Skeletal muscle mitochondrial network dynamics in metabolic disorders and aging

Trends in
Molecular Medicine

CellPress

Review

Skeletal muscle mitochondrial network dynamics in metabolic disorders and aging

Ciarán E. Fealy,[1,2] Lotte Grevendonk,[1] Joris Hoeks,[1] and Matthijs K.C. Hesselink[1,*]

With global demographics trending towards an aging population, the numbers of individuals with an age-associated loss of independence is increasing. A key contributing factor is loss of skeletal muscle mitochondrial, metabolic, and contractile function. Recent advances in imaging technologies have demonstrated the importance of mitochondrial morphology and dynamics in the pathogenesis of disease. In this review, we examine the evidence for altered mitochondrial dynamics as a mechanism in age and obesity-associated loss of skeletal muscle function, with a particular focus on the available human data. We highlight some of the areas where more data are needed to identify the specific mechanisms connecting mitochondrial morphology and skeletal muscle dysfunction.

Highlights

Global demographics suggest an aging population, prompting concerns about an increase in the numbers of individuals with an age-associated loss of independence.

Increasing adiposity is a risk factor for skeletal muscle insulin resistance, metabolic disease, and loss of skeletal muscle mass and function.

Mitochondrial dynamics may be a therapeutic target for disorders of aging with an increasing number of studies suggest the presence of altered mitochondrial morphology in aging and obesity.

Mitochondrial fragmentation is associated with metabolic disease development, while mitochondrial autophagy may be dysregulated in loss of muscle mass and strength.

There remain significant gaps in the literature; however, the development of novel methodologies is facilitating a better understanding of mitochondrial network dynamics in age- and obesity-associated skeletal muscle dysfunction.

Skeletal muscle in age and age-associated diseases

Increases in human longevity illustrate the remarkable advances achieved in biomedicine, nutrition, and healthcare over the past century. Yet, as lifespan has increased, gains in healthspan – defined as the period of life spent in good health, free from the chronic diseases and disabilities of aging [1] – have stagnated [2]. With the proportion of elderly among the global population set to reach >20% in the next decades [3], the imperative to find new solutions to the problem of age-associated loss of independence has become increasingly relevant.

One of the main obstacles to improvements in healthspan is the contemporaneous rise in overweight and obesity in the global population. Although overweight and obesity have been associated with an increase in premature death, the impact of these on mortality has declined such that recent evidence suggests that elderly individuals with overweight or mild obesity now have a life expectancy similar to normal-weight elderly [4]. However, this group is at increased risk of disability and spend more years with limitations in physical function than normal-weight elderly [5,6].

Deterioration in skeletal muscle mass, metabolic fitness, and contractile vigor are fundamental to the progression of both metabolic disease and age-associated loss of independence (Figure 1). Increased adiposity and ectopic lipid deposition, whether in the presence of obesity or not, contribute to skeletal muscle insulin resistance [7], are common features of aging [8–10], and are strong predictors of both metabolic disease progression [11,12] and muscle strength and mass [9,10]. Increasing adiposity blunts the effectiveness of insulin and amino acid stimulation of muscle protein synthesis [13]. Muscle, the primary target tissue for insulin-stimulated glucose disposal, is a key regulator of whole-body glucose homeostasis. Thus, reduced muscle mass is also associated with reduced energy expenditure [14]. Age-related decline in both physical function and metabolic health can be mitigated, in large part, by increased physical activity levels and exercise training. Thus, the underlying pathology is one of adaptation to chronic environmental stimuli and allostatic loading rather than an obligatory consequence of aging [7,8]. Progressive deterioration in mitochondrial capacity has been linked to age- and obesity-associated muscle defects although there remains little consensus on a specific mechanistic pathway to explain

[1]Department of Nutrition and Movement Sciences, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands
[2]Department of Physical Education and Sport Sciences, University of Limerick, Castletroy, Limerick, Ireland

*Correspondence:
matthijs.hesselink@maastrichtuniversity.nl (M.K.C. Hesselink).

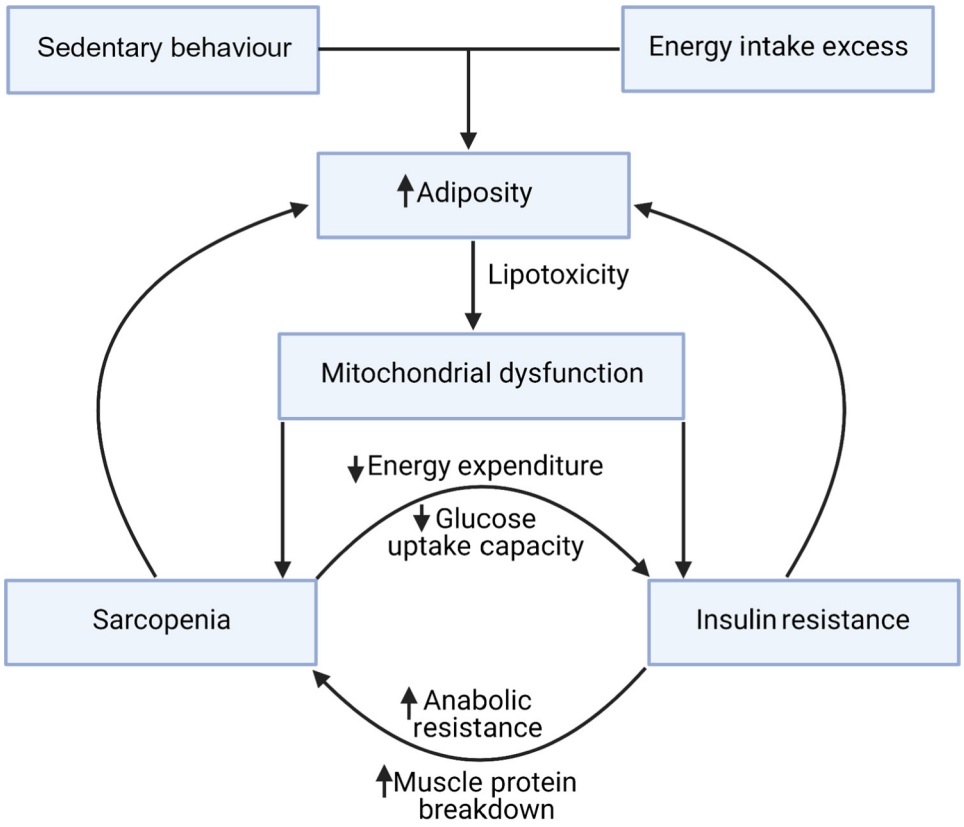


Figure 1. Reciprocal relation between muscle mass and metabolic homeostasis. Both sedentary behavior and excess energy intake during aging negatively influence body composition by increasing fat mass. Excess adiposity is associated with increased ectopic lipid deposition leading to lipotoxicity and consequent mitochondrial dysfunction. Mitochondrial dysfunction has been implicated in the onset of sarcopenia and insulin resistance. Sarcopenia and insulin resistance are likely to interact, whereby loss of muscle mass results in reduced energy expenditure and reduced capacity for glucose uptake while insulin resistance may induce anabolic resistance to insulin and amino acids and reduce insulin inhibition of muscle protein breakdown. Collectively, these mechanisms further exacerbate adiposity in a positive feedback loop resulting a progressive decline in metabolic and functional fitness in aged muscle.

this association. Recent developments in understanding the dynamic behavior of mitochondrial networks and quality control pathways have opened new prospective avenues of mitochondrial involvement in physical and metabolic decline. In this review we examine the evidence for the presence of aberrant mitochondrial network dynamics in the pathogenesis of age-associated skeletal muscle dysfunction with a focus, where available, on evidence from human research.

Skeletal muscle mitochondria are an important target to increase healthspan

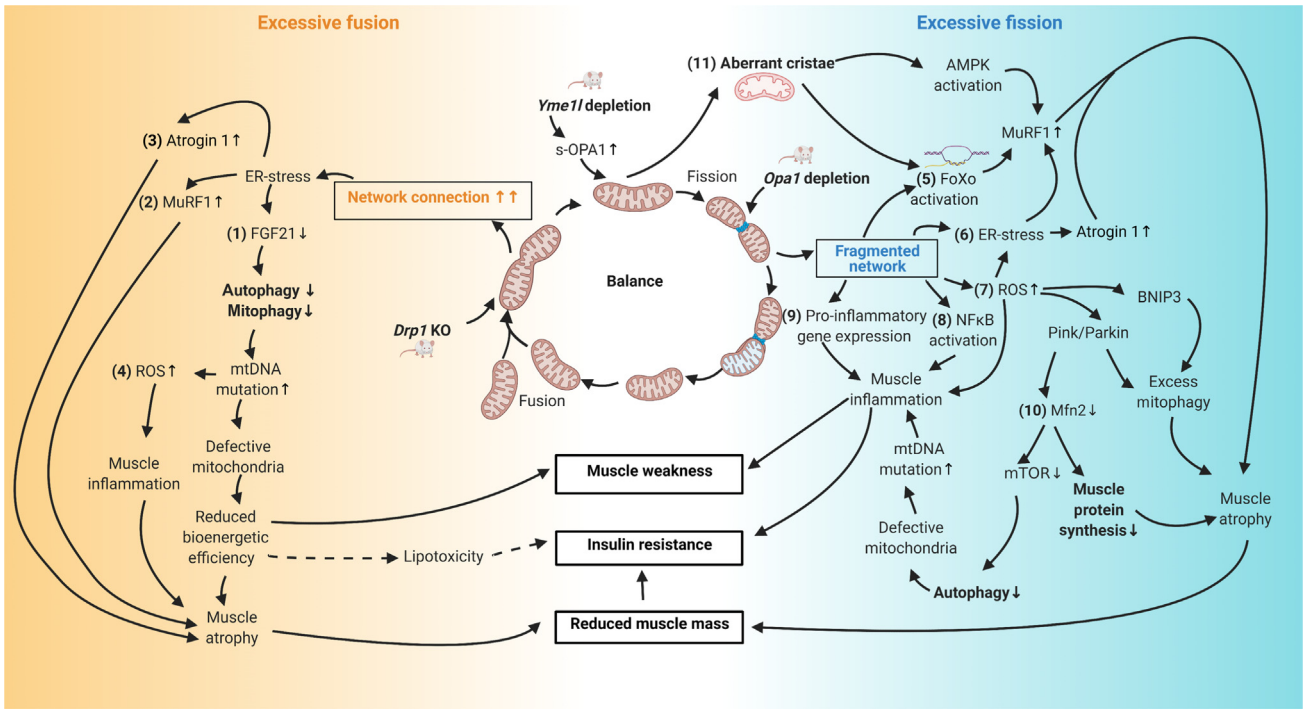
One of the early consequences of chronic **lipotoxicity** (see Glossary) in both metabolic disease and aging is a reduction in mitochondrial content and capacity [9]. However, the extent to which reduced mitochondrial capacity contributes to impaired muscle function in obesity and aging is debated. Classically, mitochondrial function has been viewed through the prism of mitochondrial density and intrinsic bioenergetic capacity; however, fresh insights into the varied functions of mitochondria necessitates an expansion of the concept. In the present view, mitochondrial function also includes the ability of mitochondria to move and physically interact with each other in dynamic reticular networks. Mitochondrial network architecture, regulated by the processes of fusion and fission (Figure 2), varies widely among cell types, and their morphology is intrinsically

Glossary

Apoptosis: programmed cell death.
Autophagy (macroautophagy): the process in which cellular contents are degraded by lysosomes or vacuoles and recycled.
Bariatric surgery: covers a variety of weight loss surgeries, including laparoscopic gastric banding surgery and Roux-en-Y gastric bypass (RYGB).
Cachexia: a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass.
Laparoscopic gastric banding surgery: a surgical procedure that involves the placement of an adjustable belt around the upper portion of the stomach using a laparoscope. The belt limits the expansion of the stomach conferring increased satiety in the patient.
Lipotoxicity: the deleterious effects of lipid accumulation in non-adipose tissue.
Mitochondrial fission: the division of a single mitochondrion into two or more independent structures.
Mitochondrial fusion: the physical merging of the outer and then the inner mitochondrial membranes of two originally distinct mitochondria.
Mitochondrial nanotunnels: thin double-membrane protrusions that connect the matrices of non-adjacent mitochondria.
Mitophagy: the selective degradation of mitochondria by autophagy.
Reactive oxygen species (ROS): oxygen-containing radicals such as the superoxide anion ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO^{\bullet}$) that can be generated by aerobic metabolism. ROS may serve as cell signaling molecules for normal biological processes; however, excessive production of ROS can result in damage to multiple cellular organelles and processes.
Roux-en-Y gastric bypass (RYGB): a surgical procedure that involves the creation of a small gastric pouch connected to a roux limb, which bypasses a large portion of the small intestine. This results in the food bypassing the majority of the stomach, the duodenum, and the first 40–50 cm of jejunum.
Sarcopenia: the age-associated loss of muscle mass and strength.

linked to the energetic and functional aspects of the cell. Thus, in dividing cells and in cells with a high mitochondrial turnover, **mitochondrial fission** predominates and the morphology shifts towards a 'fragmented' network [15]. By contrast, skeletal muscle, which requires rapid conductance of energy potentials, forms a vast interconnected reticulum that acts as a power grid to distribute membrane potential according to energetic requirements [16–18], although the degree of networking may be less apparent in humans than in mice [16]. Mitochondrial morphology and dynamics have been linked to a variety of cellular processes including cell division [19], regulation of gene expression [20], **apoptosis** [14,21] and **autophagy** [22], removal of damaged mitochondria (**mitophagy**) [23], calcium homeostasis [24], the production of **reactive oxygen species**

Type 2 diabetes (T2D): a metabolic disorder that results in hyperglycemia due to reduced effectiveness of the hormone insulin (insulin resistance) an inability of the pancreas to produce enough insulin to overcome insulin resistance.



Trends in Molecular Medicine

Figure 2. Putative pathways in response to dysregulated mitochondrial dynamics in age-related decline in muscle function and metabolism. Examples of identified putative pathways that may explain the contribution of alteration in mitochondrial morphology to the decline in metabolic and functional fitness in aged muscle. Drp1 knockout animals represent a model of excessive fusion. Excessive fusion leads to the endoplasmic reticulum (ER)-stress-mediated downregulation of fibroblast growth factor 21 (FGF21) (1). Lower levels of FGF21 result in reduced mitophagy and autophagy, which result in excessive levels of mitochondrial DNA (mtDNA) mutations, leading to inefficient mitochondria, reduced bioenergetic efficiency, and eventually muscle weakness and a greater risk of lipotoxicity-induced insulin resistance. ER stress also induces the expression of the atrogenic genes and Atrogin 1 (2) and muscle RING-finger protein-1 (MuRF1) (2), which, together with mtDNA damage-induced reactive oxygen species (ROS)-mediated (4) muscle inflammation, enhances muscle atrophy, also resulting in reduced muscle mass and consequent insulin resistance. Optic atrophy 1 (Opa1) depletion represents a model for excessive fission and a fragmented network. The fragmented network results in activation of the atrogenic forkhead box Os (FOXOs) (5), increased stress on the ER (6), increased ROS (7), nuclear factor kappa-light-chain-enhancer of activated B cells (NfκB) activation (8), and increased expression of several proinflammatory genes (9). ER stress (6) leads to upregulation of MuRF1 expression and both the activation of FOXO (5) and increased ER stress (6) upregulate the expression of MuRF1 and Atrogin 1, leading to muscle atrophy-mediated decline in muscle mass and consequent insulin resistance. ROS (7) additionally mediate the upregulation of BCL2-interacting protein 3 (BNIP3) and Parkin, which both result in excessive mitophagy and muscle atrophy. The ROS-mediated (7) activation of Parkin also leads to a decline in Mfn2 (10), which impairs muscle protein synthesis, resulting in muscle atrophy, reduced muscle mass, and consequent insulin resistance. Also, a decline in Mfn2 (10) leads to a decline in mTOR and consequently a decline in autophagy, resulting in defective mitochondria, increased mtDNA mutation, and muscle inflammation. In parallel, increased ROS production (7), NFκB activation (8), and increased expression of proinflammatory genes (9) stimulate muscle tissue inflammation, together resulting in muscle weakness and insulin resistance. ATP-dependent zinc metalloprotease YME1L (Yme1l) depletion stimulates the expression of Opa1 (short form) (S-Opa1), leading to aberrant mitochondrial cristae (11), which activates AMP-activated protein kinase (AMPK) and FOXO (5), both stimulating the expression of MuRF1 and Atrogin 1 and leading to an atrogen-mediated reduction in muscle mass and increased insulin resistance. Both excessive fusion and excessive fission can impair muscle function and metabolic health. Abbreviations: Drp1, dynamin-related protein 1; Mfn2, mitofusin-2.

(ROS) [25], and the regulation of cellular bioenergetic capacity and efficiency [26]. Mitochondrial morphology also responds to the nutrient status of cells, with fragmentation observed in conditions of high nutrient availability and fusion predominating to maintain higher bioenergetic efficiency where nutrients are scarce [27]. The major proteins that regulate mitochondrial morphology are described in Table 1. Given the ubiquity of cellular functions in which mitochondrial dynamics participate, it is hardly surprising that dysregulation of mitochondrial network dynamics has been implicated in an increasing number of disease states [23,28], including metabolic disease [25,26] and age-associated functional decline [29].

Mitochondrial network morphology in metabolic disorders

There is a growing consensus that obesity and metabolic disease are associated with mitochondrial network fragmentation in skeletal muscle. Increased mitochondrial network fragmentation was first demonstrated in the skeletal muscle of Zucker Diabetic Fatty rats [30]. Similarly, obese mice that are deficient for leptin receptors and mice with high-fat diet (HFD)-induced obesity were found to exhibit smaller and shorter mitochondria in skeletal muscle compared with lean

Table 1. Major fusion and fission proteins that regulate mitochondrial morphology

Protein		Location	Function	
Fusion proteins				
Mfn1	MFN1	Outer mitochondrial membrane	Outer mitochondrial membrane fusion	[93]
Mfn2	MFN2	Outer mitochondrial membrane ER	Outer mitochondrial membrane fusion Facilitating mitochondrial calcium influx from ER ER–mitochondrial contact site tethering Facilitates mitophagy as a Parkin receptor	[94] [95] [96] [97]
L-Opa1 (long form)	OPA1	Inner mitochondrial membrane	Inner mitochondrial membrane fusion	[98]
S-Opa1 (short form)	OPA1	Mitochondrial intermembrane space	Cristal morphogenesis (together with L-Opa1)	
YME1L	ATP-dependent zinc metalloprotease YME1L	Inner mitochondrial membrane	Cleavage of L-Opa1 into S-Opa1	
Oma1	Zinc metallopeptidase	Inner mitochondrial membrane	Cleavage of L-Opa1 during mitochondrial depolarization	
Fission proteins				
Drp1	Dynamin related protein 1	Outer mitochondrial membrane	Mitochondrial fission	[99]
Fis 1	Mitochondrial fission 1	Outer mitochondrial membrane	Putative Drp1 receptor; may facilitate Drp1-mediated mitochondrial fission	
Mff	Mitochondrial fission factor	Outer mitochondrial membrane	Mitochondrial receptor for Drp1; facilitates Drp1-mediated mitochondrial fission	
MiD49	Mitochondrial dynamics protein of 49 kDa	Outer mitochondrial membrane	Mitochondrial receptor for Drp1; may regulate the DRP1 and maintain its inactive state until fission is required	
MiD51	Mitochondrial dynamics protein of 51 kDa	Outer mitochondrial membrane	Mitochondrial receptor for Drp1; facilitates Drp1-mediated mitochondrial fission (Fis1 and Mff independent)	

mice [31]. In line with these findings, reduced propagation of mitochondrial matrix-targeted photoactivatable GFP was observed in the skeletal muscle of mice fed a HFD compared with chow-fed controls [32], indicating a reduction in the degree of mitochondrial networking. Importantly, the presence of increased mitochondrial fragmentation in skeletal muscle has also been observed in humans with obesity and **type 2 diabetes (T2D)** compared with a lean reference group. This morphological phenotype was restored 1.5 years after **bariatric surgery** [33]. Similarly, mitochondrial capacity in patients undergoing **laparoscopic gastric banding surgery** was normalized 1 year post-surgery, which was independent of changes in mitochondrial content [34]. Taken together, these studies suggest an interdependency of mitochondrial form and function in obese patients. In young, healthy humans, induction of insulin resistance by lipid infusion resulted in skeletal muscle mitochondrial fragmentation in association with activation of DRP1 by phosphorylation at serine 616 ($Drp1^{ser616}$) [35]. Strikingly, this effect was observed without a reduction in mitochondrial capacity or content, which suggests that functional defects may be secondary to chronic lipotoxicity. Primary myotubes cultured from skeletal muscle biopsies obtained from severely obese non-diabetic and T2D participants retained a more fragmented mitochondrial network relative to young, lean, healthy individuals [36]. This change in the mitochondrial network was associated with increased $Drp1^{ser616}$. Moreover, the number of fragmented mitochondria in cultured myotubes from the same population was reduced at 7 months after **Roux-en-Y gastric bypass (RYGB)** [37] concomitant with reductions in $Drp1^{ser616}$. The retention of these morphological characteristics in primary myotubes despite identical cell culture conditions suggests the involvement of transcriptional programming and is therefore not solely an acute response to a positive energy balance and lipotoxicity.

Regulation of skeletal muscle mitochondrial morphology in metabolic disorders

Mitochondrial fusion

Protein expression studies also provide insights into the role of mitochondrial dynamics in metabolic disorders. Several lines of evidence support negative transcriptional regulation of **mitochondrial fusion** as an underlying component of mitochondrial fragmentation in metabolic disease. Reduced mitofusin-2 (MFN2) mRNA and protein expression is observed in skeletal muscle in obese rodents relative to lean controls [30,32,38,39] and in humans [30,40,41]. Repression of MFN2 in a rat muscle cell line decreased pyruvate and palmitate oxidation [30], increased the production of ROS, and increased c-Jun N-terminal kinases (JNKs) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) signaling in association with reduced glucose uptake and insulin signaling (Figure 2). Conversely, MFN2 gain of function reduced ROS production and restored insulin signaling [42,43]. Similarly, in humans MFN2 mRNA correlated with glucose oxidation and increases in mRNA levels following bariatric surgery were positively correlated with the percentage change in glucose oxidation between fasted and insulin-stimulated conditions during a hyperinsulinemic–euglycemic clamp procedure [40]. In healthy premenopausal women, MFN2 protein expression was associated with ROS-emitting capacity in permeabilized skeletal muscle fibers [44]. Expression of the inner mitochondrial membrane fusion protein optic atrophy-1 (OPA1), along with the mitochondrial fission protein FIS1, was highest in athletes compared with individuals with T2D [45]. Enhanced mitochondrial networking was also apparent in the athletes [45], perhaps indicating that athletes exhibit enhanced mitochondrial turnover through both mitochondrial fusion and fission, although with fusion the predominant factor in the resting homeostatic state. Provision of a eucaloric diet along with aerobic exercise training in older obese individuals induced a significant weight loss and resulted in increased skeletal muscle OPA1 [46]. This further supports a profusion mitochondrial phenotype in response to exercise and a negative energy balance. In addition, as in a rat model [47], RYGB surgery in obese patients increased skeletal muscle MFN2 gene expression at 3 and 12 months post-surgery in obese individuals [48]. However, in contrast to the above findings, several studies reported that skeletal muscle mitochondrial fusion gene and/or protein expression were not altered

in human obesity [49] or T2D [50]. Moreover, single-fiber analysis of skeletal muscle in morbid obesity revealed that the amount of both MFN1/2 was higher in muscle fibers that displayed fragmented networks [33]. An alternative explanation for these discrepancies may relate to the noncanonical functions of MFN2 such as involvement in lipid droplet [51] and sarcoplasmic reticulum (SR)–mitochondria contact sites [52]. In particular, dysregulated SR–mitochondria interactions have been implicated in metabolic disease in animals and humans [51,53,54]. Moreover, a recent study [55] demonstrated that, in healthy individuals, fat oxidation was affected by both mitochondrial size and SR–mitochondria contact sites. It is therefore worth acknowledging that alterations in fusion proteins may also influence metabolic disease independent of their effects on mitochondrial morphology.

Mitochondrial fission

Besides reduced fusion, excessive mitochondrial fission has also been implicated in skeletal muscle metabolic dysfunction. Similar to humans [35], in C2C12 cells and rodent muscle, excess lipids resulted in mitochondrial fragmentation and reduced glucose uptake [31]. This was attenuated by inhibition of DRP1 [31]. Conversely, exercise training in obese humans results in a reduction in DRP1 activation, which was correlated with improvements in insulin sensitivity and fat oxidation [56]. Along the same lines, expression of FIS1 was elevated in the muscle of obese and T2D humans and reduced by diet and exercise and RYGB. DRP1-mediated mitochondrial fragmentation has also been implicated in ceramide-induced H_2O_2 production, impaired mitochondrial bioenergetics, and insulin resistance in C2C12 myotubes [57]. Recent evidence from liver cells supports a role for the DRP1 adaptor protein MFF in mediating ceramide-induced metabolic dysfunction [58]. Ceramide species, particularly C16:0 ceramides, are a major lipotoxic lipid class and are thought to be significant mediators of insulin resistance [59]. These data further support a link between lipotoxicity and mitochondrial fragmentation in skeletal muscle metabolic dysfunction observed in metabolic disorders.

Obviously, mitochondrial morphology is defined by a balance between the rates of fusion and fission and it may be that mitochondrial morphology *per se* modulates substrate metabolism and insulin sensitivity regardless of whether shifts in morphology are driven by alterations in either fission or fusion. Development of biomarkers of mitochondrial morphology in bigger datasets encompassing healthy and obese participants is therefore essential to advance our understanding of the role of mitochondrial dynamics in metabolic disorders. In this context, focusing on the ratio between fusion and fission protein expression rather than the individual proteins may prove to be a useful strategy. Several attempts to this effect have been made, such as OPA1-FIS1 [46] and MFN2-FIS1 [60]; however, further validation may be necessary. Nonetheless, imaging remains the gold standard for the determination of mitochondrial morphology, and advances in imaging techniques have been instrumental in enhancing our understanding of mitochondrial networks in skeletal muscle. Mitochondria in skeletal muscle observed by electron microscopy appear in tightly packed clusters with many mitochondria grouped together in subsarcolemmal regions and appearing in doublets in the intermyofibrillar regions of the muscle. 3D imaging of rodent muscle, and more recently human muscle, indicate that skeletal muscle relies on the formation of nanotunnels between mitochondria for the efficient transmission of membrane potential and transfer of mitochondrial DNA (mtDNA) and proteins [16]. Nanotunneling is reduced in obese mice [32], but data in skeletal muscle of obese humans are lacking. Nonetheless, this discovery represents an additional challenge in defining the relationship between mitochondrial dynamics and mitochondrial morphology in human disease, as the finding of increased fragmentation, despite increased **mitochondrial nanotunneling**, observed in humans with primary mtDNA mutations illustrates [16].

Table 2. Summary of literature linking mitochondrial morphology and dynamics in humans to age- and obesity- associated skeletal muscle dysfunction

Participants	Treatment/intervention	Mitochondrial morphology	Fusion gene/protein expression	Fission gene/protein expression	Functional outcome	Refs
Morbid obesity with and without T2D vs lean controls		↑ Fragmentation in obese groups	↑ Mfn1/2 in obese	↑ Drp1 in obese and T2D	Reduced insulin sensitivity in obese and T2D	[33]
	RYGB	↓ Fragmentation in some obese and T2D patients		↓ Fis1	↑ Insulin sensitivity after RYGB	
Young healthy	Lipid infusion	↑ Fragmentation	No change	↑ $Drp1^{ser616}$	Reduced insulin sensitivity No change in mitochondrial capacity Reduced mitochondrial membrane potential	[35]
Cultured myotubes from severely obese and T2D patients vs young, lean healthy		↑ Fragmentation in Obesity and T2D		↑ $Drp1^{ser616}$ in obesity and T2D		[36]
Cultured myotubes from severely obese and T2D patients	RYGB	↓ Fragmentation following surgery		↓ $Drp1^{ser616}$ following surgery		[37]
Obese and T2D vs lean controls			↓ Mfn2 in obese and T2D			[30]
Severely obese females	Bariatric surgery		↓ Mfn2 mRNA following weight loss		↓ Mfn2 mRNA correlated with improved insulin sensitivity	[40]
Elderly with heart failure vs healthy age-matched controls			↓ Mfn2 in patients with heart failure		Mfn2 was positively associated with exercise performance	[41]
Healthy premenopausal women	Hyperinsulinemic–euglycemic clamp		No change	No change	Association between Mfn2 expression and ROS	[44]
Lean healthy, obese, T2D, athletes		↑ Fragmentation in T2D relative to athletes	OPA1 highest in athletes and lowest in T2D	Fis1 highest in athletes and lowest in T2D		[45]
Older obese individuals	12 weeks diet and exercise		↑ OPA1 following intervention			[46]
Morbidly obese women	RYGB		↓ Mfn2 mRNA following weight-loss surgery		Association between increased MFN2 and glucose uptake following surgery	[48]
Obese vs age-matched lean			No difference	No difference		[49]
T2D patients vs weight-matched controls			No difference	Drp1 mRNA (DNM1L) was lower in the T2D group		[50]
Older obese individuals	12 weeks exercise		↑OPA1 mRNA after exercise training	↓ $Drp1^{ser616}$	Association between changes in Drp1ser616 and improvements in insulin sensitivity and fat oxidation	[56]
Young active, young sedentary, middle-aged sedentary, and older sedentary			↑ OPA1 and Mfn2 in young active	↑ Fis1 and Drp1 in young active ↓ Drp1 in middle aged sedentary	Negative relationship between Fusion and fission proteins with BMI	[10]

(continued on next page)

Table 2. (continued)

Participants	Treatment/intervention	Mitochondrial morphology	Fusion gene/protein expression	Fission gene/protein expression	Functional outcome	Refs
Young sedentary, high-functioning elderly, low-functioning elderly			↓ OPA1 with age, no effect of activity	No difference		[77]
Young men vs old men	12 weeks exercise		No difference between groups at baseline; Mfn1/2 increased following exercise training in both groups	No difference between groups at baseline; Fis1 increased following exercise training in both groups		[78]
Young sedentary, sedentary seniors (sarcopenic), senior sportsmen			↓ Mfn 1/2 and OPA1 in sedentary seniors compared with young sedentary and senior sportsmen	↓ Drp1 in sedentary seniors compared with young sedentary and senior sportsmen		[79]
Elderly patients with hip fracture divided into sarcopenic and non-sarcopenic groups			↓ Mfn 2 in sarcopenic elderly	No change		[80]
Older adults	10 days bed rest followed by 8 weeks resistance training		No change with bedrest or resistance training	No change with bedrest or resistance training		[89]

Aberrant mitochondrial network morphology is a hallmark of skeletal muscle myopathy

Mitochondrial morphology has long been associated with myopathy [61]. Indeed, abnormalities in muscle mitochondrial structure became a diagnostic factor for inherited skeletal muscle myopathies [62] in the latter third of the 20th century. While it is beyond the scope of this review to provide a comprehensive overview of inherited and developed myopathies with a mitochondrial defect, it is nonetheless instructive to highlight some instances that illustrate a clear link between aberrant mitochondrial morphology and skeletal muscle myopathy. In humans, mutations in the MFN2 gene result in Charcot–Marie–Tooth type 2A, a neuropathic disorder characterized by progressive muscle atrophy [63]. Autosomal-dominant optic atrophy (ADOA) mutations in the *OPA1* gene result in muscle wasting associated with reductions in ATP-generating capacity and increased mtDNA mutations in skeletal muscle [64]. More recently a novel myopathy caused by a homozygous nonsense mutation in the *MIEF2* gene encoding mitochondrial dynamics protein of 49 kDa (MID49) was associated with increased fusion, mitochondrial elongation, and aberrant cristal structure [65]. In cancer **cachexia** and chemotherapy-induced cachexia, *DRP1*, *OPA1*, and *MFN2* gene expression were dramatically suppressed [66,67].

Mitochondrial morphology in aging muscle

Sarcopenia is the age-associated loss of muscle mass and strength. In aging, muscle mass begins to decline as early as age 40 years at a rate of up to 1% per year, while strength declines even more rapidly [68]. In aged muscle, reports from rodent studies suggest that aged mitochondria are smaller in size, while others suggest an increase in mitochondrial networking. Moreover, aged skeletal muscle displays impaired mitochondrial energetics [69] and increased mitochondria-mediated apoptosis [70]. However, 2D imaging of human skeletal muscle has identified the presence of giant mitochondria in aged muscle indicating that there may be multiple mechanisms at play in the regulation of mitochondrial morphology in aged skeletal muscle.

Interestingly, giant mitochondria in cultured myoblasts display both reduced levels of OPA1 and impaired capacity for fusion and autophagy [71]. In addition, muscle of aged mice also displayed abnormal mitochondrial structure and crista formation (Figure 2) [72,73]. These morphological alterations are associated with reduced muscle cross-sectional area and reduced grip strength concomitant with reduced levels of OPA1 and DRP1 protein expression [73]. Consistent with this finding, grip strength is reduced in mice with a muscle-specific OPA knockout [74]. The discrepancies in mitochondrial morphologies reported in studies of aging may relate to nonlinear changes in fission and fusion over the lifespan [75]. These morphological alterations correlated with changes in muscle function evidenced at the different stages of life of mice. Taken together, these data illustrate a potential causal relationship between mitochondrial morphology and age-associated diseases of muscle weakness.

Mitochondrial dynamics and age-associated sarcopenia

Several studies have indicated similar mitofusin gene [76] and protein [10,77,78] expression in skeletal muscle biopsies of young versus old volunteers. In addition, skeletal muscle OPA1 expression was unchanged [10] or decreased [77,79] in elderly skeletal muscle; however, the degree to which these elderly participants were sarcopenic is not apparent. In a recent study [80], MFN2 protein expression was lower in elderly patients with sarcopenia than in non-sarcopenic elderly. This suggests that clearer characterization of sarcopenia status, or long-term prospective studies in the elderly, may be needed to determine the degree to which altered mitochondrial fusion is related to age-associated physical decline in humans. Despite this, data from preclinical studies provide robust support for a link between altered mitochondrial fusion and age-related decline in muscle health. A reduction in both MFN1 and MFN2 has been demonstrated in aged mice, while global *Mfn2* knockout in mice resulted in mitochondrial dysfunction, increased oxidative stress, a reduction in ambulatory activity, and reduced muscle cross-sectional area. Notably, *Mfn2* knockout in mice resulted in muscle atrophy by downregulation of protein synthesis via the mTOR pathway and an accumulation of autophagosomes (Figure 2) [81]. This is indicative of a reduction in autophagy, the cellular mechanism that maintains tissue quality by the removal of damaged cellular components. Reduced autophagy is a common feature of muscle atrophy [82,83] and aging [84] and has been linked to reduced MFN2 in muscle cells [85]. MFN2 repression was also associated with the accumulation of defective mitochondria despite increased mitophagy signaling via ROS-dependent upregulation of BNIP3 and Parkin (Figure 2) [81]. Interestingly, Parkin-mediated ubiquitination and degradation of MFN2 also induces muscle-disuse atrophy through excessive mitochondrial fragmentation and mitophagy (Figure 2). This can be rescued by increasing mitochondrial mass through overexpression of the mitochondrial biogenesis activator peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) [86].

Opa1 depletion leads to reduced grip strength and muscle atrophy in rodents via elevated production of ROS [87], enhanced expression of proinflammatory genes [87], activation of the atrogenic forkhead box Os (FOXOs) class of transcription factors [88], and increased muscle inflammation characterized by NFκB activation (Figure 2). Moreover, depletion of the OPA1 cleavage factor ATP-dependent zinc metalloprotease YME1L (YME1L) in myotubes results in accumulation of the OPA1 short form and the activation of AMP-activated protein kinase (AMPK) and FOXO3, thereby leading to increased muscle RING-finger protein-1 (MuRF1) (Figure 2) [88]. Reduced OPA1 has also been implicated in skeletal muscle atrophy via both endoplasmic reticulum (ER)-stress-mediated translational inhibition and upregulation of atrogenic genes such as MuRF1, Atrogin 1, and fibroblast growth factor 21 (FGF21) in rodent models (Figure 2) [79]. Collectively, these data illustrate how downregulation of profusion proteins in skeletal muscle can activate catabolic systems and ultimately cause muscle mass loss. However,

Clinician's corner

Mitochondria are multifaceted organelles that participate in a variety of cellular functions far beyond simple energy provision. The ability of mitochondria to adapt to environmental stimuli and support metabolism and cellular homeostasis is governed by shifts in mitochondrial morphology; however, increasing evidence suggests that aberrant mitochondrial morphology is present in numerous pathological conditions, including metabolic disease and age-associated skeletal muscle dysfunction. In addition, animal studies suggest that dysregulation of mitochondrial morphology may be mechanistically linked to pathogenesis. Currently, there are no pharmaceutical agents available to target these pathways; however, diet and exercise and bariatric surgery in humans and animals have been effective at normalizing mitochondrial morphology and dynamics in association with improved body composition and metabolic outcomes.

whether these findings translate to age-associated declines in muscle protein synthesis and sarcopenia in human aging remains an open question.

The evidence supporting increased mitochondrial fission in skeletal muscle sarcopenia in humans is limited, with studies showing similar protein expression of DRP1 and FIS1 in young and old sedentary adults [10,77] and following 10 days of bed rest in older adults [89]. Nonetheless, there is evidence for both knockout and overexpression of *Drp1* resulting in muscle atrophy, albeit through the activation of separate atrogenic pathways (Figure 2) [90]. Intriguingly, muscle-specific *Opa1* and *Drp1* DKO mice had a less severe phenotype than *Opa1* knockout mice, despite persistent mitochondrial dysfunction. This effect was primarily through blunted atrogenic transcriptional activity and reduced FGF21, although at the cost of translational inhibition via increased ER stress and reduced autophagy and mitophagy (Figure 2) [90]. By contrast, data from mouse cardiomyocytes [91] and *Caenorhabditis elegans* suggest that co-deletion of fission and fusion protein expression results in decreases in lifespan and metabolic health. Nonetheless, these data highlight the need to maintain the appropriate balance between fission and fusion for the preservation of skeletal muscle mass and function. Interestingly, muscle cells incubated with palmitate lose skeletal muscle integrity via modulation of mitochondrial fission gene expression, proinflammatory cytokines, and atrogenes, yet this effect was attenuated by co-incubation with oleate [92]. This suggests that the fatty acid-mediated mitochondrial redox state is a significant factor in the regulation of myotube atrophy. This finding is consistent with the effects of lipids on skeletal muscle mitochondrial fragmentation in insulin resistance in cells and animals [31] and humans [35] and therefore highlights the potential interconnectedness between muscle mitochondrial dynamics in both skeletal muscle metabolic function and quality.

Concluding remarks

There is growing evidence for impaired mitochondrial network dynamics in the etiology of skeletal muscle health in metabolic disorders and aging (Table 2). However, what this review highlights is that there remains a scarcity of detailed mechanistic studies, particularly in human models. Such studies will be essential to understand the role of altered mitochondrial networks in the pathogenesis of muscle dysfunction and for the development of new therapeutics to target mitochondrial dynamics in human disease. Nonetheless, new technologies that have emerged including advances in imaging technology with increasing application to human tissue have shed new light on the organization of mitochondrial networks in muscle; these approaches open the possibility for deeper insights into the role of skeletal muscle mitochondrial dynamics in metabolic disorders and aging (see Outstanding questions).

Outstanding questions

What is the contribution of increasing adiposity and lipotoxicity to dysregulated mitochondrial morphology and sarcopenia?

Despite increasing evidence for aberrant mitochondrial morphology underlying metabolic disease and sarcopenia, questions remain regarding the mechanistic processes that drive the pathogenesis. Alterations of morphology by targeting both fission and fusion proteins independently are sufficient to drive functional deficits in muscle; however, the question remains – can the onset of muscle dysfunction in aging and metabolic disease be attributed to fission, fusion, or the morphology itself?

How do changes in morphology mediate negative downstream effects in human aging and metabolic disease?

What is the contribution of nanotunnels and other forms of mitochondrial–organellar communication to aging and obesity associated diseases?

Are there existing pharmacological agents that can target these pathways effectively and safely or can they be developed?

Declaration of interests

No interests are declared.

References

1. Kaeberlein, M. (2018) How healthy is the healthspan concept? *Geroscience* 40, 361–364
2. Chang, V.W. *et al.* (2017) Trends in the relationship between obesity and disability, 1988–2012. *Am. J. Epidemiol.* 186, 688–695
3. Kanasi, E. *et al.* (2016) The aging population: demographics and the biology of aging. *Periodontol* 2000, 13–18
4. Mehta, T. *et al.* (2014) Obesity and mortality: are the risks declining? Evidence from multiple prospective studies in the United States. *Obes. Rev.* 15, 619–629
5. Batsis, J.A. *et al.* (2014) Sarcopenia, sarcopenic obesity and mortality in older adults: results from the National Health and Nutrition Examination Survey III. *Eur. J. Clin. Nutr.* 68, 1001–1007
6. Baumgartner, R.N. *et al.* (2004) Sarcopenic obesity predicts instrumental activities of daily living disability in the elderly. *Obes. Res.* 12, 1995–2004
7. Abbasi, F. *et al.* (2002) Relationship between obesity, insulin resistance, and coronary heart disease risk. *J. Am. Coll. Cardiol.* 40, 937–943
8. Chambers, T.L. *et al.* (2020) Skeletal muscle size, function, and adiposity with lifelong aerobic exercise. *J. Appl. Physiol. (1985)* 128, 368–378
9. St-Jean-Pelletier, F. *et al.* (2017) The impact of ageing, physical activity, and pre-frailty on skeletal muscle phenotype, mitochondrial content, and intramyocellular lipids in men. *J. Cachex. Sarcopenia Muscle* 8, 213–228

10. Distefano, G. *et al.* (2017) Chronological age does not influence *ex-vivo* mitochondrial respiration and quality control in skeletal muscle. *J. Gerontol. A Biol. Sci. Med. Sci.* 72, 535–542
11. Kang, Y.M. *et al.* (2017) Visceral adiposity index predicts the conversion of metabolically healthy obesity to an unhealthy phenotype. *PLoS One* 12, e0179635
12. Okamura, T. *et al.* (2019) Ectopic fat obesity presents the greatest risk for incident type 2 diabetes: a population-based longitudinal study. *Int. J. Obes.* 43, 139–148
13. Murton, A.J. *et al.* (2015) Obesity appears to be associated with altered muscle protein synthetic and breakdown responses to increased nutrient delivery in older men, but not reduced muscle mass or contractile function. *Diabetes* 64, 3160–3171
14. Yagi, S. *et al.* (2014) Association of lower limb muscle mass and energy expenditure with visceral fat mass in healthy men. *Diabetol. Metab. Syndr.* 6, 27
15. Gupte, T.M. (2015) Mitochondrial fragmentation due to inhibition of fusion increases cyclin B through mitochondrial superoxide radicals. *PLoS One* 10, e0126829
16. Vincent, A.E. *et al.* (2019) Quantitative 3D mapping of the human skeletal muscle mitochondrial network. *Cell Rep.* 26, 996–1009.e4
17. Glancy, B. *et al.* (2015) Mitochondrial reticulum for cellular energy distribution in muscle. *Nature* 523, 617–620
18. Glancy, B. *et al.* (2017) Power grid protection of the muscle mitochondrial reticulum. *Cell Rep.* 19, 487–496
19. Nunnari, J. *et al.* (1997) Mitochondrial transmission during mating in *Saccharomyces cerevisiae* is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. *Mol. Biol. Cell* 8, 1233–1242
20. Al-Mehdi, A.B. *et al.* (2012) Perinuclear mitochondrial clustering creates an oxidant-rich nuclear domain required for hypoxia-induced transcription. *Sci. Signal.* 5, ra47
21. Lalia, A.Z. *et al.* (2016) Predictors of whole-body insulin sensitivity across ages and adiposity in adult humans. *J. Clin. Endocrinol. Metab.* 101, 626–634
22. Toyama, E.Q. *et al.* (2016) Metabolism. AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. *Science* 351, 275–281
23. Wu, W. *et al.* (2016) FUNDC1 regulates mitochondrial dynamics at the ER–mitochondrial contact site under hypoxic conditions. *EMBO J.* 35, 1368–1384
24. Favaro, G. *et al.* (2019) DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. *Nat. Commun.* 10, 2576
25. Hung, C.H. *et al.* (2018) A reciprocal relationship between reactive oxygen species and mitochondrial dynamics in neurodegeneration. *Redox Biol.* 14, 7–19
26. Baker, N. *et al.* (2019) Linking mitochondrial dynamics, cristae remodeling and supercomplex formation: how mitochondrial structure can regulate bioenergetics. *Mitochondrion* 49, 259–268
27. Liu, Y.J. *et al.* (2020) Mitochondrial fission and fusion: a dynamic role in aging and potential target for age-related disease. *Mech. Ageing Dev.* 186, 111212
28. Zhang, Q. *et al.* (2019) ROCK1 induces dopaminergic nerve cell apoptosis via the activation of Drp1-mediated aberrant mitochondrial fission in Parkinson's disease. *Exp. Mol. Med.* 51, 1–13
29. Manczak, M. *et al.* (2011) Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. *Hum. Mol. Genet.* 20, 2495–2509
30. Bach, D. *et al.* (2003) Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. *J. Biol. Chem.* 278, 17190–17197
31. Jheng, H.F. *et al.* (2012) Mitochondrial fission contributes to mitochondrial dysfunction and insulin resistance in skeletal muscle. *Mol. Cell. Biol.* 32, 309–319
32. Liu, R. *et al.* (2014) Impaired mitochondrial dynamics and bioenergetics in diabetic skeletal muscle. *PLoS One* 9, e92810
33. Kristensen, M.D. *et al.* (2018) Obesity leads to impairments in the morphology and organization of human skeletal muscle lipid droplets and mitochondrial networks, which are resolved with gastric bypass surgery-induced improvements in insulin sensitivity. *Acta Physiol. (Oxf.)* 224, e13100
34. Vijgen, G.H. *et al.* (2013) Impaired skeletal muscle mitochondrial function in morbidly obese patients is normalized one year after bariatric surgery. *Surg. Obes. Relat. Dis.* 9, 936–941
35. Axelrod, C.L. *et al.* (2021) Lipids activate skeletal muscle mitochondrial fission and quality control networks to induce insulin resistance in humans. *Metabolism* 121, 154803
36. Gundersen, A.E. *et al.* (2020) Altered mitochondrial network morphology and regulatory proteins in mitochondrial quality control in myotubes from severely obese humans with or without type 2 diabetes. *Appl. Physiol. Nutr. Metab.* 45, 283–293
37. Kugler, B.A. *et al.* (2020) Roux-en-Y gastric bypass surgery restores insulin-mediated glucose partitioning and mitochondrial dynamics in primary myotubes from severely obese humans. *Int. J. Obes.* 44, 684–696
38. Pich, S. *et al.* (2005) The Charcot–Marie–Tooth type 2A gene product, Mfn2, up-regulates fuel oxidation through expression of OXPHOS system. *Hum. Mol. Genet.* 14, 1405–1415
39. Greene, N.P. *et al.* (2015) Mitochondrial quality control, promoted by PGC-1α, is dysregulated by Western diet-induced obesity and partially restored by moderate physical activity in mice. *Physiol. Rep.* 3, e12470
40. Mingrone, G. *et al.* (2005) Could the low level of expression of the gene encoding skeletal muscle mitofusin-2 account for the metabolic inflexibility of obesity? *Diabetologia* 48, 2108–2114
41. Molina, A.J. *et al.* (2016) Skeletal muscle mitochondrial content, oxidative capacity, and Mfn2 expression are reduced in older patients with heart failure and preserved ejection fraction and are related to exercise intolerance. *JACC Heart Fail.* 4, 636–645
42. Nie, Q. *et al.* (2014) Mitofusin 2 deficiency leads to oxidative stress that contributes to insulin resistance in rat skeletal muscle cells. *Mol. Biol. Rep.* 41, 6975–6983
43. Kong, D. *et al.* (2013) Overexpression of mitofusin 2 improves translocation of glucose transporter 4 in skeletal muscle of high-fat diet-fed rats through AMP-activated protein kinase signaling. *Mol. Med. Rep.* 8, 205–210
44. Warren, J.L. *et al.* (2017) Effects of acute hyperinsulinemia on skeletal muscle mitochondrial function, reactive oxygen species production, and metabolism in premenopausal women. *Metabolism* 77, 1–12
45. Houzelle, A. *et al.* (2021) Human skeletal muscle mitochondrial dynamics in relation to oxidative capacity and insulin sensitivity. *Diabetologia* 64, 424–436
46. Axelrod, C.L. *et al.* (2019) Exercise training remodels human skeletal muscle mitochondrial fission and fusion machinery towards a pro-elongation phenotype. *Acta Physiol. (Oxf.)* 225, e13216
47. Sacks, J. *et al.* (2018) Effect of Roux-en-Y gastric bypass on liver mitochondrial dynamics in a rat model of obesity. *Physiol. Rep.* 6, e13600
48. Gastaldi, G. *et al.* (2007) Upregulation of peroxisome proliferator-activated receptor gamma coactivator gene (PGC1A) during weight loss is related to insulin sensitivity but not to energy expenditure. *Diabetologia* 50, 2348–2355
49. Holloway, G.P. *et al.* (2008) PGC-1α's relationship with skeletal muscle palmitate oxidation is not present with obesity despite maintained PGC-1α and PGC-1β protein. *Am. J. Physiol. Endocrinol. Metab.* 294, E1060–E1069
50. Kruse, R. *et al.* (2017) Intact initiation of autophagy and mitochondrial fission by acute exercise in skeletal muscle of patients with type 2 diabetes. *Clin. Sci. (Lond.)* 131, 37–47
51. Boutant, M. *et al.* (2017) Mfn2 is critical for brown adipose tissue thermogenic function. *EMBO J.* 36, 1543–1558
52. Sebastian, D. *et al.* (2012) Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis. *Proc. Natl. Acad. Sci. U. S. A.* 109, 5523–5528
53. Rieusset, J. (2018) The role of endoplasmic reticulum-mitochondria contact sites in the control of glucose homeostasis: an update. *Cell Death Dis.* 9, 388
54. Arruda, A.P. *et al.* (2014) Chronic enrichment of hepatic endoplasmic reticulum–mitochondria contact leads to mitochondrial dysfunction in obesity. *Nat. Med.* 20, 1427–1435

55. del Campo, A. *et al.* (2014) Mitochondrial fragmentation impairs insulin-dependent glucose uptake by modulating Akt activity through mitochondrial Ca^{2+} uptake. *Am. J. Physiol. Endocrinol. Metab.* 306, E1–E13
56. Fealy, C.E. *et al.* (2014) Exercise training decreases activation of the mitochondrial fission protein dynamin-related protein-1 in insulin-resistant human skeletal muscle. *J. Appl. Physiol. (1985)* 117, 239–245
57. Smith, M.E. *et al.* (2013) Mitochondrial fission mediates ceramide-induced metabolic disruption in skeletal muscle. *Biochem. J.* 456, 427–439
58. Hammerschmidt, P. *et al.* (2019) CerS6-derived sphingolipids interact with Mff and promote mitochondrial fragmentation in obesity. *Cell* 177, 1536–1552.e23
59. Reali, F. *et al.* (2017) Mechanistic interplay between ceramide and insulin resistance. *Sci. Rep.* 7, 41231
60. Castro-Sepulveda, M. *et al.* (2020) Relative lipid oxidation associates directly with mitochondrial fusion phenotype and mitochondria-sarcoplasmic reticulum interactions in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 318, E848–E855
61. Luft, R. *et al.* (1962) A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical, and morphological study. *J. Clin. Invest.* 41, 1776–1804
62. Lombes, A. *et al.* (1989) Mitochondrial encephalomyopathies. *Rev. Neurol. (Paris)* 145, 671–689
63. Zuchner, S. *et al.* (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot–Marie–Tooth neuropathy type 2A. *Nat. Genet.* 36, 449–451
64. Delettre, C. *et al.* (2000) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat. Genet.* 26, 207–210
65. Bartsakoulia, M. *et al.* (2018) A novel mechanism causing imbalance of mitochondrial fusion and fission in human myopathies. *Hum. Mol. Genet.* 27, 1186–1195
66. Barreto, R. *et al.* (2016) Cancer and chemotherapy contribute to muscle loss by activating common signaling pathways. *Front. Physiol.* 7, 472
67. van der Ende, M. *et al.* (2018) Mitochondrial dynamics in cancer-induced cachexia. *Biochim. Biophys. Acta Rev. Cancer* 1870, 137–150
68. Mitchell, W.K. *et al.* (2012) Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Front. Physiol.* 3, 260
69. Gouspillou, G. *et al.* (2014) Mitochondrial energetics is impaired *in vivo* in aged skeletal muscle. *Aging Cell* 13, 39–48
70. Chabi, B. *et al.* (2008) Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. *Aging Cell* 7, 2–12
71. Navratil, M. *et al.* (2008) Giant mitochondria do not fuse and exchange their contents with normal mitochondria. *Exp. Cell Res.* 314, 164–172
72. Pietrangelo, L. *et al.* (2015) Age-dependent uncoupling of mitochondria from Ca^{2+} release units in skeletal muscle. *Oncotarget* 6, 35358–35371
73. Huang, D.D. *et al.* (2019) Nrf2 deficiency exacerbates frailty and sarcopenia by impairing skeletal muscle mitochondrial biogenesis and dynamics in an age-dependent manner. *Exp. Gerontol.* 119, 61–73
74. Pereira, R.O. *et al.* (2017) OPA1 deficiency promotes secretion of FGF21 from muscle that prevents obesity and insulin resistance. *EMBO J.* 36, 2126–2145
75. Del Campo, A. *et al.* (2018) Muscle function decline and mitochondria changes in middle age precede sarcopenia in mice. *Aging (Albany NY)* 10, 34–55
76. Bori, Z. *et al.* (2012) The effects of aging, physical training, and a single bout of exercise on mitochondrial protein expression in human skeletal muscle. *Exp. Gerontol.* 47, 417–424
77. Joseph, A.M. *et al.* (2013) Dysregulation of mitochondrial quality control processes contribute to sarcopenia in a mouse model of premature aging. *PLoS One* 8, e69327
78. Konopka, A.R. *et al.* (2014) Markers of human skeletal muscle mitochondrial biogenesis and quality control: effects of age and aerobic exercise training. *J. Gerontol. A Biol. Sci. Med. Sci.* 69, 371–378
79. Tezze, C. *et al.* (2017) Age-associated loss of OPA1 in muscle impacts muscle mass, metabolic homeostasis, systemic inflammation, and epithelial senescence. *Cell Metab.* 25, 1374–1389.e6
80. Marzetti, E. *et al.* (2016) Association between myocyte quality control signaling and sarcopenia in old hip-fractured patients: results from the Sarcopenia in Hip FracTure (SHIFT) exploratory study. *Exp. Gerontol.* 80, 1–5
81. Sebastian, D. *et al.* (2016) Mfn2 deficiency links age-related sarcopenia and impaired autophagy to activation of an adaptive mitophagy pathway. *EMBO J.* 35, 1677–1693
82. Masiero, E. and Sandri, M. (2010) Autophagy inhibition induces atrophy and myopathy in adult skeletal muscles. *Autophagy* 6, 307–309
83. Carnio, S. *et al.* (2014) Autophagy impairment in muscle induces neuromuscular junction degeneration and precocious aging. *Cell Rep.* 8, 1509–1521
84. Kroemer, G. (2015) Autophagy: a druggable process that is deregulated in aging and human disease. *J. Clin. Invest.* 125, 1–4
85. Ding, Y. *et al.* (2015) Mitofusin 2-deficiency suppresses cell proliferation through disturbance of autophagy. *PLoS One* 10, e0121328
86. Kang, C. and Ji, L.L. (2016) PGC-1α overexpression via local transfection attenuates mitophagy pathway in muscle disuse atrophy. *Free Radic. Biol. Med.* 93, 32–40
87. Rodriguez-Nuevo, A. *et al.* (2018) Mitochondrial DNA and TLR9 drive muscle inflammation upon Opa1 deficiency. *EMBO J.* 37, e96553
88. Lee, Y.J. *et al.* (2020) Down-regulation of the mitochondrial i-AAA protease Yme1L induces muscle atrophy via FoxO3a and myostatin activation. *J. Cell. Mol. Med.* 24, 899–909
89. Standley, R.A. *et al.* (2017) Effects of beta-hydroxy-beta-methylbutyrate on skeletal muscle mitochondrial content and dynamics, and lipids after 10 days of bed rest in older adults. *J. Appl. Physiol. (1985)* 123, 1092–1100
90. Romanello, V. *et al.* (2019) Inhibition of the fission machinery mitigates OPA1 impairment in adult skeletal muscles. *Cells* 8, 597
91. Song, M. *et al.* (2017) Abrogating mitochondrial dynamics in mouse hearts accelerates mitochondrial senescence. *Cell Metab.* 26, 872–883.e5
92. Lee, H. *et al.* (2017) Oleate prevents palmitate-induced atrophy via modulation of mitochondrial ROS production in skeletal myotubes. *Oxidative Med. Cell. Longev.* 2017, 2739721
93. Santel, A. *et al.* (2003) Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. *J. Cell Sci.* 116, 2763–2774
94. de Brito, O.M. and Scorrano, L. (2008) Mitofusin 2: a mitochondria-shaping protein with signaling roles beyond fusion. *Antioxid. Redox Signal.* 10, 621–633
95. Naon, D. *et al.* (2016) Critical reappraisal confirms that mitofusin 2 is an endoplasmic reticulum–mitochondria tether. *Proc. Natl. Acad. Sci. U. S. A.* 113, 11249–11254
96. Ainbinder, A. *et al.* (2015) Role of mitofusin-2 in mitochondrial localization and calcium uptake in skeletal muscle. *Cell Calcium* 57, 14–24
97. Chen, Y. and Dorn II, G.W. (2013) PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science* 340, 471–475
98. Del Dotto, V. *et al.* (2017) OPA1 isoforms in the hierarchical organization of mitochondrial functions. *Cell Rep.* 19, 2557–2571
99. Hu, C. *et al.* (2017) Drp1-dependent mitochondrial fission plays critical roles in physiological and pathological progresses in mammals. *Int. J. Mol. Sci.* 18, 144