REVIEW

*Journal of Cachexia, Sarcopenia and Muscle* 2017; **8**: 349–369
Published online 21 April 2017 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12178

# Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue

Kerstin Boengler[1], Maik Kosiol[1], Manuel Mayr[2], Rainer Schulz[1] & Susanne Rohrbach[1]*

[1]*Institute of Physiology, Justus Liebig University Giessen, Aulweg 129, 35392, Giessen, Germany;* [2]*King's British Heart Foundation Centre, King's College London, 125 Coldharbour Lane, London SE5 9NU, UK*

## Abstract

Age is the most important risk factor for most diseases. Mitochondria play a central role in bioenergetics and metabolism. In addition, several lines of evidence indicate the impact of mitochondria in lifespan determination and ageing. The best-known hypothesis to explain ageing is the free radical theory, which proposes that cells, organs, and organisms age because they accumulate reactive oxygen species (ROS) damage over time. Mitochondria play a central role as the principle source of intracellular ROS, which are mainly formed at the level of complex I and III of the respiratory chain. Dysfunctional mitochondria generating less ATP have been observed in various aged organs. Mitochondrial dysfunction comprises different features including reduced mitochondrial content, altered mitochondrial morphology, reduced activity of the complexes of the electron transport chain, opening of the mitochondrial permeability transition pore, and increased ROS formation. Furthermore, abnormalities in mitochondrial quality control or defects in mitochondrial dynamics have also been linked to senescence. Among the tissues affected by mitochondrial dysfunction are those with a high-energy demand and thus high mitochondrial content. Therefore, the present review focuses on the impact of mitochondria in the ageing process of heart and skeletal muscle. In this article, we review different aspects of mitochondrial dysfunction and discuss potential therapeutic strategies to improve mitochondrial function. Finally, novel aspects of adipose tissue biology and their involvement in the ageing process are discussed.

**Keywords** Mitochondria; Ageing; Heart; Skeletal muscle; Reactive oxygen species; Caloric restriction

*Received: 21 June 2016; Revised: 23 October 2016; Accepted: 24 November 2016*
**Correspondence to: Susanne Rohrbach, MD, Institute for Physiology, Justus Liebig University Giessen, Aulweg 129, 35392 Giessen, Germany. Fax: 0049-6 41 99-4 72 69, Email: susanne.rohrbach@physiologie.med.uni-giessen.de*

## Introduction

With ageing, the normal physiological functions of an organism gradually decline. Whereas the exact mechanisms responsible for senescence are not fully understood up to now, mitochondria have emerged as central regulators of the ageing process.[1] The primary function of mitochondria is to generate large quantities of ATP, but they are also involved in processes such as apoptosis, autophagy, reactive oxygen species (ROS) production, or calcium handling. Dysfunctional mitochondria generating less ATP have been observed in various aged organs including skeletal muscle, heart, and adipose tissue (AT). Indeed, mitochondrial function in aged skeletal muscle and aged myocardium is impaired at various levels including mitochondrial content and morphology, activity of the complexes of the electron transport chain (ETC), opening of the mitochondrial permeability transition pore (MPTP), ROS formation, and mitochondrial dynamics.

The prevalence of cardiovascular diseases increases with age, and dysfunctional cardiac mitochondria are considered to contribute, e.g. to myocardial ischemia/reperfusion injury, ventricular hypertrophy, cardiomyopathies, and heart failure.[2] However, cardiac mitochondrial subpopulations demonstrate significant differences in respiratory capacity or age-associated functional decline, and they also differ with respect to their ROS-generating ability and their antioxidant capacity in aged hearts. The expression of a variety of mitochondrial proteins is affected by ageing, and most of these differentially expressed proteins are involved in metabolism, respiratory chain function, or stress resistance, pointing to the central role of mitochondria in cardiac ageing. In skeletal

muscle, the aforementioned, diverse mitochondrial changes can contribute to an age-related loss in skeletal muscle mass and a decline in skeletal muscle function, a condition defined as sarcopenia.[3] Muscle mass and muscle strength begin to decline around the fourth decade, and this decline is accelerated with advancing age. Interventions such as physical activity that reduce oxidative damage and improve mitochondrial function cannot totally prevent but attenuate the age-associated rate of muscle loss as well as the functional decline. Although the number of mitochondria is lower in mature white adipocytes than in cardiac or in skeletal myocytes, mitochondrial function is essential for adipocyte function including secretion of adipokines and has an impact on distant organs. Mitochondrial dysfunction in AT triggers systemic insulin resistance and cardiac dysfunction. Furthermore, maintenance of mitochondrial function in AT is involved in the determination of lifespan, whereas obesity seems to accelerate ageing. The present review will address the different aspects of mitochondrial changes observed in ageing skeletal muscle, heart, and AT.

## Age-associated changes in the heart

### *Mitochondrial content and morphology in the aged myocardium*

The ultrastructure of the myocardium changes with ageing, and this involves alterations at the level of the mitochondria. Whereas some studies demonstrate a reduced number of mitochondria in the cytosol of aged cardiomyocytes,[4,5] others show that the mitochondrial volume fraction is unaltered during ageing.[6,7] Mitochondrial shape is altered with increasing age (less elongated and more round[8]), and the area of the mitochondrial inner membrane per mitochondrion is reduced in aged myocardium[9,10] although cristae configuration is not affected.[11]

To maintain a pool of healthy mitochondria during ageing, it is important to preserve mitochondrial structure. The serine/threonine protein kinases Pim are part of the proteins regulating mitochondrial morphology. Mice deficient in three Pim isoforms have a reduced mitochondrial area.[12] The loss of Pim kinases is associated with premature ageing, whereas the overexpression of Pim1, the predominant isoform in the heart, decreases the levels of senescence markers.[13] According to the dependence of mitochondrial function on the morphology of the organelle, the preservation of mitochondrial structure may help to delay the consequences of ageing.

### *Oxidative phosphorylation, cardiolipin, and cardiac ageing*

Due to the high-energy demand of the heart alterations in mitochondrial bioenergetics contribute to age-induced myocardial dysfunction, the changes in oxidative phosphorylation are due to alterations at different levels, e.g. the protein level and/or activity of complexes of the ETC or phospholipid composition of the inner mitochondrial membrane.

When analysing mitochondrial oxygen consumption, it has to be taken into account that cardiomyocytes contain two mitochondrial subpopulations, which differ in morphology and function: the subsarcolemmal mitochondria (SSM), which are present beneath the plasma membrane and the interfibrillar mitochondria (IFM), which are located between the myofibrils.[14] The cristae of SSM are predominantly lamelliform, whereas the cristae of IFM are mainly tubular or consist of a mixture of lamelliform and tubular structures.[15] IFM demonstrate a higher ADP-stimulated respiration and are more tolerant towards a $Ca^{2+}$ stimulus than SSM,[14,16,17] whereas SSM have a higher rate of protein synthesis than IFM.[18] Additionally, the specific ceramide distribution differs between SSM and IFM.[19] The spatial localization of mitochondria within cardiomyocytes may be associated with the need for specific responses to various physiological or pathophysiological stimuli.[20] The data obtained from the analysis of the respiratory capacity of mitochondria from aged myocardium are mainly dependent of the type of mitochondria studied. SSM isolated from aged rodent myocardium predominantly maintain their respiratory capacity,[21,22] whereas IFM consume less oxygen.[23,24] In line with the age-dependent reduction of oxygen, consumption in IFM is a decline in the activity of complexes of the ETC. Especially, the activities of respiratory complexes III and IV are reduced in IFM isolated from aged myocardium.[23–25] However, mitochondrial function is largely preserved in permeabilized aged cardiomyocytes.[26] The age-associated decline in mitochondrial function[23–25] may affect the production of cellular energy, which in turn can interfere with cardiac function. Although the ATP level may remain constant at rest, some studies indeed suggest a reduced ATP content or production.[27,28] Furthermore, mitochondrial biogenesis is impaired, and the expression of major regulators of mitochondrial biogenesis such as the peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) is reduced in the heart of aged animals and humans.[27,29–32] This can result in a further limitation of the organelle's ability to produce sufficient amounts of ATP to maintain optimal cardiac function.

Cardiolipin, a phospholipid specifically localized to the inner mitochondrial membrane, contributes to cristae structure and thereby influences the activities of ETC protein complexes.[33] The majority of studies investigating cardiolipin in the aged myocardium show decreased amounts and/or remodelling of this phospholipid.[34,35] Based on these findings, cardiolipin was considered to be a target in order to prevent ageing-induced decline in mitochondrial function. The administration of acetyl-L-carnitine, a normal component of the

mitochondrial membrane, to the aged rat heart restores the amount of cardiolipin and the ADP-stimulated respiration to the levels observed in young controls.[34] The inhibition of the rate limiting enzyme of the syntheses of the polyunsaturated fatty acids arachidonic acid and docosahexaenoic acid delta-6 desaturase (mainly expressed in brain, liver, lung, and heart,[36] indeed results in a reversal of the age-induced cardiolipin remodelling, yet oxidative phosphorylation was not affected.[37] The synthetic tetrapeptide SS-31 binds to cardiolipin and thereby protects cristae structure and enhances oxidative phosphorylation.[38,39] Although there are no data yet on oxygen consumption of mitochondria from aged myocardium, SS-31 reversed the age-related decline of mitochondrial ATP production in mitochondria from aged skeletal muscle[40] and reduced mortality in C57/BL/6 N mice subjected to transaortic constriction.[41]

## *Contribution of reactive oxygen species to myocardial ageing*

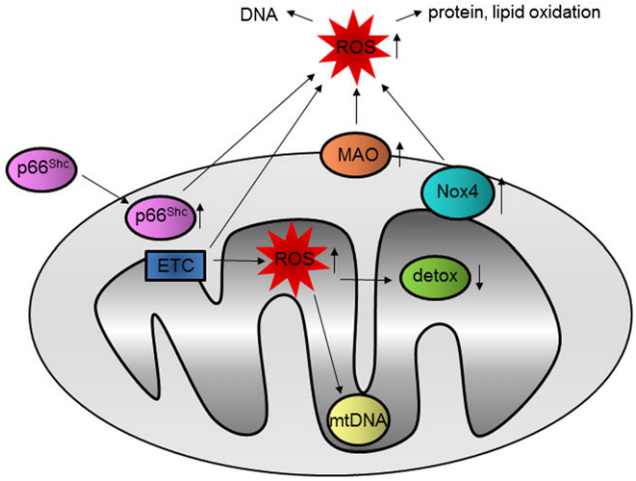
Within cardiomyocytes, ROS are generated in different compartments by different enzymes, including NADPH oxidases at the plasma membrane and xanthine oxidases in the cytosol. However, mitochondria are the most important cellular source of ROS. During ageing, activities of proteins of the ETC decline, and thus, oxidative phosphorylation is reduced. Impaired ETC complex activity is thereby directly linked to leakage of electrons from the ETC. Such electrons can reduce oxygen and thereby generate superoxide anions which in turn can be reduced to hydroxyl radicals and hydrogen peroxide. Whereas older studies indicate that around 2% of the oxygen consumed by mitochondria is used for ROS formation,[42] a more recent study shows that this value is presumably lower, i.e. 0.2% only.[43] In the heart, ROS mainly originate from ETC complexes I, II, and III.[44] In addition to the ETC, mitochondrial ROS are also produced by monoamino oxidases (MAO), which transfer electrons from amine compounds to oxygen and thereby generate hydrogen peroxide, and $p66^{Shc}$, which under physiological conditions resides in the cytosol, but translocates into the mitochondria upon stress signals.[45] Here, $p66^{Shc}$ induces the partial reduction of oxygen to hydrogen peroxide.[46] Also, a mitochondrial localization of NADPH oxidase 4 has been suggested using immunostaining of isolated cardiomyocytes.[47] In contrast, western blot analysis of purified mitochondria from mouse ventricular tissue did not detect the protein at the level of mitochondria under physiological conditions,[48] but this might change under pathophysiological conditions with ageing.[49]

Several studies detected an increase in ROS formation in aged myocardium[50–52] however, the exact origin of ROS in terms of the mitochondrial subpopulation involved is still under debate. According to Judge *et al*., hydrogen peroxide formation increases in both aged SSM and IFM, whereas the effect is more pronounced in SSM.[53] However, the enhanced level of hydrogen peroxide detected in SSM may be due to the higher antioxidant activity observed in IFM. In contrast, Suh *et al*. demonstrate increased ROS formation in old IFM,[25] whereas Hofer *et al*. detect no difference in ROS formation between aged SSM and IFM.[54] An increase in the level of mitochondrial $p66^{Shc}$ may contribute to the increased ROS formation observed in aged cardiac SSM.[55] Also, the elevation of MAO-A in the aged rat and MAO-B in the aged mouse heart may participate in cardiac ROS formation.[56] Despite the large number of studies demonstrating increased ROS formation with ageing, some studies do not show differences in ROS formation in aged myocardium.[57,58] These different findings might be explained by the diverse methods used to quantify the amounts of ROS, because the age of the animals analysed was similar between the studies and ranged mainly from 4–6 months (young animals) to 20–24 months (aged animals).

Excessive ROS formation causes detrimental effects on proteins and lipids, which induces cellular dysfunction and ultimately cell death. In addition, the proximity of the mitochondrial DNA to the site of ROS production in combination with the lack of protection of mitochondrial DNA by histones renders the mitochondrial DNA (mtDNA) highly susceptible to oxidative stress.[59] Indeed, mice with a proofreading deficient mutant of the mitochondrial polymerase $\gamma$ accumulate mutations in the mitochondrial DNA and have a reduced lifespan. Cardiomyocytes of these mice develop hypertrophy.[60] Furthermore, the induction of mitochondrial DNA mutations specifically in the heart reduces the replication of the mitochondrial DNA, the mitochondrial mass, and the antioxidant system. Mitochondrial dynamics are impaired in these mice, and the animals finally develop heart failure.[61] The use of the mitochondria-targeted ROS and electron scavenger XJB-5-131 improves respiratory function of ventricular mitochondria and renders the heart more resistant to oxidative stress during ageing.[22] Figure 1 shows a scheme of the role of ROS in myocardial ageing.

According to the free radical theory of ageing, enhanced ROS formation is associated with reduced lifespan. Indeed, mice with a mitochondrial-targeted overexpression of catalase demonstrate an attenuation of cardiac ageing[62] and extension of lifespan compared to wild-type mice.[63] In contrast, neither does the overexpression of the mitochondrial manganese superoxide dismutase-2 (MnSOD) prolong lifespan in mice[64] nor is the reduction of MnSOD to about 50% in heterozygous knockout mice associated with premature death.[65] The low hydrogen peroxide production of heart mitochondria from the long-lived pigeon is attributed to low levels of ETC complex I[66] and also complex I assembly is suggested to play a role in longevity in mice.[67] Data on the role of $p66^{Shc}$-derived ROS in longevity are controversial: whereas the initial study on $p66^{Shc}$-deficient mice shows reduced ROS formation and prolonged lifespan in this mouse strain;[68] a

**Figure 1** ROS formation in the aged myocardium. Within mitochondria, ROS are generated from the electron transport chain (ETC), from $p66^{Shc}$ in the intermembrane space, and from monoamino oxidases (MAO) in the outer mitochondrial membrane. The amount of ROS generated by the ETC increases with ageing. The expression of $p66^{Shc}$ and MAO is enhanced with ageing, whereas the mitochondrial ROS detoxifying system (detox) is decreased with ageing. NADPH oxidase 4 (Nox4) may be present in aged cardiac mitochondria under pathophysiological conditions; however, the exact mitochondrial localization of Nox4 is unclear. The amount of ROS increases with ageing and contributes to damage of the DNA and to oxidative modifications of proteins and lipids. In the mitochondrial matrix, enhanced levels of ROS induce damage of the mitochondrial DNA (mtDNA).




recent study with larger numbers of animals ($n$ = 50 per group) demonstrates no benefit of the $p66^{Shc}$ knockout on lifespan.[69] The maintenance of the animals under more natural conditions—i.e. the mice were kept in an outdoor enclosure and had to compete for food—even displays that $p66^{Shc}$ knockout mice die earlier than their wild-type littermates.[70] Therefore, the role of ROS in healthy ageing is unclear.

## *Contribution of the mitochondrial permeability transition pore to myocardial ageing*

The MPTP represents a large conductance pore in the inner mitochondrial membrane, which is predominantly closed under non-stressed conditions. An opening of the MPTP is favoured, e.g. by ROS, increased concentrations of $Ca^{2+}$, phosphate, or mitochondrial depolarization. MPTP opening induces loss of mitochondrial membrane potential, mitochondrial swelling that leads to the rupture of the outer mitochondrial membrane and thereby to a decrease in ETC activity and a release of pro-apoptotic factors. The molecular identity of the MPTP has been unclear for many years, however, recent studies indicate that the MPTP is formed of dimers of the $F_0F_1$ ATP synthase.[71]

Opening of the MPTP can be measured by subjecting permeabilized cardiomyocyte bundles or isolated mitochondria to $Ca^{2+}$-stimuli. Consecutive pulses of defined amounts of $Ca^{2+}$ can be added until mitochondria become overloaded with calcium and MPTP opening occurs. Thereby, the so-called mitochondrial calcium retention capacity—i.e. the amount of calcium that can be sequestered by mitochondria until permeability transition occurs—can be quantified. Using this approach, no difference in the calcium retention capacity is detected between permeabilized cardiomyocyte bundles from adult and senescent rats.[26] However, the time interval between the administration of a single calcium bolus and MPTP opening is shorter in permeabilized cardiomyocyte bundles from senescent rats than in young rats, indicating a greater intrinsic susceptibility to MPTP opening with ageing. In addition, the widely used MPTP inhibitor cyclosporine A delays oxidative stress-induced MPTP opening effectively in cardiomyocytes from young, but not from old rat hearts.[72] However, when analysing MPTP opening in aged hearts, the contribution of mitochondrial subpopulations has to be considered. Whereas the tolerance of SSM towards a $Ca^{2+}$-stimulus to induce MPTP opening is not altered with age,[73] IFM from aged myocardium display a reduced calcium retention capacity compared to IFM from young hearts.[54,74]

## *The role of mitochondrial dynamics and quality control in cardiac ageing*

Mitochondria are highly dynamic cell organelles that undergo morphological changes including fusion and fission and a regulated turnover. However, mitochondrial fusion and fission in cardiomyocytes may be less prominent compared with that in other cell types.[75] The recently developed MitoTimer mouse demonstrates that newly synthesized and older mitochondria are heterogeneously distributed in the heart.[76] Mitochondrial fusion and fission contributes to the segregation


DOI: 10.1002/jcsm.12178

of damaged organelles and thereby to the removal of these organelles from the mitochondrial pool. Key proteins of mitochondrial fusion include mitofusin 1 and 2 (Mfn1 and Mfn2) as well as Opa1 (optic atrophy 1). Mitochondrial fission is mediated—among other proteins—by Drp1 (dynamin-related protein 1 and a GTPase) and Fis1 (mitochondrial fission 1 protein). Damaged mitochondria separated by fission are finally removed by mitophagy. Similar to Drp1, Mfn1, and Mfn2 belong to the GTPase family of proteins, and their knockout results in embryonic lethality.[77] Also, mice with germ-line deleted Drp1 die at embryonic day 12.5 due to abnormalities in the forebrain.[78] Mitochondria of inducible cardiac-specific Drp1 knockout mice become elongated and damaged mitochondria accumulate. The mice develop mitochondrial dysfunction, left ventricular dysfunction and finally die within 13 weeks.[79] These data point to the importance of mitochondrial fusion and fission for growth and development. The appearance of so-called giant mitochondria with disorganized cristae is described with age—especially after enforced endurance training—and is considered to be a degenerative response.[80]

The analysis of the expression of proteins involved in mitochondrial fusion or fission demonstrates decreased amounts of Mfn1 and Mfn2 with age. In this study, ageing has no influence on the protein levels of Opa1 and Drp1.[81] In contrast, enhanced expression of Opa1 and Drp1with age is presented in a study by Ljubicic.[55] The discrepancies between the two studies might be explained by the different ages of the rats investigated (25 months vs. 36 months). Because a general knockout of Mfn2 results in embryonic lethality, mice with a cardiomyocyte-restricted deletion of Mfn2 were generated. These mice show an accumulation of damaged mitochondria and finally develop heart failure. The moderate expression of mitochondrial-targeted catalase induces a normalization of ROS formation and reduces the structural changes occurring in Mfn2-deficient hearts.[82] Interestingly, the expression of higher amounts of mitochondrial catalase does not improve mitochondrial function and heart failure. These data imply that no dose–effect relationship exists between local ROS formation and cardiac degeneration.

The term autophagy refers to the degradation of cytosolic components by the lysosome in order to maintain cellular homeostasis, whereas mitophagy describes a type of autophagy that sequesters dysfunctional mitochondria into double-membrane vesicles called autophagosomes and delivers them to the lysosome. The quality control system of mitophagy ensures cellular structure and function of mitochondrial proteins. Mitochondrial fission is important for mitophagy because mitochondrial fragmentation precedes mitophagy: among the triggers of mitophagy are ROS, a loss of the mitochondrial membrane potential, and MPTP opening.[83] Two well-known regulators of mitophagy are the mitochondria-targeted serine/threonine kinase Pink1 (phosphatase and tensin homologue-induced putative kinase 1) and the E3 ubiquitin ligase Parkin. Upon loss of the mitochondrial membrane potential, Pink1 accumulates on damaged mitochondria and induces the translocation of cytosolic Parkin and its subsequent activation, which finally leads to the mitophagic elimination of the organelle. Pink1-deficient mice develop left ventricular dysfunction, and in patients with end-stage heart failure, the protein levels of Pink1 are reduced.[84] The overexpression of Parkin in mice stimulates mitophagy.[85]

Besides the ubiquitin-mediated pathway, autophagy occurs via mitochondrial lipids and proteins functioning as mitophagy receptors. Here, Bnip3 (Bcl-2/adenovirus E1B 19-kDa-interacting protein 3) and Nix (Nip-like protein) are important. These proteins induce mitophagy by recruiting LC3II (a cleavage product of LC3 and the microtubule-associated protein 1 light chain 3). The protein Beclin1 localizes autophagic proteins to a pre-autophagosomal structure. A recent study shows that also Kruppel-like factor 4 is important for autophagy because its ablation leads to the accumulation of damaged mitochondria.[86]

An interrelation between mitochondrial fission/fusion and autophagy/mitophagy is observed in cardiomyocytes following the deletion of Drp1 that induces the expression of Parkin, a protein expressed only at low levels under physiological conditions.[85] Parkin-deficient mitochondria are smaller and more disorganized than wild-type mitochondria, and this effect is associated with increased expression of the fission protein Fis1.[87] The overexpression of Bnip3 in cardiomyocytes leads to the translocation of Drp1 from the cytosol to the mitochondria, and silencing of Drp1 reduces autophagy elicited by Bnip3 overexpression.[88] Bnip3 expression also reduces the protein level of the fusion protein Mfn1. Mfn2 represents a target of Pink1 and aids in the recruitment of Parkin.[89]

The efficiency of autophagy/mitophagy declines with advancing age in the heart.[90] This is suggested to increase the number of damaged proteins and/or mitochondria and thereby to contribute to the development of cardiovascular diseases.[90] Therefore, the stimulation of autophagy should delay ageing, and indeed, several studies have demonstrated increased lifespan by the activation of autophagy (reviewed in Leon and Gustafsson[91]). In contrast, cardiac-specific knockdown of Atg5 (autophagy-related protein 5), a protein contributing to autophagosomes formation, results in the accelerated onset of heart failure, and the mice die prematurely starting at the age of 6 months.[92]

However, data on autophagy/mitophagy in the ageing heart are controversial. Indeed, decreased numbers of mitochondria incorporated in autophagosomes are observed in aged mouse hearts.[93] Here, the protein expression of Pink2 and Parkin is similar in young and aged hearts; however, the translocation of Parkin is reduced with ageing. In Parkin-deficient hearts, damaged mitochondria accumulate with increasing age.[94] A decreased expression of LC3II in aged

hearts is suggested to confer a decline in mitophagic activity.[92] In contrast, increased protein levels of Beclin1 and LC3II with age as observed by Boyle *et al*. are considered to contribute to increased autophagy with age.[95] A study by Zhou *et al*.[96] also shows enhanced expression of LC3II in aged myocardium; however, Beclin1 expression is not affected by age. When analysing young and aged hearts, Inuzuka *et al*. detected increased mRNA levels of Beclin1, but no difference in the amount of LC3II between young and aged hearts.[97] The reason for the different findings is unclear, but it has to be considered that the differential expression of proteins involved in autophagy does not indicate whether or not autophagic flux is altered. A summary of the proteins involved in autophagy/mitophagy and ageing is given in Table 1.

## *Proteomic analysis of aged cardiac mitochondria*

To gain further insight into mitochondrial function and their disease-dependent[98,99] and age-dependent variations, the unbiased analysis of the mitochondrial proteome represents an important tool. During the last years, the methodological approach to identify mitochondrial proteins has been more and more refined. Currently, the human mitochondrial protein database lists about 1500 proteins, and in cardiac SSM alone, around 1000 proteins have been identified.[100]

Recently, we analysed the proteome of SSM and IFM from ventricular tissue of young (5 months) and aged (23–25 months) male C57BL/6 mice by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing (equal protein amounts of SSM and IFM were pooled and investigated). A total of 98 spots were up-regulated or down-regulated with ageing. These spots were picked and analysed by liquid chromatography-mass spectrometry/mass spectrometry. Because a protein may be detected in more than one spot due to different isoforms or post-translational modifications, it is not possible to quantify the exact change in the expression level of a protein. Therefore, we provide data on the 24 proteins that are differentially expressed between young and aged mitochondria with a ratio $>1.2$, and these proteins are listed in Table 2. Some of the proteins detected are already described to be regulated by ageing using proteomic or other approaches. Proteins central to mitochondrial energy metabolism are up-regulated by ageing, among them are malate dehydrogenase, isocitrate dehydrogenase, aconitate hydratase, and 2-oxoglutarate dehydrogenase. An enhanced amount of malate dehydrogenase in aged female hearts has already been detected using a proteomic approach,[101] and also malate dehydrogenase activity is shown to increase with age.[102] However, others also observed decreased activity of the malate dehydrogenase in aged hearts.[103] Chakravarti *et al*.[104] detected decreased amounts of the isocitrate dehydrogenase and unchanged levels of the aconitate hydratase in aged mouse myocardial mitochondria. The activity of the aconitate hydratase is found to decline with age.[105] Deviating data also exist for the 2-oxoglutarate dehydrogenase, which is described to be either down-regulated[103,106] or unchanged in aged hearts.[104] The reason for the conflicting results is unclear; however, it has to be considered that in our recent study both SSM and IFM were investigated, whereas others studied only SSM.[103] Furthermore, species differences[106] or gender differences might exist.[101]

The amount of the succinyl-CoA:3-ketoacid CoA transferase 1 (Scot1), which is involved in the breakdown of ketone bodies, is increased in aged mitochondria (Table 2).[104] In addition, increased Scot1 activity is measured in aged rat heart mitochondria; however, the amount of Scot1 is not altered in this model.[107]

Cellular stress resistance is associated with longevity, and therefore, one would expect decreased expression of heat shock proteins with ageing. Indeed, we detected lower amounts of heat shock protein 60 (Hsp60) in mitochondria from aged ventricles. This finding is in line with previous data demonstrating decreased Hsp60 mRNA and protein in aged rat hearts.[108]

The enzyme aldehyde dehydrogenase 2 (Aldh2) belongs to a family of proteins that are involved in the detoxifying process of aldehydes. Aldh2 contributes to ageing because a knockout of the protein decreases lifespan in mice.[109] The authors of this study found that ageing is associated with a decline in the cardiac Aldh2 activity, whereas the amount of Aldh2 is not affected with age. In our study using the proteomic approach, we found Aldh2 to be up-regulated. Our data

**Table 1** Factors involved in autophagy/mitophagy and their expression in ageing hearts

| Name | Species | Age | mRNA | Protein | Reference |
|---|---|---|---|---|---|
| Pink2 | Mouse | Y: 10 months<br>O: 20 months | nd | ≈ | Hoshino *et al*.[93] |
| Parkin | Mouse | Y: 10 months<br>O: 20 months | nd | ≈<br>translocation ↓ | Hoshino *et al*.[93] |
| LC3II | Mouse | Y: 10 weeks<br>O: 6, 12, and 24 months | nd | ↓ | Taneike *et al*.[92] |
| | Mouse | Y: 2 months<br>O: 18 months | ≈ | ↑ | Boyle *et al*.[95] |
| | Mouse | Y: 3 months<br>O: 12 m, 24 months | nd | ↑ | Zhou *et al*.[96] |
| | Mouse | Y: 3 months<br>O: 20–24 months | ≈ | ≈ | Inuzuka *et al*.[97] |
| Beclin1 | Mouse | Y: 2 months<br>O: 18 months | ≈ | ↑ | Boyle *et al*.[95] |
| | Mouse | Y: 10 weeks<br>O: 12 and 24 months | nd | ≈ | Zhou *et al*.[96] |
| | Mouse | Y: 3 months<br>O: 20–24 months | ↑ | nd | Inuzuka *et al*.[97] |

Y, young; O, old; nd, not determined; ≈, not affected with ageing; ↑, increased with ageing; ↓, decreased with ageing.

**Table 2** Mitochondrial proteome analysis

| UniProt ID | Protein identified | pI | Mw (kDa) | PANTHER GO-Slim biological process | Regulation with age (old/young) |
|---|---|---|---|---|---|
| COQ9_MOUSE | Ubiquinone biosynthesis protein COQ9 | 4.93 | 35.08 | Unclassified | ↓ |
| QCR2_MOUSE | Cytochrome b-c1 complex subunit 2 | 8.99 | 48.24 | Respiratory electron transport chain, proteolysis | ↑ |
| QCR1_MOUSE | Cytochrome b-c1 complex subunit 1 | 5.34 | 52.85 | Respiratory electron transport chain, proteolysis | ↑ |
| IDHP_MOUSE | Isocitrate dehydrogenase [NADP] | 8.49 | 50.91 | Unclassified | ↑ |
| ACON_MOUSE | Aconitate hydratase | 7.4 | 85.47 | Generation of precursor metabolites and energy, carbohydrate metabolic process, tricarboxylic acid cycle, cellular amino acid biosynthetic process | ↑ |
| MDHM_MOUSE | Malate dehydrogenase | 8.55 | 35.61 | Generation of precursor metabolites and energy, carbohydrate metabolic process, tricarboxylic acid cycle | ↑ |
| ODO1_MOUSE | 2-Oxoglutarate dehydrogenase | 6.05 | 116.45 | Generation of precursor metabolites and energy, primary metabolic process, cellular process | ↑ |
| MCCA_MOUSE | Methylcrotonoyl-CoA carboxylase subunit alpha | 6.65 | 79.34 | Coenzyme metabolic process, gluconeogenesis, fatty acid biosynthetic process | ↑ |
| SCOT1_MOUSE | Succinyl-CoA:3-ketoacid CoA transferase 1 | 7.01 | 55.99 | Coenzyme metabolic process, carbohydrate metabolic process, fatty acid metabolic process | ↑ |
| ECH1_MOUSE | Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase | 6.01 | 36.12 | carbohydrate metabolic process, fatty acid beta-oxidation | ↓ |
| HIBCH_MOUSE | 3-Hydroxyisobutyryl-CoA hydrolase | 6.24 | 43.04 | Coenzyme metabolic process, vitamin biosynthetic process, carbohydrate metabolic process, fatty acid beta-oxidation | ↓ |
| BCAT2_MOUSE | Branched-chain-amino-acid aminotransferase | 7.7 | 44.13 | Cellular amino acid metabolic process | ↓ |
| ODPB_MOUSE | Pyruvate dehydrogenase E1 component subunit beta | 5.39 | 38.94 | Lipid metabolic process, cellular amino acid catabolic process, lipid metabolic process | ↑ |
| ECI2_MOUSE | Enoyl-CoA delta isomerase 2 | 8.42 | 43.27 | Lipid metabolic process, lipid transport, regulation of catalytic activity | ↓ |
| SSDH_MOUSE | Succinate-semialdehyde dehydrogenase | 7.12 | 55.97 | Metabolic process | ↓ |
| SPRE_MOUSE | Sepiapterin reductase | 5.56 | 27.88 | Steroid metabolic process | ↓ |
| VDAC2_MOUSE | Voltage-dependent anion-selective channel 2 | 7.44 | 31.73 | Anion transport | ↓ |
| CH60_MOUSE | 60 kDa heat shock protein | 5.35 | 60.96 | Unclassified | ↓ |
| PGFS_MOUSE | Prostamide/prostaglandin F synthase | 6.31 | 21.67 | No PANTHER hit | ↑ |
| PARK7_MOUSE | DJ-1 | theoretical pI 6.31 | 20.02 | Transcription from RNA polymerase II promoter, proteolysis, response to stress, regulation of transcription from RNA polymerase II promoter | ↓ |
| ALDH2_MOUSE | Aldehyde dehydrogenase 2 | 6.05 | 56.54 | Metabolic process | ↑ |
| THIM_MOUSE | 3-Ketoacyl-CoA-thiolase | 8.33 | 41.83 | Protein acetylation | ↑ |
| MCEE_MOUSE | Methylmalonyl-CoA epimerase | 6.71 | 19.02 | Unclassified | ↓ |
| PRDX5_MOUSE | Peroxiredoxin-5 | 7.7 | 21.9 | Unclassified | ↑ |

Mouse ventricular mitochondrial proteins (SSM and IFM) were isolated from young (5 months) and aged (23–25 months) male C57/BL/6 mice. Equal amounts of subsarcolemmal mitochondria and interfibrillar mitochondria proteins were pooled and analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing. Spots with differential expression were picked and characterized by liquid chromatography-mass spectrometry/mass spectrometry. Proteins with a differential expression (ratio $> 1.2$), their biochemical properties (pI and molecular weight), their classification to a biological process, and their increased (↑) or decreased (↓) abundance in ageing are listed.

are confirmed by Lancaster *et al*.[101] who display enhanced amounts of the aldehyde dehydrogenase pre-protein in aged female mitochondria.

Mitochondrial ubiquinone plays a role in mitochondrial electron transport and superoxide generation. Whereas a global loss of ubiquinone shortens lifespan, the loss of ubiquinone in the heart has no influence on cardiac function.[110] In the rat heart, a decrease in the ubiquinone biosynthesis protein Coq9 is found in 28 months old, but not in 19-months-old animals. The data of our present study on 23- to 25-months-old mice confirm these data.

In addition to the aforementioned proteins, which have already been described to be dysregulated in aged hearts, some of the proteins identified in our study are found to be associated with ageing in other organs than the heart. Among these proteins is the voltage-dependent anion channel 2, which is up-regulated in skeletal muscle,[111] the branched-chain amino acid transaminase (down-regulated with age in mouse liver[112]), and the sepiapterin reductase, which is involved in tetrahydrobiopterin biosynthesis and reduced in the mesenteric arteries of aged mice.[113] Other proteins such as DJ-1, methylmalonyl-CoA epimerase, or enoyl-CoA delta isomerase 2, which we found to be present in reduced amounts in aged mitochondria have not been linked to ageing before. Further studies are required to confirm the differential expression of the proteins with independent techniques and to evaluate their roles in the process of cardiac ageing.

## Age-associated changes in skeletal muscle

### *Mitochondrial function and ROS production in aged skeletal muscle*

Sarcopenia, the atrophy of skeletal muscle and, consequently, the decline in muscle strength, is a hallmark of the ageing process. The sarcopenic phenotype is characterized by a reduction of muscle mass and quality, a shift in fibre-type distribution, changes in protein synthesis, reduced satellite cell regeneration, replacement of muscle fibres with fat, and an increase in fibrosis. Sarcopenia is partially attributed to changes in the mitochondrial compartment but also involves cytosolic pro-inflammatory mediators, proteolytic activation, and apoptosis signalling pathways.[114]

Interestingly, cachexia, a muscle wasting disease in response to a chronic disease such as cancer, shows not only some similarities in the underlying mechanisms of muscle loss but also a number of significant differences compared with sarcopenia.[114,115] Cancer-associated cachexia, which is characterized by severe muscle wasting, systemic inflammation, and malnutrition, is a complex metabolic disorder with profound mitochondrial alterations. Impaired mitochondrial biogenesis, reduced mitochondrial oxidative capacities, mitochondrial energetic inefficiency, and enhanced mitophagy and fission strongly contribute to cancer-induced muscle wasting and muscle weakness.[116–118] Furthermore, mitochondria can be affected by the toxic effects of cancer therapeutics. Among the commonly applied therapies, mitochondrial dysfunction with defective mitochondrial biogenesis and increased ROS formation occurs after doxorubicin or oxaliplatin treatment.[119] Both substances induce deleterious effects in skeletal muscle, resulting in significant reductions in muscle mass and strength in cancer patients.[119]

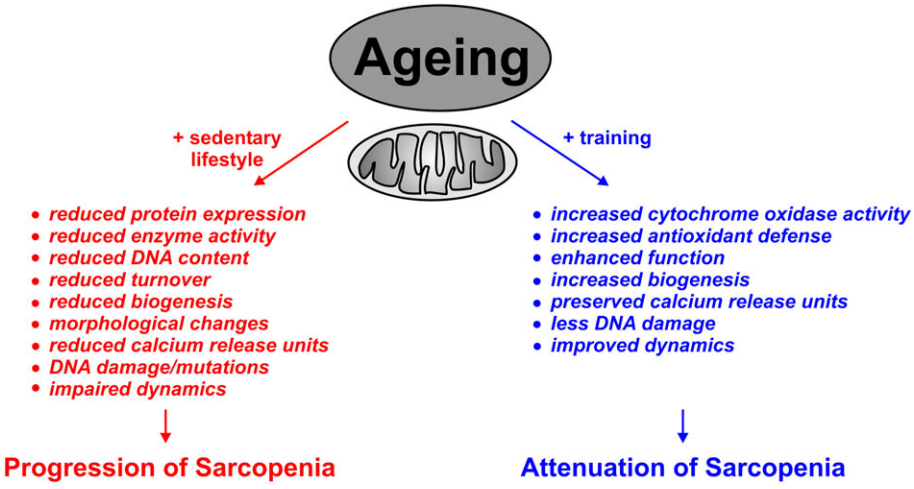
Age-associated mitochondrial changes in skeletal muscle show many similarities but only a few differences compared to the heart (Table 3). Similar to the heart, two populations of mitochondria (SSM and IFM) exist in skeletal muscle. These two subpopulations exhibit a distinct behavuior in skeletal muscle during ageing. SSM produce greater amounts of ROS and show higher rates of fragmentation and degradation, while IFM are more susceptible to apoptotic stimuli and MPTP opening.[120] Recently, the existence of these two separate subpopulations was challenged by demonstrating that SSM and IFM are physically interconnected in skeletal muscle.[121] Age-associated mitochondrial decay (Figure 2) is an important factor driving skeletal muscle ageing and sarcopenia. Slower walking speed, which is among the clinical parameters for sarcopenia case finding in older individuals, correlates with lower mitochondrial capacity and efficiency.[122] Skeletal muscles of human subjects demonstrate an age-related decline in mtDNA and mRNA abundance, mitochondrial ATP production and oxygen consumption.[120,123,124] Interestingly, the age-associated decline in ATP content and production was observed in isolated rat mitochondria from the gastrocnemius muscle but not in heart mitochondria from the same animals[125] (Table 3). Furthermore, mitochondrial content has been reported to be reduced in ageing muscle, while other studies found no change.[126] Mitochondria in aged skeletal muscle appear enlarged with matrix vacuolization and shorter cristae. A greater proportion of mitochondria in the elderly are depolarized or nonfunctional, and mitochondrial density is reduced.[127–129] Complexes I and IV activities are decreased in aged muscle, probably because these complexes contain subunits encoded by the mtDNA, which is more vulnerable to ROS derived from the respiratory chain.[127] The decline in mitochondrial function (Figure 2) is a consequence of physical inactivity and may partially be normalized by endurance training.[114,130,131]

Enhanced ROS production together with an increase in the DNA repair enzyme 8-oxoguanine glycosylase 1 occurs in rat senescent skeletal muscle.[132] This increase in ROS production is associated with a lower mitochondrial content and protein expression of PGC-1alpha together with an increased mitochondrial apoptotic susceptibility, which may all be involved in age-related sarcopenia.[132] Mice expressing a

**Table 3** Comparison of age-associated mitochondrial changes in the heart and skeletal muscle

| | Heart | Skeletal muscle |
|---|---|---|
| Mitochondrial volume (% cell) | 30–40[75,249] | 3–8[250] |
| Stem cells | -Extremely low numbers[251] | -Low numbers (satellite cells)[252]<br>-Functional decline with ageing[253] |
| | **Aged heart** | **Aged skeletal muscle** |
| Mitochondrial function | -Impaired mainly in IFM[23–25]<br>-Largely preserved[26] | -Impaired[120,124,127,129] |
| ATP production/ATP content | -Not altered[125]-Reduced[27,28] | -Reduced[123–125] |
| Mitochondrial biogenesis or expression of major regulators of mito. biogenesis | Reduced[27,29–32] | -Reduced[120,124,127–129,132] |
| Mitochondrial content | -Reduced[4,5]-No change[6,7]<br>-Increased[27] | -Reduced[123,124,129,132]<br>-No change[126] |
| Cardiolipin content | -Reduced[34,35] | -Reduced[254,255] |
| Mitochondrial shape | -Shortened, more round[8,75]<br>-Giant mitochondria[80] | -Enlarged mitochondria[128,129,144,256] |
| Mitochondrial fusion | -Decreased amounts of Mfn1 and Mfn2[81]<br>-increased Opa1 expression[55]<br>-Shortened, hypodynamic organelles lacking remodelling[75] | -Increased fusion resulting in enlarged mitochondria[128,129,144,256]<br>-Reduced fusion due to reduced Mfn2[257,258] |
| Mitochondrial fission | -Increased Drp1 expression[55] | -Smaller, fragmented mitochondria; higher expression of Fis1 and Drp1[257]<br>-Lower Fis1 expression[144] |
| Mitophagy | -Decreased[90,92,93]<br>-Increased[95,96] | -Impaired[120,137] |
| Mitochondrial ROS | -Increased[25,53,55] | -Increased[120,127–129,132] |
| Susceptibility for mPTP opening | -increased mainly in IFM[54,72,74] | -Increased[123,126,132,137] |

**Figure 2** Sarcopenia in aged individuals' role of mitochondria. A sedentary lifestyle significantly contributes to the progression of sarcopenia though various mito-based mechanisms. In particular, resistance exercise training can attenuate the progression of sarcopenia, which involves also a number of changes in mitochondrial function. Whether or not a total prevention of sarcopenia can be achieved by exercise training is still a matter of debate.




proofreading-deficient version of the mitochondrial DNA polymerase gamma (mtDNA mutator mice) accumulate mtDNA mutations and display a prematurely aged, sarcopenic phenotype of skeletal muscle.[133] In these mice, mtDNA mutations impair the assembly of functional ETC complexes, resulting in a decrease in oxidative phosphorylation, and finally the induction of skeletal muscle apoptosis and sarcopenia.[134] The involvement of the mitochondrial free radical vicious cycle

in muscle ageing in humans has also been shown in a study by Bua *et al*.: they demonstrated that the number of muscle fibres exhibiting mitochondrial electron–transport–system abnormalities increases from 6% at age 49 years to 31% at age 92 years together with a clonal expansions of mtDNA deletion mutations in electron–transport–system-abnormal regions of single fibres.[135]

## *Mitochondrial dynamics and quality control in aged skeletal muscle*

One of the consequences of mitochondrial dysfunction is the activation of skeletal muscle apoptosis. Indeed, apoptotic activation in aged skeletal muscle has been observed in various studies[132,136] and occurs even when the persons remain physically active.[137] Activation of apoptosis correlates with reduced muscle volume in older persons and slower walking speed.[138] In the mtDNA mutator mouse,[133] the accumulation of mtDNA mutations is associated with the induction of apoptotic markers not only in a skeletal muscle but also in a number of other organs.

Damaged mitochondria separated by fission are finally removed by mitophagy. The AMP-activated protein kinase (AMPK) triggers the destruction of defective, fragmented mitochondria through FoxO3-dependent mitophagy.[139,140] Accordingly, muscle atrophy involves the activation of the ubiquitin–proteasome and the autophagy–lysosome systems and requires AMPK activation.[139] Aged skeletal muscle seems to accumulate dysfunctional mitochondria with exaggerated sensitivity to MPTP opening because of impaired mitophagy,[120,137] resulting in a progressive accumulation of a non-degradable, polymeric, autofluorescent material called lipofuscin in lysosomes. This interrelated mitochondrial and lysosomal damage has been suggested to contribute to the functional impairment in skeletal muscle with advanced age.[128,141]

Inhibition of mitochondrial fusion results in an accumulation of mtDNA mutations triggering mitochondrial dysfunction, the loss of the mitochondrial genome and finally accelerated muscle loss.[142] Aged skeletal muscle has long ago been shown to contain giant mitochondria with irregularly spaced cristae and lipofuscin in close relationship with the damaged mitochondria.[143] The accumulation of such enlarged mitochondria, which may be the consequence of hyperfusion, suggests that mitochondrial dynamics are disturbed in aged skeletal muscle. While aged mouse muscles exhibit higher levels of markers of mitochondrial fusion and lower levels of markers of autophagy, muscles from mtDNA mutator mice, however, display higher mitochondrial fission and autophagy levels.[144] Thus, mtDNA-based mechanisms are unable to sufficiently explain the phenotypic changes in aged skeletal muscle and may not be the primary cause of sarcopenia.

Not only mitophagy but also the generation of new organelles via mitochondrial biogenesis is impaired in aged skeletal muscle,[120] and mitochondrial content declines with age in sedentary individuals.[124] Transcriptional complexes that contain PGC-1alpha control mitochondrial oxidative function and mitochondrial biogenesis. However, the mitochondrial biogenesis signalling activated by PGC-1alpha is reduced with increasing age.[127] AMPK promotes mitochondrial biogenesis via PGC-1alpha up-regulation and activation.[145,146] AMPK phosphorylates PGC-1alpha at Thr177 and Ser538, which is required for the PGC-1alpha dependent induction of the PGC-1alpha promoter and the mitochondrial biogenic response.[146] In addition, PGC-1alpha modulates mitochondrial turnover in skeletal muscle via Mfn2 and via degradation using the autophagy–lysosome machinery.[147,148]

## *Impact of exercise training in aged skeletal muscle*

Among the modifiable lifestyle factors, physical activity is the most effective intervention to attenuate loss of muscle strength and mass.[114,131] Several studies suggest that the decline in mitochondrial function is partially normalized by exercise training (Figure 2).[130] It increases type II muscle fibres and cytochrome oxidase activity, decreases oxidative damage to DNA, and increases the mitochondrial content in older adults.[124,129,149–151] The beneficial effects of exercise include the multifaceted activation of pathways involved in mitochondrial turnover.[152] Among those, PGC-1alpha increases mitochondrial content and mitochondrial quality by modulating mitochondrial fusion/fission and mitophagy.[147,148] PGC-1alpha also prevents the excessive activation of proteolytic systems during muscle atrophy.[153] A splice variant of the PGC-1alpha gene, PGC-1alpha4, is highly expressed in exercised skeletal muscle and controls muscle mass through induction of IGF1 and repression of myostatin without affecting 'classical' PGC-1alpha targets involved in mitochondrial biogenesis.[154] In humans, controversial results have been obtained with regard to the induction of this splice variant in skeletal muscle after exercise.[155,156] As described earlier, the mtDNA mutator mouse displays skeletal muscle sarcopenia.[133,134] Interestingly, 5 months of endurance exercise induce systemic mitochondrial biogenesis, prevent mtDNA depletion, increase mitochondrial oxidative capacity, and prevent dysfunction in various organs including skeletal muscle sarcopenia in these mtDNA mutator mice.[157] This demonstrates that endurance exercise is an effective therapeutic approach to attenuate or even prevent mitochondrial dysfunction in ageing skeletal muscle.

Exercise training causes an increase in ROS production.[158,159] These ROS play an important role in the stimulation of major signalling pathways that regulate skeletal muscle quality control and dynamics of mitochondria. Low levels of ROS mediate positive effects on muscle physiological

responses and play a crucial role in mitochondrial maintenance during physical activity including activation of autophagy.[129,158–162] Accordingly, antioxidant treatment impairs exercise tolerance in wild-type mice.[162] On the other hand, high levels of ROS contribute to contractile dysfunction resulting in muscle weakness and fatigue,[159] and mitochondrial ROS production is required to induce muscle atrophy through activation of diverse proteolytic pathways in muscle fibres exposed to prolonged inactivity.[163] In addition, an endurance training-induced increase in cellular antioxidant defence has been reported,[129,164] which may contribute to the maintenance of low-ROS levels.

However, there are also a number of unresolved questions related to the effects of endurance training in aged skeletal muscle, and the role of exercise training in reversing sarcopenia in individuals older than 80 years still remains to be determined. First, only a few studies were performed in the elderly, while most endurance exercise-related studies have examined young subjects.[124,165] In humans, skeletal muscle mitochondrial content is suggested to remain adaptable only until the age of 80 years or below[126,166,167] due to a failure to up-regulate the mitochondrial biogenesis machinery. Similarly, single muscle fibre contractile function and myosin heavy chain distribution are unaltered in very old men (>80 years) in response to progressive resistance training indicating limited muscle plasticity.[168] Furthermore, the most effective type of exercise and the frequency of exercise to attenuate or even prevent sarcopenia are still under discussion.[169,170] The specific effects of endurance exercise training vs. strength exercise training on skeletal muscle physiology in younger people are well known, but their role in reversing sarcopenia in elderly individuals over 80 years of age remains to be determined.[124] Even an interference between different types of exercise (endurance and resistance exercises), resulting in a blunted response, has been suggested,[129] while others reported that the order of exercise modes does not affect training-induced changes in mitochondrial enzyme activity or improvements in muscle function.[171]

## *Impact of caloric restriction on skeletal muscle ageing*

Caloric restriction (CR), which typically involves consuming 20–40% calories less than normal in most experimental studies, delays the age-associated loss of muscle fibres, in part, by improving mitochondrial function. Already early studies investigating the impact of CR on skeletal muscle mitochondrial function reported that the age-associated decline in activities of respiratory chain complexes was prevented with strongest effects on complex IV.[172–175] Thus, CR reduces the age-associated accumulation of complex IV-negative and complex II-hyperactive fibres.[176,177] CR augments PGC-1alpha signalling and the mitochondrial biogenic response and increases mitochondrial density and function.[178–180] AMPK, which is activated under low-nutrient conditions, directly phosphorylates PGC-1alpha, resulting in a mitochondrial biogenic response in skeletal muscle.[146] Accordingly, a significant increase in mitochondrial biogenesis occurs in multiple tissues in mice after CR, a condition with chronically low nutrients.[181] The mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) signalling pathway is also critically involved in physiological adaptations to nutrient supply and considered a main player mediating CR effects. Inhibition of mTOR robustly extends the lifespan of model organisms including mice. Furthermore, mTORC1 has been identified to influence mitochondrial content and function in skeletal muscle.[182–184] Muscle-specific inactivation of mTOR leads to impaired oxidative metabolism and altered mitochondrial biogenesis,[182,183] while TORC1 activation promotes mitochondrial biogenesis.[184]

CR also induces a reduction in mitochondrial ROS generation, a lower amount of oxidatively damaged mitochondrial proteins and less mtDNA mutations in aged animals.[125,185–189] CR animals from different species are characterized by an attenuation of the age-related impairment of autophagy or ubiquitin–proteasome activity[190,191] and reduced susceptibility for apoptotic cell death.[190,192,193] Furthermore, CR prevents the age-related decline in skeletal muscle aerobic function[173] and increases insulin-stimulated glucose uptake in skeletal muscle,[194] and CR-fed rats retain motor activity even in old age.[188] Even when started late in life, CR is sufficient to inhibit ageing-induced muscle loss through changes in mitochondrial biogenesis and apoptotic proteins.[195] Interestingly, these protective effects appear to occur in a fibre type-specific manner with glycolytic muscle being more responsive to CR.[195]

Thus, experimental data suggest that the impact of ageing on skeletal muscle and skeletal muscle mitochondria can be delayed. Controlled trials on the effects of long-term CR on skeletal muscle function in humans are lacking for obvious reasons including unresolved safety issues or difficulties in lifelong observation of participants. The Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy (CALERIE) trials systematically investigate the effects of CR in healthy, non-obese human beings.[196] Phase 1 of CALERIE used short-term CR (6–12 months), while phase 2 of CALERIE is a randomized, multicentre study that uses dietary and behavioural interventions to achieve 25% CR for 2 years.[196] However, currently, there are no comprehensive data available related to mitochondrial parameters from the skeletal muscle of patients from these controlled trials. Another study performed in humans shows that the skeletal muscle transcriptional profile of voluntary CR practitioners resembles that of younger individuals.[197] Furthermore, a shift in skeletal muscle gene expression towards oxidative metabolism including a set of genes related to long-term CR has been reported in obese patients after weight loss.[198] CR in

young overweight adults results in an increased expression of genes involved in mitochondrial biogenesis and function, an increase in muscle mitochondrial DNA in association with a decrease in DNA damage compared to controls.[199] Similar to the results obtained in animals, CR also reduces the susceptibility for apoptotic cell death in human skeletal muscle.[200] For this limitations and undisputed hazards of CR such as hypotension, loss of libido, menstrual irregularities, infertility, osteoporosis, cold sensitivity, slower wound healing, depression, or emotional deadening to be overcome,[201] pharmacological approaches to mimic the effects of CR such as resveratrol, metformin, or rapamycin have been proposed.[202]

### *Impact of obesity on skeletal muscle ageing*

Obesity and type 2 diabetes mellitus accelerate ageing or induce a prematurely aged phenotype in humans in various organs such as liver,[203] heart,[30] AT,[204] or skeletal muscle,[205] and telomere length is inversely associated with obesity.[204] The ETC activity and mtDNA content are reduced in the skeletal muscle of type 2 diabetics and in obese patients compared with lean subjects.[206] Furthermore, healthy subjects with a family history of type 2 diabetes have reduced mtDNA content and high-fat diet-induced fat oxidation.[207] They also demonstrate a metabolic inflexibility, suggesting that reduced mitochondrial capacity may be a cause rather than a consequence of insulin resistance.[207] Accordingly, impaired mitochondrial activity not only in skeletal muscle but also in AT (see below) could predispose to obesity and induce a premature ageing process. In skeletal muscle, obesity is often accompanied by sarcopenia and vice versa, a scenario termed sarcopenic obesity. Obesity appears to be a sarcopenia promoting factor, but the underlying mechanisms are poorly understood.[208] Sarcopenia and obesity both pose a health risk for elderly people, but in combination, they synergistically increase the risk for negative health outcomes.[209]

### *Therapeutic strategies to target mitochondria*

Recent studies suggest that maintenance of mitochondrial function is beneficial in the delay of age-associated diseases. Experimental strategies to target mitochondria range from regulation of mitochondrial biogenesis, targeting of mitochondrial dynamics, enhancement of respiratory chain function to scavenging of toxic substances. The pan-PPAR agonist bezafibrate increases mitochondrial biogenesis and oxidative phosphorylation (OXPHOS) activity.[210] In addition, certain hormones such as estrogens, thyroid hormone or erythropoietin, and various AMPK activators such as AICAR, A-769662, metformin, resveratrol, quercetin, or hydroxytyrosol mediate some of their protective effects through increased mitochondrial biogenesis in various organs.[211,212] However, more work is warranted to substantiate their therapeutic potential in aged muscular tissues.

The use of untargeted antioxidant compounds including lipoic acid, vitamin C, vitamin E, or ubiquinol has so far failed to demonstrate benefits in larger clinical trials and some preclinical models. Mitochondria-targeted antioxidants such as MitoQ, MitoTEMPO, SS-31, or Tiron were shown to improve mitochondrial function in preclinical settings, but larger clinical applications have not yet been performed. Homologues of coenzyme Q10 such as idebenone or Epi-743 are known to enhance mitochondrial function, the latter being successfully used in patients with inherited mitochondrial disease.[213]

As mitochondrial dynamics influence mitochondrial function, pharmacological approaches to target the involved pathways are increasingly attracting interest. Specific inhibitors of mitochondrial fission (mdivi-1, Dynasore, and P110) or activators of fusion (M1-hydrazone and 15-Oxospriramilactone) have been developed. Inhibition of Drp1-mediated mitochondrial fission by usage of Dynasore, P110, or mdivi-1 has been shown to confer cardioprotection in various preclinical models.[214] However, inhibition of mitochondrial fission with mdivi-1 was also shown to induce a reduction in mitochondrial mass and impair myogenic differentiation.[215] Furthermore, prolonged treatment with these fission inhibitors can result in mitochondrial hyperfusion with deleterious consequences. Thus, a balance between the rates of fission and fusion or a partial reduction of mitochondrial fission appears to be necessary for normal mitochondrial adaptations. With better understanding of the molecular mechanisms in aged muscular tissues, more therapeutics can be developed to modulate mitochondrial dynamics. Given the major impact of mitochondrial dysfunction in cancer-induced muscle wasting as well as cancer therapy-induced toxicity, the aforementioned novel strategies that target mitochondrial biogenesis, dynamics, or ROS could also turn out to be useful in cancer-induced mitochondrial defects. In addition, anti-inflammatory therapies and exercise training constitute promising therapeutic countermeasures to cancer-associated cachexia, in part by improving mitochondrial function.

## Adipose tissue

### *Lipotoxicity*

Adipose tissue is a key organ in the regulation of energy balance, participating in both energy storage and energy expenditure.[216] However, it is now also considered as an endocrine organ through the release of various adipokines, orchestrating crucial interactions with other organs including heart and skeletal muscle. Similar to other cells, mitochondria

represent the main site of ATP production in adipocytes. Adipocyte development and differentiation are associated with increases in mtDNA content and mtDNA encoded components of the OXPHOS system.[217] However, the number of mitochondria in mature white adipocytes is significantly lower than during differentiation.[217] Although the number of mitochondria is low, mitochondrial function is essential for adipocyte function including secretion of adipokines such as adiponectin.[218] The mitochondria in AT play an important role in lipogenesis by providing key intermediates (glycerol 3-phosphate and acetyl-CoA) for the synthesis of triglycerides, and mtDNA content is strongly related to lipogenesis in white adipocytes.[219] Impaired mitochondrial activity in adipocytes is usually associated with reduced fatty acid oxidation, leading to an increase in cytosolic free fatty acids that can cause deterioration in other organs function. The AT expandability hypothesis[220] states that AT possesses a limited expandability, resulting in limited oxidative capacity and storage capacity of adipocytes. The capacity of AT to expand seems to be influenced by genes, environmental factors, and the individual's age.[220,221] Once AT storage capacity is exceeded, lipids will be deposited ectopically in skeletal muscle or cardiac myocytes, hepatocytes, or pancreatic beta cells. Ectopic lipid deposition can cause toxic effects such as insulin resistance and cardiovascular complications.[222] This lipotoxicity can be initiated through entrance of fatty acids into deleterious pathways such as ceramide production, which causes apoptosis of lipid-loaded cells. In addition, changes in the mitochondrial phosphoproteome caused by alterations in kinase activities have been suggested to play a major role in the initiation of cellular dysfunction in lipotoxicity.[223] Lipotoxicity and lipoapoptosis can be prevented by caloric restriction, PPARgamma agonist treatment, or leptin.[222,224,225] The PPARgamma agonist rosiglitazone triggers mitochondrial biogenesis in white adipocytes from leptin-deficient mice, accompanied by a remodelling of adipocyte mitochondria in shape, size, and function.[226]

### *Potential role in the ageing process*

Adipose tissue is also involved in the determination of lifespan and whole body metabolisms.[227,228] Obesity is associated with a poor performance of mitochondria in WAT, accelerates ageing, and induces a prematurely aged phenotype in AT.[204] Telomere length in AT is inversely associated with obesity.[204] Oxygen consumption of human and rat AT is negatively related to age and the degree of obesity.[229,230] Furthermore, mitochondrial content, copy number of mtDNA, and expression of genes for mitochondrial proteins in WAT are reduced in obese patients and animals.[231,232]

There is growing evidence that the insulin/insulin-like growth factor (IGF) signalling pathway is important in controlling the rate of ageing in mammals.[233,234] Mice with a fat-specific insulin receptor knockout (FIRKO), which show increases in median and maximum lifespans, have reduced fat mass and are protected against age-related obesity and its subsequent metabolic abnormalities despite a normal or even increased food intake.[227,235] Furthermore, white adipose tissue (WAT) of FIRKO mice shows a high expression of nuclear-encoded mitochondrial genes involved in glycolysis, tricarboxylic acid cycle, fatty acid oxidation, and oxidative phosphorylation even at high age, while wild-type mice show a decline in many of these genes with increasing age.[236] In addition, old FIRKO mice demonstrate signs of increased mitochondrial activity and an increased number or mass of mitochondria in WAT,[236] suggesting that maintenance of mitochondrial function in AT may be an important contributor to the increased lifespan. Similarly, genetically induced, severe mitochondrial dysfunction in AT with decreased expression and OXPHOS activity in adipocytes not only results in whole body insulin resistance but also induces hypertension and cardiac dysfunction.[228]

### *Brown adipose tissue*

Brown adipose tissue (BAT) is abundant in humans during early postnatal development, but absent or present only in small amounts in adults. It is located in interscapular and supraclavicular regions of the adult thorax. BAT originates from the myogenic (Myf5þ) lineage, while WAT has a mesenchymal origin. Brown adipocytes are thermogenic cells and maintain the balance between energy storage and energy expenditure through matching oxidative phosphorylation and dissipation of the proton gradient. The high-oxidative capacity of BAT is due to its high mitochondrial density. WAT can undergo a process known as browning where WAT takes on characteristics of BAT such as expression of uncoupling protein 1 and an increase in mitochondria and oxidative metabolism,[237] resulting in higher energy expenditure. These inducible or beige adipocytes have unique molecular and developmental characteristics compared to classical brown adipocytes,[238,239] but both increase energy expenditure through the uncoupling of oxidative phosphorylation from ATP production as a result of a transmembrane proton leak mediated by uncoupling protein 1. Browning of WAT can be induced by chronic cold exposure, PPARgamma agonists, leptin, natriuretic peptides, or beta-adrenergic stimulation.[240] The three core transcriptional regulators of inducible brown fat are PPARgamma, PGC-1$\alpha$, and the PR domain zinc finger protein 16.[237,238] The activity of BAT negatively correlates with BMI,[241] and browning of WAT has been shown to have anti-obesity and anti-diabetic effects in rodent models.[238] Conversely, genetically induced, severe mitochondrial dysfunction in AT results in whitening of BAT.[228] The prevalence and glucose-

uptake activity of BAT is negatively correlated with patient's age[242] and with obesity.[243] CR on the other hand increases BAT activity and attenuates the age-related decline in mitochondrial mass and mitochondrial function in BAT of rats.[244,245] Manipulations that increase BAT activity have also been shown to increase cellular stress resistance.[246] Thus, brown fat activation results in increased energy expenditure and limits weight gain. Browning of WAT through targeted pharmaceutical interventions may be an efficient way to increase energy consumption also in humans, making AT a good candidate organ to treat obesity and possibly also to slow the ageing process. However, parathyroid hormone-related protein (PTHrP)-regulated and IL-6-regulated browning of AT also occurs in cancer patients.[247,248] Here, it enhances energy dissipation and thus contributes to the progression of cancer-associated cachexia.

## Summary

Mitochondria are central regulators of the ageing process in the heart, in skeletal muscle. A decline in mitochondrial content and mitochondrial function plays a major role in ageing heart and skeletal muscle, contributing to the development of cardiac dysfunction or sarcopenia, respectively. However, the exact mechanisms by which aged mitochondria affect cardiac or skeletal muscle function are diverse, but the following effects can be envisioned: the reduced respiratory capacity can result in an energetic deficit of cardiac and skeletal myocytes. An increased susceptibility of MPTP opening could increase apoptotic cell death of cardiomyocytes or skeletal muscle cells. Replacement of cardiomyocytes by fibroblast in the heart as well as the low regenerative capacity of aged skeletal muscle could then facilitate functional impairment of heart and muscle. Furthermore, an increase in ROS production by mitochondria could evoke an increase of mitochondrial damage and consequently removal of these damaged organelles, again resulting in an energetic deficit.

Even in AT, which exhibits a much lower mitochondrial density than both muscular tissues, mitochondria have emerged as major regulators of the ageing process. Impaired mitochondrial activity in adipocytes is associated with alterations in AT metabolism, differentiation, and adipokine release. In addition, mitochondrial dysfunction in AT can cause deterioration in other organs' function and has an impact on lifespan. However, exact mechanisms involved in the latter effect remain to be fully elucidated.

## Acknowledgements

The authors certify that they comply with the ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle.[259] The study was funded by the German Research Foundation (BO2955/2-1 and SCHU843/9-1).

## Conflict of interest

All authors declare that they have no conflict of interest.

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