancy (257). FGF21 also displays a cardioprotective role against pathologic cardiac hypertrophy, myocardial ischemia, hypertensive heart disease, and atherosclerosis (248). FGF21 might prevent cardiomyopathic cardiac remodeling by regulating the mitochondrial integrated stress response through ERK1/2, ATF5, and c-Myc activation only under mild-to-moderate mitochondrial dysfunction, while during severe mitochondrial dysfunction FGF21 effects in the heart are dispensable (258).

Aerobic exercise in mice induced the cardiac expression of β-klotho, the co-receptor for FGF21 (259), and as such, the heart became more susceptible to FGF21. Indeed, aerobic exercise reduced diabetes-induced cardiac dysfunction in wild-type mice, but not in mice with dysfunctional FGF21 signaling (259). In particular, mitochondrial damage was reduced and increased activities of mitochondrial enzymes in hearts via the expression of the mitochondrial deacetylase SIRT3 by AMPK-evoked phosphorylation of FOXO3 (259). FGF21 further prevented lipotoxicity-induced mitochondrial dysfunction and oxidative stress by induction of the AMPK/FOXO3/SIRT3 signaling axis in human induced pluripotent stem cell–derived cardiomyocytes (259).

Chronic induction of the integrated stress response is not protective for skeletal muscle in the context of mitochondrial myopathy (139). FGF21 in mitochondrial myopathy is a circulating mediator of mitochondrial dysfunction from muscle to other organs, inducing weight loss and brain metabolic defects (252). Children with severe early lethal mitochondrial myopathy caused by mtDNA defects have higher FGF21 concentration (200-fold) than adults with mitochondrial myopathy (2- to 10-fold) (252).

As such, FGF21 is a specific serum biomarker of muscle-specific mitochondrial disorders caused by mtDNA defects (252, 260), subclinical atherosclerosis (261), and aging (33, 262). Overall, higher FGF21 concentrations are associated with worsening of overall health and reduced life expectancy in older people (263). A chronic increase of FGF21 circulating levels is associated with age-related diseases, such as obesity, cardiovascular disease, insulin resistance (through altered glucose uptake), type 2 diabetes (247), and cancer cachexia (264). In addition, high circulating FGF21 is associated with worsening health parameters and mortality in aging and during severe acute COVID-19 infection (265).

In conclusion, a mild or transient increase in circulating FGF21 results in adaptive responses to local and transient mitochondrial stress. A dramatic or chronic increase can under certain pathological conditions be detrimental for overall health. A healthy or unhealthy FGF21 outcome on aging and survival can also be explained by considering that the inflammatory response induction might synergize with FGF21-induced senescence. Future studies are required to understand the role of mitochondrial stress–derived FGF21 on whole-body physiology in various cardiac and skeletal muscle-related conditions. Further, how the positive effects of low-grade, acute FGF21 turn into a negative consequence for overall cell metabolism after chronic (high dose) exposure is currently unknown.

Growth Differentiation Factor 15

Growth differentiation factor 15 (GDF15) is a distant member of the transforming growth factor β (TGF-β) superfamily with pleiotropic functions (266). GDF15 expression in striated muscle increases with different stresses and stimuli, including mitochondrial dysfunction and the mitochondrial unfolding protein response and integrated stress response. GDF15 in striated muscle acts locally through autocrine/paracrine signaling or spreads from muscles to other organs in an endocrine fashion. As with FGF21, the outcome of GDF15 critically depends on the capacity of the myomitokines to overcome the stress condition and restore homeostasis. Therefore, skeletal muscle GDF15 induction can be associated with improved mitochondrial function, or it can be associated with chronic mitochondrial dysfunction (51, 267).

GDF15-treated C2C12 myoblasts show increased mitochondrial respiration and improved mitochondrial fatty acid oxidation (268), confirming the positive autocrine and paracrine effect of GDF15. However, it has been reported that the contamination of commercial recombinant GDF15 with low levels of TGF-β caused GDF15-dependent activation of the SMAD pathway (269). Metabolic phenotype improved after treating obese mice with GDF15, as genes involved in lipolysis, fatty acid mobilization, and oxidation, such as PGC-1α and adipose triglyceride lipase, increased (268). Muscle-derived GDF15 plays protective roles against diet-induced obesity and insulin resistance (268). Moreover, GDF15 displays cardioprotective effects in aging and age-related disorders (243). Mild mitochondrial uncoupling, specifically in skeletal muscle, results in increased muscle-derived FGF21 and GDF15, eliciting effective adaptive stress responses with beneficial effects on the systemic energy metabolism (270-272), and ultimately to an increased lifespan in mice (273). The beneficial systemic metabolic effects on metabolic flexibility and insulin sensitivity depend on the diurnal action of GDF15 (267), while FGF21 signaling is dispensable (274).

Cardiac release of GDF15 regulates liver growth hormone signaling and thus, body growth in pediatric heart disease (275). In addition, GDF15 has cardioprotective autocrine/paracrine effects in a model of ischemia/reperfusion injury (276, 277) and cardiac hypertrophy (278). Besides a direct link between GDF15 and mitochondrial metabolism, recent studies have highlighted the role of GDF15 in reducing appetite in the brain, leading to reduced food intake (279). The GFRAL receptor mediates GDF15 effects as an appetite inhibitor (reviewed in (266)).

Despite these positive effects, a chronic activation and higher concentration of circulating GDF15 has a negative effect on overall cell function. Serum concentrations of GDF15 increase with age (263, 280, 281) and predict all-cause mortality (282-285). Moreover, GDF15 is an established biomarker of cardiac hypertrophy, atherosclerosis, and heart failure (286). The increased serum GDF15 levels in age-related disorders such as diabetes, cardiovascular diseases, sarcopenia, and cancer, are probably due to an unsuccessful attempt to reestablish homeostasis and slow disease progression (243).

Gene expression and protein concentrations of GDF15 are elevated in serum and skeletal muscle from patients with intensive care unit–acquired weakness, suggesting an association between GDF15 and muscle wasting in the critically ill (287). GDF15 is involved in the activation of the ubiquitin-mediated protein degradation in skeletal muscle, potentially via suppressed microRNA (miR) expression of miR-1, miR-499, miR-181a, and increased atrogin 1 levels (287). MiR-1 inactivates circulating myostatin, a major suppressor of muscle protein synthesis, and a reduction in miR-1 contributes to lower myosin heavy chain expression (287). GDF15-induced suppression of these microRNAs sensitizes skeletal muscle to TGF-β signaling and primes muscle atrophy (287), suggesting a role for GDF15 in muscle wasting. Indeed, GDF15 muscle and serum levels inversely correlate with muscle mass and force in people with impairments in lower limb mobility (288), cardiometabolic (288) and lung diseases (289). Moreover, the overexpression of GDF15 in muscle is sufficient to induce muscle atrophy (289), likely by FoxO1 and SMAD3 activation (290). However, it has been reported that GDF15-dependent activation of the SMAD pathway might be caused by the contamination of commercial recombinant GDF15 with low levels of TGF-β (269).

The inhibition of GDF15 by targeting its receptor GFRAL in brainstem neurons of tumor-bearing mice identified a novel strategy to treat cancer cachexia (269). This approach preserves weight loss independently of food intake, white adipose tissue, muscle force and reverses tumor-induced muscle wasting by reducing the atrophy-related genes Atrogin1, Gadd45a, and Bnip3 (291).

The expression of the GFRAL receptor is limited to the area postrema and the nucleus of the solitary tract in the hindbrain

(292-295). It therefore remains unclear whether the effects of GDF15 on muscle catabolism are due to a direct action on skeletal muscle since the only GDF15 receptor identified so far is the GFRAL receptor. It has not been clarified if the direct contribution of mitochondrial stress–induced GDF15 is to cause whole-body adaptation via a loss of appetite, since most research to date has been performed on transgenic GDF15 mice or monitoring the effects of injections with supraphysiological concentrations of GDF15.

Mitochondrial DNA

Mitochondria not only share electrochemical information with the mitochondrial reticulum but can also share mtDNA through membrane contact points between adjacent mitochondria. However, mitochondrial stress can result in the release of mitochondrial damage–associated proteins (mtDAMPs) in the cytosol, extracellular matrix, and blood (296), and include several mitochondrial components such as mtDNA (297). This may be one way to share contents, for example subunits of the oxidative phosphorylation system, with other mitochondria. However, mtDNA was also recently suggested to both sense genotoxic stress as well as act as a signaling factor to enhance nuclear DNA repair (298). The release of cell-free mtDNA into the circulation following cellular injury initiates systemic pro-inflammatory processes and activation of the immune system (296). This may be related to the similarity of mtDNA to bacterial DNA, and subsequently, the immune system recognizes mtDNA and elicits a similar response pattern used to fight bacteria (299). The link between mtDNA and inflammatory response has been shown to be through the activation of polymorphonuclear neutrophils secretion by mtDNA/Toll-like receptor (TLR)-9 (296, 300) and NFk-B pathway activation (300). OPA1 inhibition in skeletal muscle causes muscle inflammation, mediated by mtDNA-TLR-9 recognition and activation of NF-kB activation inflammatory program (52). By these means, striated muscle mitochondrial dysfunction is sufficient to induce a local inflammatory response.

Notably, the release of cell-free mtDNA into the circulation has been observed following myocardial infarction (301) and cell hypoxia and reoxygenation (302) and is related to accelerated myocardial injury (301). Moreover, postoperative elevated circulating mtDNA following cardiac surgery (cardiopulmonary bypass) is associated with systemic inflammatory response, increased platelet activation (299), and increased risk of atrial fibrillation (303). Elevated plasma mtDNA levels in hospitalized critically ill patients are prospectively associated with an approximately 6-fold increased risk of mortality (304). Whereas monitoring cell-free circulating mtDNA is an important prognostic biomarker for patient survival following myocardial infarction and cardiac arrest (305). Cell-free circulating mtDNA may also be associated with the severity of atrial fibrillation and patients at risk for recurrence of atrial fibrillation after treatment (306). Circulating mtDNA levels were also correlated to the success of cardiac transplantation (307). Increased circulating mtDNA has also been shown during hypertension, and was associated with activation of innate immunity, via TLR-9 (308), possibly indicating an origin of vascular dysfunction and hypertension.

As both low-grade inflammation and the disruption of mitochondrial quality control pathways are key in many pathological conditions, including aging, the role of mtDNA in this crosstalk should be further explored. For instance, it is currently unknown whether extracellular mtDNA from exercise-induced muscle damage serves as a local attractor of immune cells. Similarly, the clinical relevance of mtDNA for cardiovascular diseases is currently not well explored.

General Discussion and Outstanding Questions

Mitochondrial communication can be studied at various levels, from cell to whole-body studies. How mitochondria communicate with other organelles and organs is likely best understood as an intricate system of interacting components. As such, to better understand mitochondrial communication in striated muscle, this should be assessed by focusing on various organ(elle)s in response to temporary stressors, such as physical (in)activity, infections, nutritional alterations, or mental stress. This requires a combined understanding of endocrinology and molecular signaling, where not only traditional hormones, or myokines, exert autocrine, paracrine, or endocrine effects.

Understanding Mitochondrial Communication Within Cells

To understand mitochondrial communication at the cellular level, signaling pathways coming from and going to the mitochondria, independent of phosphocreatine (PCr) redox balance or ATP energy levels, should be annotated. Metabolic flux and pathway analyses are popular tools to study metabolism. In general, intracellular metabolite fluxes can be measured using isotope labeling of substrates, after which isotopomer distribution in metabolites can be quantified (3, 24, 309). Future studies are required to perform subcellular location of mitochondrial signaling metabolites and/or molecules. More studies are required to identify the cargo content and transfer of mitochondrial-derived vesicles and elucidate their physiological role but will require improved markers and/or specific antibodies (109). Similarly, it is currently unknown how other intracellular communicators, such as various metabolites and growth factors, communicate with other organelles and other cells. This likely requires more sophisticated technology, for instance, performing metabolomics on subcellular fractionations and new imaging techniques (310). Importantly, the continuous advancement of microscopy imaging techniques, including FRET, fluorescence lifetime imaging, and 3D/immuno-electron microscopy already provide unprecedented detail of mitochondria structure and function in skeletal and cardiac muscle (96, 311). The use of optical nanoscopy for live imaging of mitochondria (39) and cristae dynamics during metabolic adaptation in striated muscle may provide the missing links for understanding of the regulation of cristae function, dynamic assembly of the respiratory complexes, and local (intracellular) communication.

Some of this state-of-the-art equipment requires the use of cultured cells. In the field of striated muscle, the current frequently used models for skeletal and cardiac muscle cells are C2C12 myoblasts/myotubes, HL1 cells, or primary cell lines such as neonatal cardiomyocytes or (human) skeletal muscle cells. More recently, induced pluripotent stem cells (iPSCs) or 3D scaffolds have been cultured as a model to understand

metabolism in striated muscle cultures (312). In that regard, it should be noted that currently none of these models have the same spatial organization of myofibrils, sarcomeres, and mitochondrial network as intact myofibers isolated from intact muscles (312). While these models are sufficient to study certain aspects of mitochondrial communication and metabolism, independent of involvement of extracellular signaling pathways—such as the involvement of systemic inflammation (313) or confounding influence of (mito)myokines (314)—these models can currently not recapitulate all intracellular aspects of communication between mitochondria and other organelles. Particularly, the use of antibiotics, such as streptomycin and doxycycline, in cell culture has been linked to a lower force-generating capacity in engineered muscle cells (315), altered gene expression (316), and mitochondrial function (24, 317, 318). This is due to the working mechanism of certain classes of antibiotics that target particularly circular (bacterial) DNA, including mitochondrial DNA. Clearly, studying mitochondrial-nuclear communication will be complicated by a selective inhibition of these classes of antibiotics (317). Similarly, transient doxycycline-induced knockout cell models likely exhibit various adaptations in mitochondrial communication, so that it remains difficult to interrogate cause and consequence (317).

Understanding Mitochondrial Endocrine Communication in Whole-Body Physiology

The continued use of (transgenic) model organisms to understand mitochondrial communication is vital. Over the last decades, model organisms such as transgenic fruit flies and mice, but also large animal models such as pigs, have provided a wealth of information about the endocrine effects of FGF21 and GDF15 exerted in other organs. In that regard, many tissue-specific knockout models for mitochondrial proteins often have unexpected systemic side effects. One such example is that circulating FGF21 levels were higher after muscle-specific OPA1 deletion, which has consequences for understanding the development of obesity (76). These side effects can blur the understanding of the function of the protein of interest. It is likely that many of the currently known muscle-specific knockout models for mitochondrial proteins have "off-target" effects in other organs, via mitomyokines. The use of vehicle-treated animals is a semi-optimal solution, as the combination of the altered expression of a gene of interest together with the downstream effects of mitochondrial dysfunction (or systemic off-target effects of tamoxifen/doxycycline) can aggravate the combined overall phenotype of the animal and makes the physiological interpretation from these models sometimes difficult (317). Knowledge of how mitochondrial dysfunction, overall ATP levels and energy production rates, and/or the (indirect) circulating effects of mitochondria-derived myomitokines can cause downstream alterations in gene/protein expression or cell function, therefore, is a prerequisite for the correct understanding of such in vivo models. Crossbreeding with different knockout models may provide valuable future information about this, but this in turn requires large amounts of funding, time, and expertise.

The continued development of novel isotope tracers for functional imaging using positron emission tomography (PET) (319) will be of importance for in vivo studies to investigate roles of interorgan communication of mitochondria in human physiology and during pathology. Noninvasive in vivo quantification of mitochondrial membrane potential using hybrid PET and magnetic resonance (MR)-imaging has been shown to have various clinical applications, for example, through assessing the response to therapy (319, 320). Moreover, this would further the study of endocrine responses to mitochondrial stress.

Mitochondria in immune cells are increasingly recognized as being at the center of innate immune signaling. Increased focus is warranted on mitochondrial functions in target organs, such as striated muscle, during an immune response (313, 320, 321) as well as their contribution to a general alarm response (alarmins) when mitochondrial components, such as mtDNA, are released to the systemic circulation.

Muscle-derived exosomes (238) are relatively recently described forms of interorgan communication. These exosomes can derive from mitochondria, but also from other cellular organelles. Extracellular vesicles contain peptides and nucleic acids, which can be hydrophobic or have a very short half-life time in the blood. As such, exosomes can facilitate the exchange of peptides, microRNA, mRNA, and mtDNA between cells and tissues. That circulatory extracellular vesicle content increases in an intensity-dependent manner after endurance exercise suggests that these exosomes are important new features in exercise endocrinology that deserve further study (238). What these muscle-derived exosomes exactly contain, at what time point, and under which experimental or environmental conditions is work that will have to be conducted in the future.

Medical Horizons, Therapeutic Potential, and Translational Aspects

Harnessing mitochondrial signaling may provide ample opportunities for therapeutic interventions, although not without challenges. Mitochondrial dynamics and mitochondrial quality control are closely interconnected; therefore, interventions acting on one specific pathway inevitably impinge on the activation/inhibition of the other components. This critical interplay for the mitochondrial network function, should be considered in studies aiming to develop therapeutic strategies to maintain mitochondrial homeostasis in muscle diseases. Moreover, this field of research is further complicated by the observation that the communication of mitochondria with other organelles is interrelated, and therefore difficult to target individually. For instance, interfering with the gene expression at the mitochondrial DNA level using certain classes of antibiotics not only reduces mitochondrial respiration in the heart, but also affects mitochondrial morphology, and ultimately, cardiac contractile function (24). Similarly, muscle-derived GDF15 protects against diet-induced obesity and insulin resistance via a reduced appetite (321), but this also contributes to an altered substrate handling and metabolic flexibility of skeletal muscle mitochondria. These inter-relationships clearly complicate the simple translational application of circulating compounds, such as myomitokines, FGF21, and GDF15 and/or extracellular vesicles, for medical interventions or exercise mimetics of mitochondrial myopathies, metabolic and cardiovascular diseases, and sarcopenia.

A similar potential opportunity for mitochondrial communication for therapy lies in the application of mitochondrial-derived vesicles. In a proof-of-concept study using cell culture, mitochondrial-derived vesicles obtained from a hemorrhagic shock rat model inhibited hypoxia-induced cardiomyocyte apoptosis (322). At least in theory, this would suggest that

cargo from mitochondrial-derived vesicles (whether or not therapeutically administered) could improve cell fate during disease conditions. Full mitochondrial transplantation via extracellular vesicles is an upcoming therapeutic possibility (323, 324) but to fully understand the underlying process, and benefit from its full potential to improve cardiac metabolic function under various conditions, requires more fundamental and translational research (325). Thus, our deeper understanding of striated muscle physiology in health and disease requires increased knowledge of the multifaceted roles of the mitochondria.

The use of circulating cell-free mitochondrial DNA can provide new avenues for understanding physiological stress. Outstanding questions include whether circulating cell-free mtDNA can be transported in vesicles, bound to proteins, or unbound in an intact or fragmented form (326), and whether the difference in transportation affects the signal transduction pathways. This is likely the case, as cell-free mtDNA packed inside mitochondria that are transported in extracellular vesicles is not in direct contact with receptors on immune cells, and therefore does not possess the same means of mitochondrial communication as unbound fragments of cell-free mtDNA. More experimental standardization on how to isolate, measure, and interpret cell-free mtDNA is required, to fully understand the biological consequences of circulating cell-free mtDNA (326).

Concluding Remarks

In summary, mitochondrial communication is driven by various inputs that dynamically modulate the biochemical, genetic, ultrastructural, and physiological properties of mitochondria (49). Novel roles of striated muscle mitochondria in interorganelle and systemic signaling are increasingly being discovered. Recent advances in mitochondrial communication research have only partly elucidated the role of mitochondria in optimizing overall cell function via improvements in the mitochondrial reticulum and communication with various organelles such as the nucleus, peroxisomes, and SR/ER, and also glycogen and lipid species. Mitochondrial endocrinology highlights how myomitokines such as FGF21, GDF15, mtDNA, and mitochondrial-derived vesicles can alter whole-body physiology and metabolism. In whole-body physiology, the whole is more than the sum of its parts. It is therefore likely that combinations of different myo(mito)kines exert more than additive effects on interorgan communication, possibly even by confounding other factors such as the immune system. As our knowledge of mitochondrial endocrinology advances, we have demonstrated the importance of integrative physiology and interorgan mitochondrial communication. We envision that the complete extent of how mitochondrial signaling mediates endocrine activity during physiology and disease will become clearer in the near future.

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References

1. Glancy B, Hartnell LM, Malide D, *et al.* Mitochondrial reticulum for cellular energy distribution in muscle. *Nature*. 2015;523-(7562):617-620.
2. Romanello V, Sandri M. Mitochondrial biogenesis and fragmentation as regulators of protein degradation in striated muscles. *J Mol Cell Cardiol*. 2013;55:64-72.
3. Smith RL, Soeters MR, Wüst RCI, Houtkooper RH. Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. *Endocr Rev*. 2018;39(4):489-517.
4. Maack C, O'Rourke B. Excitation-contraction coupling and mitochondrial energetics. *Basic Res Cardiol*. 2007;102(5):369-392.
5. Chakrabarty RP, Chandel NS. Beyond ATP, new roles of mitochondria. *Biochem (Lond)*. 2022;44(4):2-8.
6. Romanello V. The interplay between mitochondrial morphology and myomitokines in aging sarcopenia. *Int J Mol Sci*. 2020;22(1):E91.
7. Friedman JR, Nunnari J. Mitochondrial form and function. *Nature*. 2014;505(7483):335-343.
8. Vincent AE, Ng YS, White K, *et al.* The spectrum of mitochondrial ultrastructural defects in mitochondrial myopathy. *Sci Rep*. 2016;6(1):30610.
9. Eisner V, Picard M, Hajnóczky G. Mitochondrial dynamics in adaptive and maladaptive cellular stress responses. *Nat Cell Biol*. 2018;20(7):755-765.
10. Bakeeva LE, YuS C, Skulachev VP. Mitochondrial framework (reticulum mitochondriale) in rat diaphragm muscle. *Biochim Biophys Acta*. 1978;501(3):349-369.
11. Amchenkova AA, Bakeeva LE, Chentsov YS, Skulachev VP, Zorov DB. Coupling membranes as energy-transmitting cables. I. Filamentous mitochondria in fibroblasts and mitochondrial clusters in cardiomyocytes. *J Cell Biol*. 1988;107(2):481-495.
12. Kurz FT, Derungs T, Aon MA, O'Rourke B, Armoundas AA. Mitochondrial networks in cardiac myocytes reveal dynamic coupling behavior. *Biophys J*. 2015;108(8):1922-1933.
13. Chung DJ, Madison GP, Aponte AM, *et al.* Metabolic design in a mammalian model of extreme metabolism, the North American least shrew (Cryptotis parva). *J Physiol*. 2022;600(3):547-567.
14. Schon EA, DiMauro S, Hirano M. Human mitochondrial DNA: roles of inherited and somatic mutations. *Nat Rev Genet*. 2012;13(12):878-890.
15. Glancy B, Balaban RS. Energy metabolism design of the striated muscle cell. *Physiol Rev*. 2021;101(4):1561-1607.
16. Aon MA, Cortassa S, Marbán E, O'Rourke B. Synchronized whole cell oscillations in mitochondrial metabolism triggered by a local release of reactive oxygen species in cardiac myocytes. *J Biol Chem*. 2003;278(45):44735-44744.
17. Aon MA, Cortassa S, O'Rourke B. The fundamental organization of cardiac mitochondria as a network of coupled oscillators. *Biophys J*. 2006;91(11):4317-4327.
18. Cortassa S, Aon MA, Winslow RL, O'Rourke B. A mitochondrial oscillator dependent on reactive oxygen species. *Biophys J*. 2004;87(3):2060-2073.
19. Glancy B, Hartnell LM, Combs CA, *et al.* Power grid protection of the muscle mitochondrial reticulum. *Cell Rep*. 2017;19(3):487-496.
20. Kurz FT, Aon MA, O'Rourke B, Armoundas AA. Spatio-temporal oscillations of individual mitochondria in cardiac myocytes reveal modulation of synchronized mitochondrial clusters. *Proc Natl Acad Sci U S A*. 2010;107(32):14315-14320.
21. Bouda E, Stapon A, Garcia-Diaz M. Mechanisms of mammalian mitochondrial transcription. *Protein Sci*. 2019;28(9):1594-1605.
22. Picard M, McManus MJ, Csordás G, *et al.* Trans-mitochondrial coordination of cristae at regulated membrane junctions. *Nat Commun*. 2015;6(1):6259.
23. Couvillion MT, Soto IC, Shipkovenska G, Churchman LS. Synchronized mitochondrial and cytosolic translation programs. *Nature*. 2016;533(7604):499-503.
24. Wüst RCI, Coolen BF, Held NM, *et al.* The antibiotic doxycycline impairs cardiac mitochondrial and Contractile function. *Int J Mol Sci*. 2021;22(8):4100.

25. Balaban RS. The role of Ca(2+) signaling in the coordination of mitochondrial ATP production with cardiac work. *Biochim Biophys Acta*. 2009;1787(11):1334-1341.
26. Picard M, Gentil BJ, McManus MJ, *et al.* Acute exercise remodels mitochondrial membrane interactions in mouse skeletal muscle. *J Appl Physiol*. 2013;115(10):1562-1571.
27. Wüst RCI, de Vries HJ, Wintjes LT, Rodenburg RJ, Niessen HWM, Stienen GJM. Mitochondrial complex I dysfunction and altered NAD(P)H kinetics in rat myocardium in cardiac right ventricular hypertrophy and failure. *Cardiovasc Res*. 2016;111(4):362-372.
28. Song M, Dorn GW. Mitoconfusion: noncanonical functioning of dynamism factors in static mitochondria of the heart. *Cell Metab*. 2015;21(2):195-205.
29. Suen D-F, Norris KL, Youle RJ. Mitochondrial dynamics and apoptosis. *Genes Dev*. 2008;22(12):1577-1590.
30. Twig G, Elorza A, Molina AJA, *et al.* Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J*. 2008;27(2):433-446.
31. Tsushima K, Bugger H, Wende AR, *et al.* Mitochondrial reactive oxygen Species in lipotoxic hearts induce post-translational modifications of AKAP121, DRP1, and OPA1 that promote mitochondrial fission. *Circ Res*. 2018;122(1):58-73.
32. Leduc-Gaudet J-P, Hussain SNA, Barreiro E, Gouspillou G. Mitochondrial dynamics and mitophagy in skeletal muscle health and aging. *Int J Mol Sci*. 2021;22(15):8179.
33. Tezze C, Romanello V, Desbats MA, *et al.* Age-Associated loss of OPA1 in muscle impacts muscle mass, metabolic homeostasis, systemic inflammation, and epithelial senescence. *Cell Metab*. 2017;25(6):1374-1389.e6.
34. Huang X, Sun L, Ji S, *et al.* Kissing and nanotunneling mediate intermitochondrial communication in the heart. *Proc Natl Acad Sci U S A*. 2013;110(8):2846-2851.
35. Lavorato M, Iyer V. R, Dewight W, *et al.* Increased mitochondrial nanotunneling activity, induced by calcium imbalance, affects intermitochondrial matrix exchanges. *Proc Natl Acad Sci U S A*. 2017;114(5):E849-E858.
36. Picard M, White K, Turnbull DM. Mitochondrial morphology, topology, and membrane interactions in skeletal muscle: a quantitative three-dimensional electron microscopy study. *J Appl Physiol*. 2013;114(2):161-171.
37. Fujioka H, Tandler B, Haldar SM, Jain MK, Hoppel CL. String mitochondria in mouse soleus muscle. *Microsc Res Tech*. 2013;76(3):237-241.
38. Cao Y, Xu C, Ye J, *et al.* Miro2 regulates inter-mitochondrial communication in the heart and protects against TAC-induced cardiac dysfunction. *Circ Res*. 2019;125(8):728-743.
39. Opstad IS, Godtliebsen G, Ahluwalia BS, *et al.* Mitochondrial dynamics and quantification of mitochondria-derived vesicles in cardiomyoblasts using structured illumination microscopy. *J Biophotonics*. 2022;15(2):e202100305.
40. Vincent AE, Turnbull DM, Eisner V, Hajnóczky G, Picard M. Mitochondrial nanotunnels. *Trends Cell Biol*. 2017;27(11):787-799.
41. Lavorato M, Formenti F, Franzini-Armstrong C. The structural basis for intermitochondrial communications is fundamentally different in cardiac and skeletal muscle. *Exp Physiol*. 2020;105(4):606-612.
42. Vincent AE, White K, Davey T, *et al.* Quantitative 3D mapping of the human skeletal muscle mitochondrial network. *Cell Rep*. 2019;26(4):996-1009.e4.
43. Lavorato M, Huang T-Q, Iyer VR, *et al.* Dyad content is reduced in cardiac myocytes of mice with impaired calmodulin regulation of RyR2. *J Muscle Res Cell Motil*. 2015;36(2):205-214.
44. Wang C, Du W, Su QP, *et al.* Dynamic tubulation of mitochondria drives mitochondrial network formation. *Cell Res*. 2015;25(10):1108-1120.
45. Cadete VJJ, Deschênes S, Cuillerier A, *et al.* Formation of mitochondrial-derived vesicles is an active and physiologically relevant mitochondrial quality control process in the cardiac system. *J. Physiol*. 2016;594(18):5343-5362.
46. Colina-Tenorio L, Horten P, Pfanner N, Rampelt H. Shaping the mitochondrial inner membrane in health and disease. *J Intern Med*. 2020;287(6):645-664.
47. Liu X, Weaver D, Shirihai O, Hajnóczky G. Mitochondrial 'kiss-and-run': interplay between mitochondrial motility and fusion-fission dynamics. *EMBO J*. 2009;28(20):3074-3089.
48. Chen H, Vermulst M, Wang YE, *et al.* Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. *Cell*. 2010;141(2):280-289.
49. Picard M, Shirihai OS. Mitochondrial signal transduction. *Cell Metab*. 2022;34(11):1620-1653.
50. Favaro G, Romanello V, Varanita T, *et al.* DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. *Nat Commun*. 2019;10(1):2576.
51. Romanello V, Scalabrin M, Albiero M, Blaauw B, Scorrano L, Sandri M. Inhibition of the fission machinery mitigates OPA1 impairment in adult skeletal muscles. *Cells*. 2019;8(6):E597.
52. Rodríguez-Nuevo A, Díaz-Ramos A, Noguera E, *et al.* Mitochondrial DNA and TLR9 drive muscle inflammation upon Opa1 deficiency. *EMBO J*. 2018;37(10):e96553.
53. Romanello V, Sandri M. The connection between the dynamic remodeling of the mitochondrial network and the regulation of muscle mass. *Cell Mol Life Sci*. 2021;78(4):1305-1328.
54. Chen H, Chomyn A, Chan DC. Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *J Biol Chem*. 2005;280(28):26185-26192.
55. Frezza C, Cipolat S, Martins de Brito O, *et al.* OPA1 Controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell*. 2006;126(1):177-189.
56. Disatnik M-H, Ferreira JCB, Campos JC, *et al.* Acute inhibition of excessive mitochondrial fission after myocardial infarction prevents long-term cardiac dysfunction. *J Am Heart Assoc*. 2013;2(5):e000461.
57. Giacomello M, Pyakurel A, Glytsou C, Scorrano L. The cell biology of mitochondrial membrane dynamics. *Nat Rev Mol Cell Biol*. 2020;21(4):204-224.
58. Cogliati S, Enriquez JA, Scorrano L. Mitochondrial cristae: where beauty meets functionality. *Trends Biochem Sci*. 2016;41(3):261-273.
59. McBride H, Soubannier V. Mitochondrial function: OMA1 and OPA1, the grandmasters of mitochondrial health. *Curr Biol*. 2010;20(6):R274-R276.
60. Eisner V, Cupo RR, Gao E, *et al.* Mitochondrial fusion dynamics is robust in the heart and depends on calcium oscillations and contractile activity. *Proc Natl Acad Sci U S A*. 2017;114(5):E859-E868.
61. Picard M, Turnbull DM. Linking the metabolic state and mitochondrial DNA in chronic disease, health, and aging. *Diabetes*. 2013;62(3):672-678.
62. Spiegel R, Saada A, Flannery PJ, *et al.* Fatal infantile mitochondrial encephalomyopathy, hypertrophic cardiomyopathy and optic atrophy associated with a homozygous OPA1 mutation. *J Med Genet*. 2016;53(2):127-131.
63. Boardman NT, Pedersen TM, Rossvoll L, Hafstad AD, Aasum E. Diet-induced obese mouse hearts tolerate an acute high-fatty acid exposure that also increases ischemic tolerance. *Am. J. Physiol. Heart Circ. Physiol*. 2020;319(3):H682-H693.
64. Makrecka-Kuka M, Liepinsh E, Murray AJ, *et al.* Altered mitochondrial metabolism in the insulin-resistant heart. *Acta Physiol. Oxf. Engl*. 2020;228:e13430.
65. Boudina S, Sena S, Theobald H, *et al.* Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. *Diabetes*. 2007;56(10):2457-2466.
66. Ding M, Liu C, Shi R, *et al.* Mitochondrial fusion promoter restores mitochondrial dynamics balance and ameliorates diabetic

cardiomyopathy in an optic atrophy 1-dependent way. *Acta Physiol. Oxf. Engl.* 2020;229:e13428.
67. Wang D, Wang J, Bonamy GMC, *et al.* A small molecule promotes mitochondrial fusion in mammalian cells. *Angew. Chem. Int. Ed Engl.* 2012;51(37):9302-9305.
68. Varanita T, Soriano ME, Romanello V, *et al.* The OPA1-dependent mitochondrial cristae remodeling pathway controls atrophic, apoptotic, and ischemic tissue damage. *Cell Metab.* 2015;21(6):834-844.
69. Cogliati S, Frezza C, Soriano ME, *et al.* Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell.* 2013;155(1):160-171.
70. Zhou J, Chong SY, Lim A, *et al.* Changes in macroautophagy, chaperone-mediated autophagy, and mitochondrial metabolism in murine skeletal and cardiac muscle during aging. *Aging.* 2017;9(2):583-599.
71. Ibebunjo C, Chick JM, Kendall T, *et al.* Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the neuromuscular junction driving rat sarcopenia. *Mol Cell Biol.* 2013;33(2):194-212.
72. Romanello V, Guadagnin E, Gomes L, *et al.* Mitochondrial fission and remodelling contributes to muscle atrophy. *EMBO J.* 2010;29(10):1774-1785.
73. Dulac M, Leduc-Gaudet J-P, Reynaud O, *et al.* Drp1 knockdown induces severe muscle atrophy and remodelling, mitochondrial dysfunction, autophagy impairment and denervation. *J Physiol.* 2020;598(17):3691-3710.
74. Zorzano A, Liesa M, Palacín M. Role of mitochondrial dynamics proteins in the pathophysiology of obesity and type 2 diabetes. *Int J Biochem Cell Biol.* 2009;41(10):1846-1854.
75. Heinonen I, Sorop O, van Dalen BM, *et al.* Cellular, mitochondrial and molecular alterations associate with early left ventricular diastolic dysfunction in a porcine model of diabetic metabolic derangement. *Sci Rep.* 2020;10(1):13173.
76. Pereira RO, Tadinada SM, Zasadny FM, *et al.* OPA1 Deficiency promotes secretion of FGF21 from muscle that prevents obesity and insulin resistance. *EMBO J.* 2017;36(14):2126-2145.
77. Dulac M, Leduc-Gaudet J-P, Cefis M, *et al.* Regulation of muscle and mitochondrial health by the mitochondrial fission protein Drp1 in aged mice. *J Physiol.* 2021;599(17):4045-4063.
78. Chen Y, Dorn GW. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science.* 2013;340(6131):471-475.
79. Song M, Mihara K, Chen Y, Scorrano L, Dorn GW. Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts. *Cell Metab.* 2015;21(2):273-286.
80. Patten DA, Wong J, Khacho M, *et al.* OPA1-dependent cristae modulation is essential for cellular adaptation to metabolic demand. *EMBO J.* 2014;33(22):2676-2691.
81. Baker N, Patel J, Khacho M. Linking mitochondrial dynamics, cristae remodeling and supercomplex formation: how mitochondrial structure can regulate bioenergetics. *Mitochondrion.* 2019;49:259-268.
82. Stephan T, Brüser C, Deckers M, *et al.* MICOS assembly controls mitochondrial inner membrane remodeling and crista junction redistribution to mediate cristae formation. *EMBO J.* 2020;39(14):e104105.
83. Eydt K, Davies KM, Behrendt C, Wittig I, Reichert AS. Cristae architecture is determined by an interplay of the MICOS complex and the F1FO ATP synthase via Mic27 and Mic10. *Microb Cell Graz Austria.* 2017;4(8):259-272.
84. Harner M, Körner C, Walther D, *et al.* The mitochondrial contact site complex, a determinant of mitochondrial architecture. *EMBO J.* 2011;30(21):4356-4370.
85. Gottschalk B, Klec C, Leitinger G, *et al.* MICU1 controls cristae junction and spatially anchors mitochondrial Ca2 + uniporter complex. *Nat Commun.* 2019;10(1):3732.
86. Mukherjee I, Ghosh M, Meinecke M. MICOS and the mitochondrial inner membrane morphology—when things get out of shape. *FEBS Lett.* 2021;595(8):1159-1183.
87. Tsai P-I, Lin C-H, Hsieh C-H, *et al.* PINK1 phosphorylates MIC60/mitofilin to control structural plasticity of mitochondrial crista junctions. *Mol Cell.* 2018;69(5):744-756.e6.
88. Wang L-J, Hsu T, Lin H-L, Fu C-Y. Drosophila MICOS knockdown impairs mitochondrial structure and function and promotes mitophagy in muscle tissue. *Biol Open.* 2020;9:bio054262.
89. Kojima R, Kakimoto Y, Furuta S, *et al.* Maintenance of cardiolipin and crista structure requires cooperative functions of mitochondrial dynamics and phospholipid transport. *Cell Rep.* 2019;26(3):518-528.e6.
90. Pacher P, Hajnóczky G. Propagation of the apoptotic signal by mitochondrial waves. *EMBO J.* 2001;20(15):4107-4121.
91. Scorrano L, Ashiya M, Buttle K, *et al.* A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. *Dev Cell.* 2002;2(1):55-67.
92. Glytsou C, Calvo E, Cogliati S, *et al.* Optic atrophy 1 is epistatic to the core MICOS component MIC60 in mitochondrial cristae shape control. *Cell Rep.* 2016;17(11):3024-3034.
93. Nielsen J, Gejl KD, Hey-Mogensen M, *et al.* Plasticity in mitochondrial cristae density allows metabolic capacity modulation in human skeletal muscle. *J Physiol.* 2017;595(9):2839-2847.
94. Broskey NT, Boss A, Fares E-J, *et al.* Exercise efficiency relates with mitochondrial content and function in older adults. *Physiol Rep.* 2015;3(6):e12418.
95. Greggio C, Jha P, Kulkarni SS, *et al.* Enhanced respiratory chain supercomplex formation in response to exercise in human skeletal muscle. *Cell Metab.* 2017;25(2):301-311.
96. Glancy B. Visualizing mitochondrial form and function within the cell. *Trends Mol Med.* 2020;26(1):58-70.
97. Wolf DM, Segawa M, Kondadi AK, *et al.* Individual cristae within the same mitochondrion display different membrane potentials and are functionally independent. *EMBO J.* 2019;38(22):e101056.
98. Hackenbrock CR. Chemical and physical fixation of isolated mitochondria in low-energy and high-energy states. *Proc Natl Acad Sci U S A.* 1968;61(2):598-605.
99. Brandt T, Mourier A, Tain LS, Partridge L, Larsson N-G, Kühlbrandt W. Changes of mitochondrial ultrastructure and function during ageing in mice and drosophila. *eLife.* 2017;6:e24662.
100. Bertero E, Kutschka I, Maack C, Dudek J. Cardiolipin remodeling in Barth syndrome and other hereditary cardiomyopathies. *Biochim Biophys Acta Mol Basis Dis.* 2020;1866(8):165803.
101. Hwang H, Bowen BP, Lefort N, *et al.* Proteomics analysis of human skeletal muscle reveals novel abnormalities in obesity and type 2 diabetes. *Diabetes.* 2010;59(1):33-42.
102. Kondadi AK, Anand R, Reichert AS. Cristae membrane dynamics—a paradigm change. *Trends Cell Biol.* 2020;30(12):923-936.
103. König T, Nolte H, Aaltonen MJ, *et al.* MIROs and DRP1 drive mitochondrial-derived vesicle biogenesis and promote quality control. *Nat Cell Biol.* 2021;23(12):1271-1286.
104. Soubannier V, McLelland G-L, Zunino R, *et al.* A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. *Curr Biol.* 2012;22(2):135-141.
105. Neuspiel M, Schauss AC, Braschi E, *et al.* Cargo-selected transport from the mitochondria to peroxisomes is mediated by vesicular carriers. *Curr Biol.* 2008;18(2):102-108.
106. Sugiura A, McLelland G-L, Fon EA, McBride HM. A new pathway for mitochondrial quality control: mitochondrial-derived vesicles. *EMBO J.* 2014;33(19):2142-2156.
107. Sugiura A, Mattie S, Prudent J, McBride HM. Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. *Nature.* 2017;542(7640):251-254.
108. Vasam G, Nadeau R, Cadete VJJ, Lavallée-Adam M, Menzies KJ, Burelle Y. Proteomics characterization of mitochondrial-derived vesicles under oxidative stress. *FASEB J.* 2021;35:e21278.

109. Peng T, Xie Y, Sheng H, Wang C, Lian Y, Xie N. Mitochondrial-derived vesicles: gatekeepers of mitochondrial response to oxidative stress. *Free Radic Biol Med*. 2022;188:185-193.
110. Amari L, Germain M. Mitochondrial extracellular vesicles—origins and roles. *Front Mol Neurosci*. 2021;14:767219.
111. Cadete VJJ, Vasam G, Menzies KJ, Burelle Y. Mitochondrial quality control in the cardiac system: an integrative view. *Biochim Biophys Acta Mol Basis Dis*. 2019;1865(4):782-796.
112. Stahl PD, Raposo G. Exosomes and extracellular vesicles: the path forward. *Essays Biochem*. 2018;62(2):119-124.
113. Picca A, Beli R, Calvani R, *et al.* Older adults with physical frailty and sarcopenia show increased levels of circulating small extracellular vesicles with a specific mitochondrial signature. *Cells*. 2020;9(4):973.
114. Joshi AU, Minhas PS, Liddelow SA, *et al.* Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. *Nat Neurosci*. 2019;22(10):1635-1648.
115. Phinney DG, Di Giuseppe M, Njah J, *et al.* Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat Commun*. 2015;6(1):8472.
116. Todkar K, Chikhi L, Desjardins V, *et al.* Selective packaging of mitochondrial proteins into extracellular vesicles prevents the release of mitochondrial DAMPs. *Nat Commun*. 2021;12(1):1971.
117. Spees JL, Olson SD, Whitney MJ, Prockop DJ. Mitochondrial transfer between cells can rescue aerobic respiration. *Proc Natl Acad Sci U S A*. 2006;103(5):1283-1288.
118. McLelland G-L, Lee SA, McBride HM, Fon EA. Syntaxin-17 delivers PINK1/Parkin-dependent mitochondrial vesicles to the endolysosomal system. *J Cell Biol*. 2016;214(3):275-291.
119. Matheoud D, Cannon T, Voisin A, *et al.* Intestinal infection triggers Parkinson's disease-like symptoms in Pink1-/- mice. *Nature*. 2019;571(7766):565-569.
120. Matheoud D, Sugiura A, Bellemare-Pelletier A, *et al.* Parkinson's disease-related proteins PINK1 and Parkin repress mitochondrial antigen presentation. *Cell*. 2016;166(2):314-327.
121. Vendelin M, Eimre Margus, Seppet Evelin, *et al.* Intracellular diffusion of adenosine phosphates is locally restricted in cardiac muscle. *Mol Cell Biochem*. 2004;256–257(1/2):229-241.
122. Siegel MP, Kruse Shane E., Percival Justin M., *et al.* Mitochondrial targeted peptide rapidly improves mitochondrial energetics and skeletal muscle performance in aged mice. *Aging Cell*. 2013;12(5):763-771.
123. Jannig PR, Dumesic PA, Spiegelman BM, Ruas JL. Snapshot: regulation and biology of PGC-1α. *Cell*. 2022;185(8):1444-1444.e1.
124. Lira VA, Benton CR, Yan Z, Bonen A. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. *Am J Physiol Endocrinol Metab*. 2010;299(2):E145-E161.
125. Gineste C, Hernandez A, Ivarsson N, *et al.* Cyclophilin D, a target for counteracting skeletal muscle dysfunction in mitochondrial myopathy. *Hum Mol Genet*. 2015;24(23):6580-6587.
126. Wredenberg A, Wibom Rolf, Wilhelmsson Hans, *et al.* Increased mitochondrial mass in mitochondrial myopathy mice. *Proc Natl Acad Sci U S A*. 2002;99(23):15066-15071.
127. Lu X, Thai PN, Lu S, Pu J, Bers DM. Intrafibrillar and perinuclear mitochondrial heterogeneity in adult cardiac myocytes. *J Mol Cell Cardiol*. 2019;136:72-84.
128. Albus CA, Berlinguer-Palmini R, Hewison C, *et al.* Mitochondrial translation occurs preferentially in the peri-nuclear mitochondrial network of cultured human cells. *Biology (Basel)*. 2021;10(10):1050.
129. Jenkins EC, Chattopadhyay M, Germain D. Folding mitochondrial-mediated cytosolic proteostasis into the mitochondrial unfolded protein response. *Front Cell Dev Biol*. 2021;9:715923.
130. Wang YT, Lim Y, McCall MN, *et al.* Cardioprotection by the mitochondrial unfolded protein response requires ATF5. *Am J Physiol Heart Circ Physiol*. 2019;317(2):H472-H478.
131. Smyrnias I, Gray SP., Okonko DO., *et al.* Cardioprotective effect of the mitochondrial unfolded protein response during chronic pressure overload. *J Am Coll Cardiol*. 2019;73(14):1795-1806.
132. Zhang B, Tan Y, Zhang Z, *et al.* Novel PGC-1α/ATF5 axis partly activates UPRmt and mediates cardioprotective role of tetrahydrocurcumin in pathological cardiac hypertrophy. *Oxid Med Cell Longev*. 2020;2020:9187065.
133. Lee H, Ha TY, Jung CH, *et al.* Mitochondrial dysfunction in skeletal muscle contributes to the development of acute insulin resistance in mice. *J Cachexia Sarcopenia Muscle*. 2021;12(6):1925-1939.
134. Cordeiro AV, Peruca GF, Braga RR, *et al.* High-intensity exercise training induces mitonuclear imbalance and activates the mitochondrial unfolded protein response in the skeletal muscle of aged mice. *GeroScience*. 2021;43(3):1513-1518.
135. Pirinen E, Cantó C, Jo YS, *et al.* Pharmacological inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. *Cell Metab*. 2014;19(6):1034-1041.
136. van de Weijer T, Phielix E, Bilet L, *et al.* Evidence for a direct effect of the NAD+ precursor acipimox on muscle mitochondrial function in humans. *Diabetes*. 2015;64(4):1193-1201.
137. Kaspar S, Oertlin C, Szczepanowska K, *et al.* Adaptation to mitochondrial stress requires CHOP-directed tuning of ISR. *Sci Adv*. 2021;7(22):eabf0971.
138. Bárcena C, Mayoral P, Quirós PM. Mitohormesis, an antiaging paradigm. *Int Rev Cell Mol Biol*. 2018;340:35-77.
139. Khan NA, Nikkanen J, Yatsuga S, *et al.* mTORC1 regulates mitochondrial integrated stress response and mitochondrial myopathy progression. *Cell Metab*. 2017;26(2):419-428.e5.
140. Picard M, Jung Boris, Liang Feng, *et al.* Mitochondrial dysfunction and lipid accumulation in the human diaphragm during mechanical ventilation. *Am J Respir Crit Care Med*. 2012;186-(11):1140-1149.
141. Vigh-Larsen JF, Ørtenblad N, Emil Andersen O, *et al.* Fibre type- and localisation-specific muscle glycogen utilisation during repeated high-intensity intermittent exercise. *J Physiol*. 2022;600-(21):4713-4730.
142. Nielsen J, Dubillot P, Stausholm M-LH, Ørtenblad N. Specific ATPases drive compartmentalized glycogen utilization in rat skeletal muscle. *J Gen Physiol*. 2022;154(9):e202113071.
143. Nielsen J, Mogensen M, Vind BF, *et al.* Increased subsarcolemmal lipids in type 2 diabetes: effect of training on localization of lipids, mitochondria, and glycogen in sedentary human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2010;298(3):E706-E713.
144. Zhang Y, Taufalele Paul V, Cochran Jesse D, *et al.* Mitochondrial pyruvate carriers are required for myocardial stress adaptation. *Nat Metab*. 2020;2(11):1248-1264.
145. Kanungo S, Wells K, Tribett T, El-Gharbawy A. Glycogen metabolism and glycogen storage disorders. *Ann Transl Med*. 2018;6-(24):474.
146. Rossi A, Ruoppolo M, Formisano P, *et al.* Insulin-resistance in glycogen storage disease type Ia: linking carbohydrates and mitochondria? *J Inherit Metab Dis*. 2018;41(6):985-995.
147. Glatz JFC, Nabben M, Luiken JJFP. CD36 (SR-B2) as master regulator of cellular fatty acid homeostasis. *Curr Opin Lipidol*. 2022;33(2):103-111.
148. Shaw CS, Swinton C, Morales-Scholz MG, *et al.* Impact of exercise training status on the fiber type-specific abundance of proteins regulating intramuscular lipid metabolism. *J Appl Physiol (Bethesda)*. 2020;128(2):379-389.
149. Pan DA, Lillioja S, Kriketos AD, *et al.* Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes*. 1997;46(6):983-988.

150. Daemen S, Gemmink A, Brouwers B, *et al.* Distinct lipid droplet characteristics and distribution unmask the apparent contradiction of the athlete's paradox. *Mol Metab*. 2018;17:71-81.
151. Knottnerus SJG, Bleeker JC, Wüst RCI, *et al.* Disorders of mitochondrial long-chain fatty acid oxidation and the carnitine shuttle. *Rev Endocr Metab Disord*. 2018;19(1):93-106.
152. Exil VJ, Roberts RL, Sims H, *et al.* Very-long-chain acyl-coenzyme a dehydrogenase deficiency in mice. *Circ Res*. 2003;93(5):448-455.
153. Nguyen TB, Louie SM, Daniele JR, *et al.* DGAT1-dependent lipid droplet biogenesis protects mitochondrial function during starvation-induced autophagy. *Dev Cell*. 2017;42(1):9-21.e5.
154. Benador IY, Veliova M, Mahdaviani K, *et al.* Mitochondria bound to lipid droplets have unique bioenergetics, composition, and dynamics that support lipid droplet expansion. *Cell Metab*. 2018;27(4):869-885.e6.
155. Wang H, Sreenivasan U, Hu H, *et al.* Perilipin 5, a lipid droplet-associated protein, provides physical and metabolic linkage to mitochondria. *J Lipid Res*. 2011;52(12):2159-2168.
156. Mason RR, Mokhtar R, Matzaris M, *et al.* PLIN5 deletion remodels intracellular lipid composition and causes insulin resistance in muscle. *Mol Metab*. 2014;3(6):652-663.
157. Harris L-ALS, Skinner JR, Shew TM, Pietka TA, Abumrad NA, Wolins NE. Perilipin 5-driven lipid droplet accumulation in skeletal muscle stimulates the expression of fibroblast growth factor 21. *Diabetes*. 2015;64(8):2757-2768.
158. Wang H, Sreenivasan U, Gong DW, *et al.* Cardiomyocyte-specific perilipin 5 overexpression leads to myocardial steatosis and modest cardiac dysfunction1[S]. *J Lipid Res*. 2013;54(4):953-965.
159. MacPherson REK, Peters SJ. Piecing together the puzzle of perilipin proteins and skeletal muscle lipolysis. *Appl Physiol Nutr Metab*. 2015;40(7):641-651.
160. Conte M, Franceschi C, Sandri M, Salvioli S. Perilipin 2 and age-related metabolic diseases: a new perspective. *Trends Endocrinol Metab*. 2016;27(12):893-903.
161. Conte M, Vasuri F, Trisolino G, *et al.* Increased Plin2 expression in human skeletal muscle is associated with sarcopenia and muscle weakness. *PloS One*. 2013;8(8):e73709.
162. Conte M, Armani A, Conte G, *et al.* Muscle-specific perilipin2 down-regulation affects lipid metabolism and induces myofiber hypertrophy. *J Cachexia Sarcopenia Muscle*. 2019;10(1):95-110.
163. Parry HA, Glancy B. Energy transfer between the mitochondrial network and lipid droplets in insulin resistant skeletal muscle. *Curr Opin Physiol*. 2021;24:100487.
164. Fransen M, Lismont C, Walton P. The peroxisome-mitochondria connection: how and why? *Int J Mol Sci*. 2017;18(6):E1126.
165. Lismont C, Nordgren M, Van Veldhoven PP, Fransen M. Redox interplay between mitochondria and peroxisomes. *Front Cell Dev Biol*. 2015;3:35.
166. Wanders RJA, Waterham HR, Ferdinandusse S. Metabolic interplay between peroxisomes and other subcellular organelles including mitochondria and the endoplasmic Reticulum. *Front Cell Dev Biol*. 2015;3:83.
167. Agrimi G, Russo A, Scarcia P, Palmieri F. The human gene SLC25A17 encodes a peroxisomal transporter of coenzyme A, FAD and NAD+. *Biochem J*. 2012;443(1):241-247.
168. Bennett CF, O'Malley KE, Perry EA, *et al.* Peroxisomal-derived ether phospholipids link nucleotides to respirasome assembly. *Nat Chem Biol*. 2021;17(6):703-710.
169. Delille HK, Alves R, Schrader M. Biogenesis of peroxisomes and mitochondria: linked by division. *Histochem Cell Biol*. 2009;131(4):441-446.
170. Schrader M, Bonekamp NA, Islinger M. Fission and proliferation of peroxisomes. *Biochim Biophys Acta*. 2012;1822(9):1343-1357.
171. Mao K, Liu X, Feng Y, Klionsky DJ. The progression of peroxisomal degradation through autophagy requires peroxisomal division. *Autophagy*. 2014;10(4):652-661.
172. Silva BSC, DiGiovanni L, Kumar R, *et al.* Maintaining social contacts: the physiological relevance of organelle interactions. *Biochim Biophys Acta Mol Cell Res*. 2020;1867(11):118800.
173. Andrade-Navarro MA, Sanchez-Pulido L, McBride HM. Mitochondrial vesicles: an ancient process providing new links to peroxisomes. *Curr Opin Cell Biol*. 2009;21(4):560-567.
174. Shai N, Yifrach E, van Roermund CWT, *et al.* Systematic mapping of contact sites reveals tethers and a function for the peroxisome-mitochondria contact. *Nat Commun*. 2018;9(1):1761.
175. Vallese F, Catoni C, Cieri D, *et al.* An expanded palette of improved SPLICS reporters detects multiple organelle contacts in vitro and in vivo. *Nat Commun*. 2020;11(1):6069.
176. Fan J, Li X, Issop L, Culty M, Papadopoulos V. ACBD2/ECI2-mediated peroxisome-mitochondria interactions in Leydig cell steroid biosynthesis. *Mol Endocrinol*. 2016;30(7):763-782.
177. Peeters A, Shinde AB, Dirkx R, *et al.* Mitochondria in peroxisome-deficient hepatocytes exhibit impaired respiration, depleted DNA, and PGC-1α independent proliferation. *Biochim Biophys Acta*. 2015;1853(2):285-298.
178. Salpietro V, Phadke R, Saggar A, *et al.* Zellweger syndrome and secondary mitochondrial myopathy. *Eur J Pediatr*. 2015;174(4):557-563.
179. Sarnat HB, Machin G, Darwish HZ, Rubin SZ. Mitochondrial myopathy of cerebro-hepato-renal (Zellweger) syndrome. *Can J Neurol Sci*. 1983;10(3):170-177.
180. Cheng H, Gang X, He G, *et al.* The molecular mechanisms underlying mitochondria-associated endoplasmic reticulum membrane-induced insulin resistance. *Front Endocrinol (Lausanne)*. 2020;11:592129.
181. Franzini-Armstrong C, Boncompagni S. The evolution of the mitochondria-to-calcium release units relationship in vertebrate skeletal muscles. *J Biomed Biotechnol*. 2011;2011:830573.
182. De La Fuente S, Lambert JP., Nichtova Z, *et al.* Spatial separation of mitochondrial calcium uptake and extrusion for energy-efficient mitochondrial calcium signaling in the heart. *Cell Rep*. 2018;24(12):3099-3107.e4.
183. Castro-Sepulveda M, Jannas-Vela S, Fernández-Verdejo R, *et al.* Relative lipid oxidation associates directly with mitochondrial fusion phenotype and mitochondria-sarcoplasmic reticulum interactions in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2020;318(6):E848-E855.
184. Zhao YG, Chen Y, Miao G, *et al.* The ER-localized transmembrane protein EPG-3/VMP1 regulates SERCA activity to control ER-isolation membrane contacts for autophagosome formation. *Mol Cell*. 2017;67(6):974-989.e6.
185. Nieblas B, Pérez-Treviño P, García N. Role of mitochondria-associated endoplasmic reticulum membranes in insulin sensitivity, energy metabolism, and contraction of skeletal muscle. *Front Mol Biosci*. 2022;9:959844.
186. Verfaillie T, Rubio N, Garg AD, *et al.* PERK Is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. *Cell Death Differ*. 2012;19(11):1880-1891.
187. Simmen T, Aslan JE, Blagoveshchenskaya AD, *et al.* PACS-2 controls endoplasmic reticulum-mitochondria communication and bid-mediated apoptosis. *EMBO J*. 2005;24(4):717-729.
188. Franzini-Armstrong C. ER-mitochondria communication. How privileged? *Physiology (Bethesda)*. 2007;22:261-268.
189. García-Pérez C, Hajnóczky G, Csordás G. Physical coupling supports the local Ca2 + transfer between sarcoplasmic reticulum subdomains and the mitochondria in heart muscle. *J Biol Chem*. 2008;283(47):32771-32780.
190. De La Fuente S, Fernandez-Sanz C, Vail Cn, *et al.* Strategic positioning and biased activity of the mitochondrial calcium uniporter in cardiac muscle. *J Biol Chem*. 2016;291(44):23343-23362.
191. Al-Qusairi L, Laporte J. T-tubule biogenesis and triad formation in skeletal muscle and implication in human diseases. *Skelet Muscle*. 2011;1(1):26.
192. Boncompagni S, Rossi AE., Micaroni M, *et al.* Mitochondria are linked to calcium stores in striated muscle by developmentally

regulated tethering structures. *Mol Biol Cell*. 2009;20(3):1058-1067.
193. Eisner V, Csordás G, Hajnóczky G. Interactions between sarco-endoplasmic reticulum and mitochondria in cardiac and skeletal muscle—pivotal roles in Ca^{2+} and reactive oxygen species signaling. *J Cell Sci*. 2013;126(Pt 14):2965-2978.
194. Wüst RCI, Helmes M, Martin JL., *et al*. Rapid frequency-dependent changes in free mitochondrial calcium concentration in rat cardiac myocytes. *J Physiol*. 2017;595(6):2001-2019.
195. Sancak Y, Markhard AL., Kitami T, *et al*. EMRE is an essential component of the mitochondrial calcium uniporter complex. *Science*. 2013;342(6164):1379-1382.
196. Mallilankaraman K, Doonan P, Cárdenas C, *et al*. MICU1 Is an essential gatekeeper for MCU-mediated mitochondrial Ca(2+) uptake that regulates cell survival. *Cell*. 2012;151(3):630-644.
197. Lambert JP, Luongo TS, Tomar D, *et al*. MCUB regulates the molecular composition of the mitochondrial calcium uniporter channel to limit mitochondrial calcium overload during stress. *Circulation*. 2019;140(21):1720-1733.
198. Csordás G, Golenár T, Seifert EL, *et al*. MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca^{2+} uniporter. *Cell Metab*. 2013;17(6):976-987.
199. Glancy B, Balaban RS. Role of mitochondrial Ca2 + in the regulation of cellular energetics. *Biochemistry*. 2012;51(14):2959-2973.
200. del Arco A, Satrústegui J. Identification of a novel human subfamily of mitochondrial carriers with calcium-binding domains. *J Biol Chem*. 2004;279(23):24701-24713.
201. Nosek MT, Dransfield DT, Aprille JR. Calcium stimulates ATP-Mg/Pi carrier activity in rat liver mitochondria. *J Biol Chem*. 1990;265(15):8444-8450.
202. Hopper RK, Carroll S, Aponte AM, *et al*. Mitochondrial matrix phosphoproteome: effect of extra mitochondrial calcium. *Biochemistry*. 2006;45(8):2524-2536.
203. Tarasov AI, Griffiths EJ, Rutter GA. Regulation of ATP production by mitochondrial Ca(2+). *Cell Calcium*. 2012;52(1):28-35.
204. Rossi AE, Boncompagni S, Wei L, Protasi F, Dirksen RT. Differential impact of mitochondrial positioning on mitochondrial Ca(2+) uptake and Ca(2+) spark suppression in skeletal muscle. *Am J Physiol Cell Physiol*. 2011;301(5):C1128-C1139.
205. Mammucari C, Gherardi G, Zamparo I, *et al*. The mitochondrial calcium uniporter controls skeletal muscle trophism in vivo. *Cell Rep*. 2015;10(8):1269-1279.
206. Logan CV, Szabadkai G, Sharpe JA, *et al*. Loss-of-function mutations in MICU1 cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling. *Nat Genet*. 2014;46(2):188-193.
207. Feno S, Munari F, Reane DV, *et al*. The dominant-negative mitochondrial calcium uniporter subunit MCUb drives macrophage polarization during skeletal muscle regeneration. *Sci Signal*. 2021;14(707):eabf3838.
208. Bauer TM, Murphy E. Role of mitochondrial calcium and the permeability transition pore in regulating cell death. *Circ Res*. 2020;126(2):280-293.
209. Magalhães J, Fraga M, Lumini-Oliveira J, *et al*. Eccentric exercise transiently affects mice skeletal muscle mitochondrial function. *Appl Physiol Nutr Metab*. 2013;38(4):401-409.
210. Dorn GW. Mitofusins as mitochondrial anchors and tethers. *J Mol Cell Cardiol*. 2020;142:146-153.
211. Chen Y, Csordás G, Jowdy C, *et al*. Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via interorganelle Ca(2+) crosstalk. *Circ Res*. 2012;111(7):863-875.
212. Schrepfer E, Scorrano L. Mitofusins, from mitochondria to metabolism. *Mol. Cell*. 2016;61(5):683-694.
213. De la Fuente S, Sheu S-S. SR-mitochondria communication in adult cardiomyocytes: a close relationship where the Ca2 + has a lot to say. *Arch Biochem Biophys*. 2019;663:259-268.
214. García-Pérez C, Schneider TG, Hajnóczky G, Csordás G. Alignment of sarcoplasmic reticulum-mitochondrial junctions with mitochondrial contact points. *Am J Physiol Heart Circ Physiol* 2011;301(5):H1907-H1915.
215. de Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature*. 2008;456(7222):605-610.
216. Naon D, Zaninello M, Giacomello M, *et al*. Critical reappraisal confirms that mitofusin 2 is an endoplasmic reticulum-mitochondria tether. *Proc Natl Acad Sci U S A*. 2016;113(40):11249-11254.
217. Castro-Sepulveda M, Fernández-Verdejo R, Tuñón-Suárez M, *et al*. Low abundance of Mfn2 protein correlates with reduced mitochondria-SR juxtaposition and mitochondrial cristae density in human men skeletal muscle: examining organelle measurements from TEM images. *FASEB J*. 2021;35(4):e21553.
218. Tubbs E, Chanon S, Robert M, *et al*. Disruption of mitochondria-associated endoplasmic reticulum membrane (MAM) integrity contributes to muscle insulin resistance in mice and humans. *Diabetes*. 2018;67(4):636-650.
219. Cheng H, Gang X, He G, *et al*. The molecular mechanisms underlying mitochondria-associated endoplasmic reticulum membrane-induced insulin resistance. *Front Endocrinol*. 2020;11:592129.
220. Thoudam T, Ha C-M, Leem J, *et al*. PDK4 Augments ER-mitochondria contact to dampen skeletal muscle insulin signaling during obesity. *Diabetes*. 2019;68(3):571-586.
221. Vance JE. MAM (mitochondria-associated membranes) in mammalian cells: lipids and beyond. *Biochim Biophys Acta*. 2014;1841(4):595-609.
222. Hailey DW, Rambold AS., Satpute-Krishnan P, *et al*. Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell*. 2010;141(4):656-667.
223. Silva-Palacios A, Zazueta C, Pedraza-Chaverri J. ER Membranes associated with mitochondria: possible therapeutic targets in heart-associated diseases. *Pharmacol Res*. 2020;156:104758.
224. Tasseva G, *et al*. Phosphatidylethanolamine deficiency in mammalian mitochondria impairs oxidative phosphorylation and alters mitochondrial morphology. *J Biol Chem*. 2013;288(6):4158-4173.
225. Monteiro-Cardoso VF, Rochin L, Arora A, *et al*. ORP5/8 And MIB/MICOS link ER-mitochondria and intra-mitochondrial contacts for non-vesicular transport of phosphatidylserine. *Cell Rep*. 2022;40(12):111364.
226. Xu C, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest*. 2005;115(10):2656-2664.
227. Bravo R, Vicencio JM, Parra V, *et al*. Increased ER-mitochondrial coupling promotes mitochondrial respiration and bioenergetics during early phases of ER stress. *J Cell Sci*. 2011;124(13):2143-2152.
228. Michelucci A, Liang C, Protasi F, Dirksen RT. Altered Ca2+ handling and oxidative stress underlie mitochondrial damage and skeletal muscle dysfunction in aging and disease. *Metabolites*. 2021;11(7):424.
229. Yang Y-F, Yang W, Liao Z-Y, *et al*. MICU3 Regulates mitochondrial Ca2+-dependent antioxidant response in skeletal muscle aging. *Cell Death Dis*. 2021;12(12):1115.
230. Miranda-Silva D, Wüst RCI, Conceição G, *et al*. Disturbed cardiac mitochondrial and cytosolic calcium handling in a metabolic risk-related rat model of heart failure with preserved ejection fraction. *Acta Physiol (Oxf)*. 2020;228:e13378.
231. Suarez J, Cividini F, Scott BT, *et al*. Restoring mitochondrial calcium uniporter expression in diabetic mouse heart improves mitochondrial calcium handling and cardiac function. *J Biol Chem*. 2018;293(21):8182-8195.
232. Baartscheer A, Schumacher CA, Wüst RCI, *et al*. Empagliflozin decreases myocardial cytoplasmic Na+ through inhibition of the cardiac Na+/H+ exchanger in rats and rabbits. *Diabetologia*. 2017;60(3):568-573.
233. Kapadia P, Bikkina P, Landicho MA, *et al*. Effect of anti-hyperglycemic drugs on endoplasmic reticulum (ER) stress in

human coronary artery endothelial cells. *Eur J Pharmacol*. 2021;907:174249.
234. Campeau M-A, Leask RL. Empagliflozin mitigates endothelial inflammation and attenuates endoplasmic reticulum stress signaling caused by sustained glycocalyx disruption. *Sci Rep*. 2022;12(1): 12681.
235. Bugga P, Mohammed SA, Alam MJ, *et al*. Empagliflozin prohibits high-fructose diet-induced cardiac dysfunction in rats via attenuation of mitochondria-driven oxidative stress. *Life Sci*. 2022;307:120862.
236. Varghese M, Kimler VA, Ghazi FR, *et al*. Adipocyte lipolysis affects perilipin 5 and cristae organization at the cardiac lipid droplet-mitochondrial interface. *Sci Rep*. 2019;9(1):4734.
237. Cuello F, Knaust AE, Saleem U, *et al*. Impairment of the ER/mitochondria compartment in human cardiomyocytes with PLN p.Arg14del mutation. *EMBO Mol Med*. 2021;13(6):e13074.
238. Safdar A, Saleem A, Tarnopolsky MA. The potential of endurance exercise-derived exosomes to treat metabolic diseases. *Nat Rev Endocrinol*. 2016;12(9):504-517.
239. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat Rev Endocrinol*. 2012;8(8):457-465.
240. Chrétien D, Bénit P, Ha H-H, *et al*. Mitochondria are physiologically maintained at close to 50 °C. *PLoS Biol*. 2018;16(1): e2003992.
241. Nakano M, Arai Y, Kotera I, Okabe K, Kamei Y, Nagai T. Genetically encoded ratiometric fluorescent thermometer with wide range and rapid response. *PloS One*. 2017;12(2):e0172344.
242. Sawicka-Gutaj N, Erampamoorthy A, Zybek-Kocik A, *et al*. The role of thyroid hormones on skeletal muscle thermogenesis. *Metabolites*. 2022;12(4):336.
243. Fujita Y, Taniguchi Y, Shinkai S, Tanaka M, Ito M. Secreted growth differentiation factor 1 5 as a potential biomarker for mitochondrial dysfunctions in aging and age-related disorders. *Geriatr Gerontol Int*. 2016;16(Suppl 1):17-29.
244. Romanello V, Sandri M. Implications of mitochondrial fusion and fission in skeletal muscle mass and health. *Semin Cell Dev Biol*. 2022;S1084-9521(22)00050–7.
245. Oost LJ, Kustermann M, Armani A, Blaauw B, Romanello V. Fibroblast growth factor 21 controls mitophagy and muscle mass. *J Cachexia Sarcopenia Muscle*. 2019;10(3):630-642.
246. Planavila A, Redondo I, Hondares E, *et al*. Fibroblast growth factor 21 protects against cardiac hypertrophy in mice. *Nat Commun*. 2013;4(1):2019.
247. Tezze C, Romanello V, Sandri M. FGF21 as modulator of metabolism in health and disease. *Front Physiol*. 2019;10:419.
248. Planavila A, Redondo-Angulo I, Villarroya F. FGF21 and cardiac physiopathology. *Front Endocrinol*. 2015;6:133.
249. Vandanmagsar B, Warfel JD, Wicks SE, *et al*. Impaired mitochondrial fat oxidation induces FGF21 in muscle. *Cell Rep*. 2016;15(8):1686-1699.
250. Liu SQ, Roberts D, Kharitonenkov A, *et al*. Endocrine protection of ischemic myocardium by FGF21 from the liver and adipose tissue. *Sci Rep*. 2013;3(1):2767.
251. Sun H, Sherrier M, Li H. Skeletal muscle and bone—emerging targets of fibroblast growth factor-21. *Front Physiol*. 2021;12: 625287.
252. Lehtonen JM, Forsström S, Bottani E, *et al*. FGF21 is a biomarker for mitochondrial translation and mtDNA maintenance disorders. *Neurology*. 2016;87(22):2290-2299.
253. Kim KH, Jeong YT, Oh H, *et al*. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. *Nat Med*. 2013;19(1):83-92.
254. Zhang Y, Xie Y, Berglund ED, *et al*. The starvation hormone, fibroblast growth factor-21, extends lifespan in mice. *eLife*. 2012;1:e00065.
255. Mashili FL, Austin RL, Deshmukh AS, *et al*. Direct effects of FGF21 on glucose uptake in human skeletal muscle: implications for type 2 diabetes and obesity. *Diabetes Metab Res Rev*. 2011;27(3):286-297.
256. Forsström S, Jackson CB., Carroll CJ., *et al*. Fibroblast growth factor 21 drives dynamics of local and systemic stress responses in mitochondrial myopathy with mtDNA deletions. *Cell Metab*. 2019;30(6):1040-1054.e7.
257. Redondo-Angulo I, Mas-Stachurska A, Sitges M, *et al*. Fgf21 is required for cardiac remodeling in pregnancy. *Cardiovasc Res*. 2017;113(13):1574-1584.
258. Croon M, Szczepanowska K, Popovic M, *et al*. FGF21 Modulates mitochondrial stress response in cardiomyocytes only under mild mitochondrial dysfunction. *Sci Adv*. 2022;8(14):eabn7105.
259. Jin L, Geng L, Ying L, *et al*. FGF21-Sirtuin 3 axis confers the protective effects of exercise against diabetic cardiomyopathy by governing mitochondrial integrity. *Circulation*. 2022;146(20): 1537-1557.
260. Suomalainen A, Elo JM, Pietiläinen KH, *et al*. FGF-21 as a biomarker for muscle-manifesting mitochondrial respiratory chain deficiencies: a diagnostic study. *Lancet Neurol*. 2011;10(9): 806-818.
261. Chow WS, Xu A, Woo YC, *et al*. Serum fibroblast growth factor-21 levels are associated with carotid atherosclerosis independent of established cardiovascular risk factors. *Arterioscler Thromb Vasc Biol*. 2013;33(10):2454-2459.
262. Hanks LJ, Gutiérrez OM, Bamman MM, *et al*. Circulating levels of fibroblast growth factor-21 increase with age independently of body composition indices among healthy individuals. *J Clin Transl Endocrinol*. 2015;2(2):77-82.
263. Conte M, Ostan R, Fabbri C, *et al*. Human aging and longevity are characterized by high levels of mitokines. *J Gerontol A Biol Sci Med Sci*. 2019;74(5):600-607.
264. Franz K, Ost M, Otten L, *et al*. Higher serum levels of fibroblast growth factor 21 in old patients with cachexia. *Nutrition*. 2019;63–64:81-86.
265. Ajaz S, McPhail MJ, Singh KK, *et al*. Mitochondrial metabolic manipulation by SARS-CoV-2 in peripheral blood mononuclear cells of patients with COVID-19. *Am J Physiol Cell Physiol*. 2021;320(1):C57-C65.
266. Wang D, Day EA, Townsend LK, *et al*. GDF15: emerging biology and therapeutic applications for obesity and cardiometabolic disease. *Nat Rev Endocrinol*. 2021;17(10):592-607.
267. Ost M, Igual Gil C, Coleman V, *et al*. Muscle-derived GDF15 drives diurnal anorexia and systemic metabolic remodeling during mitochondrial stress. *EMBO Rep*. 2020;21(3):e48804.
268. Chung HK, Ryu D, Kim KS, *et al*. Growth differentiation factor 15 is a myomitokine governing systemic energy homeostasis. *J Cell Biol*. 2017;216(1):149-165.
269. Breit SN, Brown DA, Tsai VW-W. The GDF15-GFRAL pathway in health and metabolic disease: friend or foe? *Annu Rev Physiol*. 2021;83(1):127-151.
270. Keipert S, Ost M, Johann K, *et al*. Skeletal muscle mitochondrial uncoupling drives endocrine cross-talk through the induction of FGF21 as a myokine. *Am J Physiol Endocrinol Metab*. 2014;306(5):E469-E482.
271. Keipert S, Voigt A, Klaus S. Dietary effects on body composition, glucose metabolism, and longevity are modulated by skeletal muscle mitochondrial uncoupling in mice. *Aging Cell*. 2011;10(1):122-136.
272. Gates AC, Bernal-Mizrachi C, Chinault SL, *et al*. Respiratory uncoupling in skeletal muscle delays death and diminishes age-related disease. *Cell Metab*. 2007;6(6):497-505.
273. Wang X, Chrysovergis K, Kosak J, *et al*. hNAG-1 increases lifespan by regulating energy metabolism and insulin/IGF-1/mTOR signaling. *Aging*. 2014;6(8):690-704.
274. Ost M, Coleman V, Voigt A, *et al*. Muscle mitochondrial stress adaptation operates independently of endogenous FGF21 action. *Mol Metab*. 2016;5(2):79-90.

275. Wang T, Liu J, McDonald C, *et al.* GDF15 Is a heart-derived hormone that regulates body growth. *EMBO Mol Med*. 2017;9(8):1150-1164.
276. Zhang Y, Moszczynski Lisa A, Liu Qing, *et al.* Over-expression of growth differentiation factor 15 (GDF15) preventing cold ischemia reperfusion (I/R) injury in heart transplantation through Foxo3a signaling. *Oncotarget*. 2017;8(22):36531-36544.
277. Kempf T, Eden M, Strelau J, *et al.* The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. *Circ Res*. 2006;98(3):351-360.
278. Xu J, Kimball TR., Lorenz JN, *et al.* GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. *Circ Res*. 2006;98(3):342-350.
279. Baek SJ, Eling T. Growth differentiation factor 15 (GDF15): a survival protein with therapeutic potential in metabolic diseases. *Pharmacol Ther*. 2019;198:46-58.
280. Johnson AA, Shokhirev MN, Wyss-Coray T, Lehallier B. Systematic review and analysis of human proteomics aging studies unveils a novel proteomic aging clock and identifies key processes that change with age. *Ageing Res. Rev*. 2020;60:101070.
281. Moon JS, Goeminne LJE, Kim JT, *et al.* Growth differentiation factor 15 protects against the aging-mediated systemic inflammatory response in humans and mice. *Aging Cell*. 2020;19(8):e13195.
282. Wiklund FE, Bennet AM, Magnusson PKE, *et al.* Macrophage inhibitory cytokine-1 (MIC-1/GDF15): a new marker of all-cause mortality. *Aging Cell*. 2010;9(6):1057-1064.
283. Daniels LB, Clopton P, Laughlin GA, Maisel AS, Barrett-Connor E. Growth-differentiation factor-15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: the Rancho Bernardo study. *Circulation*. 2011;123(19):2101-2110.
284. Lajer M, Jorsal A, Tarnow L, Parving H-H, Rossing P. Plasma growth differentiation factor-15 independently predicts all-cause and cardiovascular mortality as well as deterioration of kidney function in type 1 diabetic patients with nephropathy. *Diabetes Care*. 2010;33(7):1567-1572.
285. Eggers KM, Kempf T, Wallentin L, Wollert KC, Lind L. Change in growth differentiation factor 15 concentrations over time independently predicts mortality in community-dwelling elderly individuals. *Clin Chem*. 2013;59(7):1091-1098.
286. Adela R, Banerjee SK. GDF-15 as a target and biomarker for diabetes and cardiovascular diseases: A translational prospective. *J. Diabetes Res*. 2015;2015:490842.
287. Bloch SAA, Lee JY, Syburra T, *et al.* Increased expression of GDF-15 may mediate ICU-acquired weakness by down-regulating muscle microRNAs. *Thorax*. 2015;70(3):219-228.
288. Oba K, Ishikawa Joji, Tamura Y, *et al.* Serum growth differentiation factor 15 level is associated with muscle strength and lower extremity function in older patients with cardiometabolic disease. *Geriatr Gerontol Int*. 2020;20(10):980-987.
289. Patel MS, Lee J, Baz M, *et al.* Growth differentiation factor-15 is associated with muscle mass in chronic obstructive pulmonary disease and promotes muscle wasting in vivo. *J Cachexia Sarcopenia Muscle*. 2016;7(4):436-448.
290. Tang H, Inoki K, Brooks SV, *et al.* mTORC1 underlies age-related muscle fiber damage and loss by inducing oxidative stress and catabolism. *Aging Cell*. 2019;18(3):e12943.
291. Suriben R, Chen M, Higbee J, *et al.* Antibody-mediated inhibition of GDF15-GFRAL activity reverses cancer cachexia in mice. *Nat Med*. 2020;26(8):1264-1270.
292. Emmerson PJ, Wang Feng, Du Yong, *et al.* The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. *Nat Med*. 2017;23(10):1215-1219.
293. Hsu J-Y, Crawley Suzanne, Chen Michael, *et al.* Non-homeostatic body weight regulation through a brainstem-restricted receptor for GDF15. *Nature*. 2017;550(7675):255-259.
294. Mullican SE, Lin-Schmidt X, Chin C-N, *et al.* GFRAL Is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. *Nat Med*. 2017;23(10):1150-1157.
295. Yang L, Chang C-C, Sun Z, *et al.* GFRAL Is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. *Nat Med*. 2017;23(10):1158-1166.
296. Zhang Q, Raoof M, Chen Y, *et al.* Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*. 2010;464(7285):104-107.
297. Nakayama H, Otsu K. Mitochondrial DNA as an inflammatory mediator in cardiovascular diseases. *Biochem J*. 2018;475(5):839-852.
298. Wu Z, Oeck S, West AP, *et al.* Mitochondrial DNA stress signalling protects the nuclear genome. *Nat Metab*. 2019;1(12):1209-1218.
299. Qin C, Gu J, Hu J, *et al.* Platelets activation is associated with elevated plasma mitochondrial DNA during cardiopulmonary bypass. *J Cardiothorac Surg*. 2016;11(1):90.
300. Bliksøen M, Mariero LH, Torp MK, *et al.* Extracellular mtDNA activates NF-κB via toll-like receptor 9 and induces cell death in cardiomyocytes. *Basic Res Cardiol*. 2016;111(4):42.
301. Bliksøen M, Mariero LH, Ohm IK, *et al.* Increased circulating mitochondrial DNA after myocardial infarction. *Int J Cardiol*. 2012;158(1):132-134.
302. Mariero LH, Torp M-K, Heiestad CM, *et al.* Inhibiting nucleolin reduces inflammation induced by mitochondrial DNA in cardiomyocytes exposed to hypoxia and reoxygenation. *Br J Pharmacol*. 2019;176(22):4360-4372.
303. Sandler N, Kaczmarek E, Itagaki K, *et al.* Mitochondrial DAMPs are released during cardiopulmonary bypass surgery and are associated with postoperative atrial fibrillation. *Heart Lung Circ*. 2018;27(1):122-129.
304. Nakahira K, Gazourian L, Youn S, *et al.* Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. *PLoS Med*. 2013;10(12):e1001577.
305. Arnalich F, Codoceo R, López-Collazo E, Montiel C. Circulating cell-free mitochondrial DNA: a better early prognostic marker in patients with out-of-hospital cardiac arrest. *Resuscitation*. 2012;83(7):e162-e163.
306. Wiersma M, van Marion DMS, Bouman EJ, *et al.* Cell-free circulating mitochondrial DNA: a potential blood-based marker for atrial fibrillation. *Cells*. 2020;9(5):E1159.
307. Longnus SL, Rutishauser N, Gillespie MN, Reichlin T, Carrel TP, Sanz MN. Mitochondrial damage-associated molecular patterns as potential biomarkers in DCD heart transplantation: lessons from myocardial infarction and cardiac arrest. *Transplant Direct*. 2022;8(1):e1265.
308. McCarthy CG, Wenceslau CF, Goulopoulou S, *et al.* Circulating mitochondrial DNA and toll-like receptor 9 are associated with vascular dysfunction in spontaneously hypertensive rats. *Cardiovasc Res*. 2015;107(1):119-130.
309. Bequette BJ, Sunny NE, El-Kadi SW, Owens SL. Application of stable isotopes and mass isotopomer distribution analysis to the study of intermediary metabolism of nutrients. *J Anim Sci*. 2006;84(suppl_13):E50-E59.
310. Bayraktar EC, Baudrier L, Özerdem C, *et al.* MITO-Tag Mice enable rapid isolation and multimodal profiling of mitochondria from specific cell types in vivo. *Proc Natl Acad Sci U S A*. 2019;116(1):303-312.
311. Rieger B, Shalaeva DN, Söhnel A-C, *et al.* Lifetime imaging of GFP at CoxVIIIa reports respiratory supercomplex assembly in live cells. *Sci Rep*. 2017;7(1):46055.
312. Vesga-Castro C, Aldazabal J, Vallejo-Illarramendi A, Paredes J. Contractile force assessment methods for in vitro skeletal muscle tissues. *eLife*. 2022;11:e77204.
313. Eggelbusch M, Shi A, Broeksma BC, *et al.* The NLRP3 inflammasome contributes to inflammation-induced morphological and metabolic alterations in skeletal muscle. *J Cachexia Sarcopenia Muscle*. 2022;13:3048-3061.

314. Sugimoto T, Nakamura T, Yokoyama S, *et al*. Investigation of brain function-related myokine secretion by using contractile 3D-engineered muscle. *Int J Mol Sci*. 2022;23(10):5723.
315. Khodabukus A, Baar K. Glucose concentration and streptomycin alter in vitro muscle function and metabolism. *J Cell Physiol*. 2015;230(6):1226-1234.
316. Ryu AH, Eckalbar WL, Kreimer A, Yosef N, Ahituv N. Use antibiotics in cell culture with caution: genome-wide identification of antibiotic-induced changes in gene expression and regulation. *Sci Rep*. 2017;7(1):7533.
317. Wüst RCI, Houtkooper RH, Auwerx J. Confounding factors from inducible systems for spatiotemporal gene expression regulation. *J Cell Biol*. 2020;219(7):e202003031.
318. Chatzispyrou IA, Held NM, Mouchiroud L, Auwerx J, Houtkooper RH. Tetracycline antibiotics impair mitochondrial function and its experimental use confounds research. *Cancer Res*. 2015;75(21):4446-4449.
319. Pelletier-Galarneau M, Detmer FJ, Petibon Y, *et al*. Quantification of myocardial mitochondrial membrane potential using PET. *Curr Cardiol Rep*. 2021;23(6):70.
320. Pelletier-Galarneau M, Petibon Y, Ma C, *et al*. In vivo quantitative mapping of human mitochondrial cardiac membrane potential: a feasibility study. *Eur J Nucl Med Mol Imaging*. 2021;48(2):414-420.
321. Patel S, Alvarez-Guaita A, Melvin A, *et al*. GDF15 provides an endocrine signal of nutritional stress in mice and humans. *Cell Metab*. 2019;29(3):707-718.e8.
322. Li B, Zhao H, Wu Y, *et al*. Mitochondrial-derived vesicles protect cardiomyocytes against hypoxic damage. *Front Cell Dev Biol*. 2020;8:214.
323. Chen Y, Yang F, Chu Y, Yun Z, Yan Y, Jin J. Mitochondrial transplantation: opportunities and challenges in the treatment of obesity, diabetes, and nonalcoholic fatty liver disease. *J Transl Med*. 2022;20(1):483.
324. Shin B, Cowan DB, Emani SM, Del Nido PJ, McCully JD. Mitochondrial transplantation in myocardial ischemia and reperfusion injury. *Adv Exp Med Biol*. 2017;982:595-619.
325. Bertero E, Maack C, O'Rourke B. Mitochondrial transplantation in humans: 'magical' cure or cause for concern? *J Clin Invest*. 2018;128(12):5191-5194.
326. Trumpff C, Michelson J, Lagranha CJ, *et al*. Stress and circulating cell-free mitochondrial DNA: a systematic review of human studies, physiological considerations, and technical recommendations. *Mitochondrion*. 2021;59:225-245.