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REVIEW ARTICLE

Mitochondrial theory of skeletal muscle ageing – new facts, new doubts

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Abstract

For many years, scientists have been pursuing research on skeletal muscle ageing both in humans and animals. Studies on animal models have extended our knowledge of this mechanism in humans. Most researchers agree that the major processes of muscle ageing occur in the mitochondria as the major energy production centres in muscle cells. It is believed that decisive changes occur at the enzymatic activity level as well as in protein synthesis and turnover ability. Deregulation of ion channels and oxidative stress also play significant roles. In particular, in recent years the free radical theory of ageing has undergone considerable modification; researchers are increasingly highlighting the partly positive effects of free radicals on processes occurring in cells. In addition, the influence of diet and physical activity on the rate of muscle cell ageing is widely debated as well as the possibility of delaying it through appropriate physical exercise and diet programmes. Numerous studies, especially those related to genetic processes, are still being conducted, and in the near future the findings could provide valuable information on muscle ageing. The results of ongoing research could answer the perennial question of whether and how we can influence the rate of ageing both in animals and humans.

Keywords: muscle, ageing, mitochondria, enzymatic activity, ROS.

Introduction

Ageing is associated with a gradual loss of muscle mass and strength in both humans and animals. It is thought that skeletal muscle mitochondria play a significant role in this process. The free radical theory of ageing was formulated nearly six decades ago. In recent years, however, many scientists have questioned the crucial role of mitochondrial reactive oxygen species (ROS) in governing the ageing process. In turn, new evidence points to other aspects of age-related mitochondrial dysfunction, suggesting that mitochondria retain a crucial role in the complex network of processes that lead to the ageing of cells and organisms. Many animal and human studies have shown that skeletal muscle mitochondria change during the ageing process. These changes mainly include increased mitochondrial DNA (mtDNA) mutations, decreased activity of selected mitochondrial enzymes, and changes in the respiratory chain, along with reduced maximum capacity (56).

Although this process is still not fully understood, many scientists believe that it starts with molecular damage, which leads to dysfunction of cells, tissues, and eventually organs. Recently, there has been a lot of controversy over objective methods of measurement of mitochondrial energy production. This can mainly be explained by the differences in research methodology, and above all, by whether or not the study subjects' physical activity is controlled, which turns out to have considerable influence on the rate of ageing of the muscles. However, that proper diet and exercise programmes may reduce oxidative damage and improve mitochondrial function is above controversy. Although these programmes may not completely prevent the primary effects of ageing, they may help to delay the process and alleviate the consequences (31).

The result of the latest research involving genetically modified mice is a new theory of ageing, which has a much broader and more comprehensive view of the pathogenesis of ageing processes and the

role of mitochondria and free radicals in this. High accumulation of ROS can cause substantial damage to cell membranes and organelles, DNA, and proteins, however, moderate amounts of ROS are necessary to maintain many biological processes, including gene expression (21). Our work presents current scientific reports on these topics, including the emerging controversy (34).

Mitochondria as centres of intracellular energy production. One of the most critical components of the mitochondria is the respiratory chain, which contains five complexes with many subunits, the last of which is adenosine triphosphate (ATP) synthase. Highly specialised membrane conveyors transport protons and electrons, which ultimately reach oxygen as their acceptor, resulting in the formation of a water molecule. During this transport, energy is generated, which is then used for the production of ATP. It appears, however, that the electron transport system (ETS) is imperfect, and during the transfer of protons and electrons there may be a significant but highly variable leak of protons and hydrogen ions back to the matrix space, but it does not occur through the complex V of the respiratory chain (Fig. 1).

Such a proton transfer mechanism may not be coupled to the ATP phosphorylation process, resulting in an increased demand for reducing equivalents. Based on this, it can be concluded that mitochondria have a much higher ability to generate energy in the form of ATP than is usually required (24).

Studies have shown that complexes I (NADH dehydrogenase) and III (coenzyme Q:cytochrome *c* oxidoreductase) are the main sources of ROS (50). Until recently, it was thought that this excessive expansion of electron transport was a source of increased oxidative damage, which in turn was caused by the increased synthesis of free radicals. However, it has been proved that electron separation actually reduces ROS production. It was found that mitochondrial DNA has about a 100-fold higher mutation rate than nuclear DNA. There are plural factors that affect this. The most important of them is the location of the mitochondrial genome on the inner mitochondrial membrane adjacent to the respiratory chain. The respiratory chain is, as is known, the main source of intracellular ROS production, and the membrane's proximity to this can easily lead to damage to the mitochondrial genome. Also, the mitochondrial genome is not equipped with protective histones, and the mtDNA repair mechanisms are less efficient compared to those of the nuclear genome (50). These observations caused scientists to conclude that somatically acquired mtDNA mutations in ageing were caused mainly by oxygen free radical damage. The underlying assumption of the mitochondrial theory of ageing is that the accumulation of mtDNA mutations leads in consequence to abnormal activity of mitochondrial proteins in the respiratory chain, and this, in turn, results in a partial uncoupling of electron transport in the respiratory chain. As a result of these processes, there is a gradual but progressive increase in the production of ROS and the number of mtDNA mutations. The logic of the vicious cycle theory applies, according to which there is an exponential rather than a linear trajectory of the increase in the mtDNA mutational burden. There would be a domino effect with initial mutations triggering other mutations.

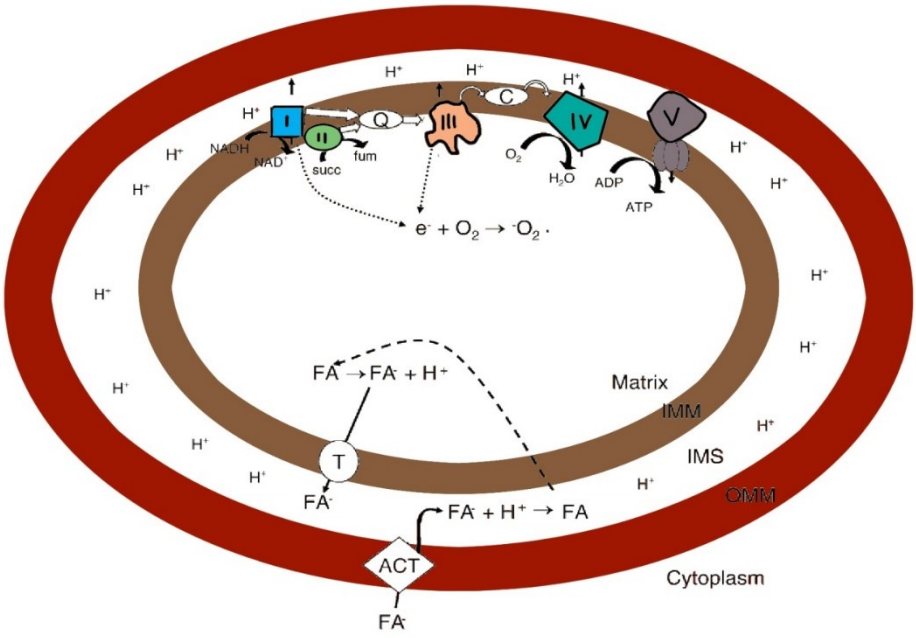

Fig. 1. Electron transport, proton leak, and ROS production in animal cells. Adapted from Staples J.F., Buck L.T.: Matching cellular metabolic supply and demand in energy-stressed animals. Comp Biochem Physiol A Physiol 2009, 95–105

However, there are doubts as to whether this is indeed the case. Recent research in this field indicates that the mtDNA mutation burden does not have to increase at all during ageing. This statement undermines the current model based on the free-radical theory of cell ageing, and also fails to confirm naturally-occurring, age-dependent mtDNA mutations (54). However, recently collected data have highlighted the importance of naturally occurring replication errors in the development of age-related mtDNA mutations. Large deletions are a typical example of mtDNA mutation in post-mitotic tissues such as muscles and neurons (8). Mutations of this type usually remove several kilobase pairs (kbs) of the mitochondrial genome. As the genome consists almost entirely of coding genes, it is highly probable that such mutations are of great functional importance. Recent research suggests that the once-deleted mtDNA species are stable over time. Until recently, it was thought that the homologous repetitions within single-stranded DNA (ssDNA) played the dominant roles in deletion processes. However, new scientific reports strongly argue that there is damage to both strands (double-strand breaks, DSBs), and this may be the driving force. These changes may result from many naturally occurring cellular processes such as oxidative damage, replication, or UV radiation. When a DSB is formed, the mtDNA molecule is repaired by increased exonuclease activity. In the first stage, ssDNA is formed, which in turn leads to homologous repeats and ultimately to mtDNA deletion (33).

The mutator mice model and the significance of early mutations. Studies on genetically engineered mice have revealed valuable information on the ageing of mitochondria. Researchers created homozygous knock-in mice (PolgD257A / D275A) that express a proof-reading-deficient version of PolgA, the nucleus-encoded catalytic subunit of mtDNA polymerase. DNA polymerase γ (POLG) is responsible for replication of thousands of copies of the 16-kilobase mitochondrial genome (mtDNA) in each human cell. The holoenzyme DNA polymerase γ (pol γ) is comprised of the catalytic subunit (encoded by POLG in the chromosomal locus 15q25) and the dimeric form of its subunit (encoded by POLG2 in the chromosomal 17q24.1 locus). MtDNA encodes 13 proteins that are necessary for the electron transport chain that provides the majority of ATP in the cell. Thus, mtDNA replication is vital to life, as demonstrated by the embryonic mortality of POLG – knockout mice. Scientists have identified over 200 mutations in POLG associated with certain mitochondrial diseases over the last ten years in humans (69). Through these mutations, the scientists showed a significant increase in the accumulation of somatic mtDNA mutation during a lifetime. This is probably due to a decrease in the lifespan and a clear progeroid phenotype. This type of phenotype combines the vast majority of the typical features of physiological ageing, such as reduced fertility, kyphosis, testicular atrophy, stem cell atrophy, hemopoietic impairment, cardiomyopathy, and general weakness (34). It is believed that a somatic mutation is at least initially unique. Therefore, a justified question is how a sufficient level of heteroplasmy can be achieved to cause a functional defect. It is already known that a process called clonal expansion is responsible for this. It can occur selectively when the mutant forms of mtDNA expand preferentially at the expense of the so-called wild-type or occur neutrally; selective expansion being based on the size difference and possible in the case of large-scale mutations. There is already evidence in the form of *in vitro* tests confirming their occurrence (16). Recently, it has been assumed that mtDNA is constantly reversible in non-dividing cells (relaxed replication), and this is termed "the neutral theory of clone extension". It is believed that the number of mutated forms of mtDNA is increased to a significant level by random drift. Such processes have a slow and progressive nature for decades, but they ultimately affect the functional significance of mutations that arose at an early stage of the organism's life (29). Mitochondrial DNA replication is the driving force of clonal expansion, and therefore even the negligible initial burden of mutations may eventually lead to functional defects in older people and animals as a result of multiple replications.

In forming such a thesis, one should take into account the extended period over which the clonal expansion processes occur. Recent research suggests that the natural history of mtDNA mutations in the ageing processes is related to progressive clonal expansion of the limited pool of early mutations rather than to ongoing mutagenesis. The research results discussed here again indicate the importance of ROS in stimulating an increased amount of mtDNA mutations in old age in humans and animals. However, if early mutations are assumed to be important, then how may that be reconciled with the fact that low-level seeding mutations are not somatic at all, but hereditary? In order to explain this phenomenon, a pair of related and a pair of unrelated persons were examined using parallel, deep resequencing. It was shown that many seemingly somatic mtDNA mutations are actually transmitted by the mother (54). Similar final results were obtained in mutator mice where it was observed that the wild offspring of the heterozygous mutator mother had germline mtDNA mutations. The tested mice showed a moderate progeroid phenotype while maintaining the normal function of gamma polymerase (60).

Hayashi *et al.* (22) came to very interesting conclusions in their work. They showed that age-related diseases, including respiratory disorders of older human fibroblasts, do not result from mtDNA mutation, but from the epigenetic regulation of genes encoded by the nucleus, as evidenced by the fact that the normal respiratory function is restored by reprogramming older fibroblasts. One crucial issue that needs to be addressed is whether older people actually accumulate such amounts of somatic mtDNA mutations as the mtDNA

mutator mice do in their tissue, and this potential disproportionality in our opinion, could also apply to other species of animals whose lifespans and ageing processes are markedly different from short-lived mice.

The mutation rates and intraspecific divergences of the mitochondrial genome of *Pristionchus pacificus* were studied by Molnar *et al*. (48). According to their calculations, the overall rate of mutation in *P. pacificus* is 7×10^{-8} per site per generation and is less than one order of magnitude different from the estimates in *C. elegans* and *Drosophila.* Using the estimation of this mutation compared to the mitochondrial genome of nine *P. pacificus* isolates, they calculated the minimum time to the last common ancestor as 10^{5}–10^{6} generations. This experiment shows that there are not only interspecific but also intraspecific differences in the rate of mutations (48). Moreover, the variability of the mitochondrial genome may influence the frequency of mutations, and the induced mutations more significantly affect male than female reproductive function. The potential for haplotype-specific influences on the nuclear mutation rate is of great significance regarding evolutionary dynamics such as the accumulation of the genetic charge, the adaptive potential, and the evolution of sexual dimorphism (76).

Increased oxidative damage due to ageing – is it true? The destructive abilities of ROS, including O_2^- and H_2O_2 mainly produced by mitochondria, result not only from their quantity but also from the fact that their main place of production is located near particularly vulnerable mtDNA. Oxidation processes stimulated by free radicals lead to the synthesis of defective proteins, oxidized lipids, and mtDNA mutations. Such a wide spectrum of changes can ultimately lead to serious dysfunction of cells and mitochondria. These are further evidence of the mitochondrial theory of ageing which maintains that the accumulation of ROS damage causes age-related mitochondrial dysfunction. However, as mentioned at the beginning of this review, the dominant role of ROS in ageing processes is currently being discussed, which is related to the emergence of new research findings. It has been shown that ROS production increases in old muscles both in the subsarcolemmal and intermyofibrillar pools of mitochondria (14). Oxidative damage has a clear tendency to accumulate and may be partly related to the reduction of ETS activity. The decline in ETS activity extends the potential time that electrons remain in complexes I and III, and thus increases the possibility of electron transfer to oxygen GPx, which in turn intensifies ROS production. Theoretically, it may also be associated with decreased antioxidant defence activity and manganese superoxide dismutase (MnSOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes contribute to the conversion of O_2^- to H_2O_2, which is then reduced to H_2O (35).

Data on antioxidant enzyme activity in the ageing process are conflicting. Some studies showed increased activity (4), while others showed a decline in enzymatic activity (57). According to the data obtained until now, oxidative stress clearly increases with age, which is not accompanied by a significant increase in antioxidant activity and may be related to mitochondrial dysfunction. Exercise programmes may attenuate age-related oxidative stress by increasing the antioxidant activity of the enzymes, even when the content of antioxidant proteins decreases. Another way to slow down the ageing of mitochondria is endurance exercises. Interestingly, in the initial stage, these exercises cause an increase in the level of ROS, which cannot be fully compensated by stimulating biogenesis and increasing the scavenging of free radicals (61). It appears that intracellular ROS may play an essential role in signalling processes that may favourably affect the ageing process (78). In this way, we are approaching the modern view that not all ROS are necessarily detrimental, and that the balance between the production and deletion of ROS is probably the key to understanding their intracellular role. If ROS is vital in the ageing process, we expect that additional antioxidants will be beneficial. In fact, human studies have shown that excess antioxidants can be damaging and do not produce the expected positive effect initially (7). In studies conducted on transgenic rodents, attempts have been made to determine whether excessive expression of antioxidants is helpful in mitochondrial protection. The results were ambiguous. In one model where antioxidant expression was not targeted to mitochondria, no effect was shown, whereas targeted antioxidants, such as catalase, decreased the rate of ageing (40). On the other hand, mice with antioxidant deficiency in mitochondria had a very unfavourable phenotype, with premature death due to mitochondrial dysfunction and neurodegeneration. In mutant mice, ROS production and oxidative damage were shown to not always be as prominent as previously assumed. These results suggest that, in addition to ROS, other degenerative factors are also responsible for the age-related loss of muscle cells. This also raises the question of whether by regulating the oxidation state of cells, and thus the reduction of oxidative stress, the ageing process can be affected (30). Researchers conducted a study of mitochondrial function to explain this phenomenon. Mitochondrial function was evaluated both *in vitro* and *in vivo* in healthy, lean, young wild mice aged 3–6 months and older but similar animals at 15–18 months and compared to that in MCAT transgenic mice of the same ages. While older wild mice showed all typical harmful metabolic disorders, including significant insulin resistance (~35%) and increased oxidative damage, mitochondrial number (~30%) and function, and intramuscular lipids (~70%), older MCAT mice resembled young mice and did not show any of the age-related disorders. These data suggest that increasing ROS capture and reduction of oxidative status in the cell can prevent many age-related deficits (40).

Changes in mitochondrial mDNA and mRNA and levels of protein that accompany ageing. Discovered in 1963, human mtDNA is a small circular

double-stranded DNA molecule approximately 16.5 thousand base pairs in size (5). It encodes 37 genes, including two ribosomal RNAs (rRNA), 22 types of RNA transporting molecules (tRNA), and 13 proteins that are subunits of respiratory chain complexes. The discovery of that and its function has caused a lot of controversy and questions about the actual influence of mutations within mtDNA on ageing processes, and whether these mutations have such a huge impact on these processes as previously thought. A large part of the research (38) shows that the number of copies of mtDNA decreases over the years in skeletal muscle, but there is also research not supporting this trend (5). Since the decrease in the number of copies of mtDNA is greater in oxidative-type fibres, it is believed that this may be at least partially caused by oxidative damage (3). In many studies, there was a correlation between the decrease in the amount of mtDNA and the increase in damage to the mtDNA structure. This mainly applies to deletions and oxidative damage, although tandem duplications, rearrangements, and point mutations are equally frequent (30) since mtDNA is located near the main production site of ROS, and the lack of protective histones and hence weaker mechanisms for DNA repair leave this DNA particularly susceptible to oxidative damage. There is growing evidence that the majority of mutations are related to the intrinsic, inherent error level of gamma polymerase (pol gamma) mtDNA (39). Again, to prove this, a mutator mouse model was used. This time, the mice had defects in the function of pol gamma correction, which significantly accelerated the rate and degree of observed mutational changes within the mtDNA. These mice showed more abnormal mitochondria, premature ageing, sarcopenia, and shorter lifespans (33). Song *et al.* (67) discovered that normal intramitochondrial deoxynucleotide triphosphate (dNTP) pools in rat tissues are highly asymmetric, and *in vitro* fidelity studies indicate that these pools can activate base substitution and frameshift mutations with a substitution pattern that corresponds with mitochondrial substitution mutations *in vivo*. These data suggest that normal intramitochondrial dNTP pool asymmetries may contribute to mitochondrial mutagenesis and possibly cause mitochondrial diseases.

However, scientific disaccord over how significantly the damage to mtDNA affects the functioning of muscle cells still persists and especially in relation to age-related muscular dystrophy (30, 39). The basic question is whether mtDNA damage is a consequence or the cause of the ageing process. It has been shown that decreases in mitochondrial energy efficiency occur before mutations within the mtDNA. However, research has emerged that reveals a strong relationship between the speed of mtDNA mutation and bioenergetic decline (usually complex IV-cytochrome c oxidase) and muscle fibre atrophy. Interestingly, specific point mutations are often focused at the mtDNA replication control sites. This localisation of mutational changes can reduce gene transcription and be at least partially responsible for the reduction of protein production, which is observed with the ageing of muscle cells (2, 11). While the majority of mitochondrial genes, including cytochrome c, do not change with age, it is noticeable that the transcription of specific genes slows down. This applies to several components of polypeptide complexes I, IV and V. However, in the case of complexes II (succinate dehydrogenase) and III, decreases are noticeable only for a few components, while most of them remain unchanged (46).

The levels of proteins are controlled by the balance between the synthesis and the degradation of proteins, and interestingly, age-related changes in mRNA may not cause similar changes in protein amounts (47). It has been proven that with the advancement of age, both the synthesis of mitochondrial proteins and proteolysis through ubiquitin-proteasome systems and lysosomal enzymes decrease. A large number of mitochondrial proteins, including the polypeptide components of ETS complexes, do not change with age (27). There is a counterfinding in a study where proteomic analysis was performed, which showed in the case of complexes I, III and V the presence of abnormal polypeptides increasing with age in skeletal muscle in humans (20) and in rodents (51). It appears that proteins involved in glycolysis do not change with age. This time the study result is consistent with the observed transition from glycogenesis to more oxidative metabolism. However, information on changes in other protein groups including mitochondrial tricarboxylic acid (TCA) (in particular citrate synthase and isocyte dehydrogenase) is not sufficient for any firm conclusions to be drawn (20, 38, 51).

As is known, there are two main mechanisms to repair damaged mitochondria and control their quality. The first is the mitochondrial unfolded protein response (UPRmt), which protects the mitochondria against the harmful effects of stress stimulus. The second mechanism is called mitophagy, or the process of elimination of damaged mitochondria. The UPRmt pathway is activated by misfolded mitochondrial proteins. Also noteworthy are the two recessive Parkinson's disease (PD) genes, PINK1 (induced by PTEN protein 1 or PARK6) and parkin (PARK2). An investigation of PINK1 and PARK2 has delivered direct evidence of the involvement of damaged mitochondria in PD pathology. Parkin is a cytosolic E3 ubiquitin ligase, and PINK1 is the only known protein kinase with a mitochondrial targeting domain. These two proteins are involved in a common pathway regulating the mitochondrial quality control and promoting the selective autophagy of depolarised mitochondria (mitophagy). Pathogenic mutations in these genes lead to the loss of this pathway of quality control and the concentration of impaired mitochondria, which are thought to be a source of toxic ROS and contribute to the death of nerve cells and onset of PD (64).

An equally important role in cell degradation processes is played by inflammasomes, first described

over a decade ago. Similarly to the apoptosome that activates the apoptotic cascade, inflammasomes activate the inflammatory cascade. Inflammasomes are macromolecular protein complexes present in the cellular cytoplasm that are capable of recognising and reacting to external cellular risk factors and have an important role in the innate immune response of the body. Activation of the inflammasome complex enables the maturation and secretion of proinflammatory cytokines interleukin-1 (IL-1) and interleukin-18 (IL-18). The secretion of these cytokines leads to the death of a cell by a different process from apoptosis, which is programmed pro-inflammatory cell death called pyroptosis (19). If there is a dysfunction in regulating inflammasome, uncontrolled cell damage may occur, which in turn may lead to many serious diseases. For example, it has been shown that excessive activation of the NRLP3 inflammasome plays a huge role in the pathogenesis of many diseases, including type 2 diabetes, gout, rheumatoid arthritis, Alzheimer's disease, and cancer. It appears that instead of blocking the action of cytokines which are later on the signalling pathway than the NRLP3 inflammasome, selective control of the activity of this inflammasome may have enormous therapeutic potential in the future (26).

Is there a decrease in the production of mitochondrial energy with age? There are still doubts as to whether age-related changes affect the level of mitochondrial energy production. It appears that significant decreases in the activity of mitochondrial enzymes are observed when physical activity is not a criterion for the selection of research objects. Much of the research on human skeletal muscle indicates that the activity of complexes I and IV is significantly reduced. Both of these complexes have more subunits encoded by more fragile mtDNA than other complexes, and this may be a reason for their activity decline (12). Very similar results were reported in rodents and dogs (23). However, there are studies that fail to support this rule in humans (9) and in rodents (51). The discrepant results of this research can be partly explained by the difference in the impact of ageing on various muscle types. This thesis was confirmed by a study in rats that showed a decrease in activity in complex IV in the gastrocnemius muscle of the lateral calf, but not in the middle one (3). It was also shown that divergent results might also result from differences in the isolation of the research material, different research methodologies, or different normalisations of enzymatic activity. *In vivo* measurements of oxidative activity in skeletal muscle are conducted with phosphorus magnetic resonance spectroscopy (31P-MRS). With this research method, the phosphocreatine kinetic processes and the time of its recovery after muscle contraction are analysed. Some of the studies performed *in vivo* in older people (27), and at least one in mice (44) showed that with age, the rate of the maximum ATP flow declines, but also this time there are studies that do not confirm this fact (2, 63). More and more researchers believe that the majority of decreases in mitochondrial function attributed to chronological age are the result of a lack of physical activity.

When experiments are conducted in which the same physical activity is investigated in young and old people, the results no longer show such pronounced changes in the activity of mitochondrial enzymes, mitochondrial breathing or age-related ATP flow (38, 47, 61). Similar conclusions were drawn from studies in mice (18). *In vivo* MRS in a subject with an active lifestyle showed no significant change in mitochondrial oxidation capacity – in this case the maximum ATP flux between young and older individuals (37). This relationship was not confirmed in an MRS *in vivo* study in sedentary subjects. This study showed a decrease in base oxidation and phosphorylation with age (55). A somewhat interesting finding is that physical activity can reverse the reductions in age-related mitochondrial function in most, but not all, markers of energy production in the mitochondria (38). These results are vital, but it should be emphasised that only a particular part of the enzymatic processes and decreases in the mitochondrial function are reversible, while other decreases do not depend on the level of physical activity and cannot be inhibited.

Decreased mitochondrial biogenesis and ageing process. The large variability of mitochondrial functions and their structure allows them to efficiently manage cellular processes, including apoptosis, the deregulation of which is considered a key factor in sarcopenia. Mitochondrial biogenesis can occur in two ways. The first one expands the content of mitochondrial cells and the second divides the existing cells. When the increase in energy demand exceeds breathing capacity – mainly in response to physical effort, stress, hypoxia, availability of nutrients, hormones (including insulin), ROS production or temperature, a rescue mechanism of mitochondrial biogenesis is initiated. Once it is launched, the nuclear genome begins to produce mitochondrial regulatory factors. After transporting them inside the mitochondria, they play the role of initiators of mtDNA replication and transcription processes, which ultimately results in the expansion of the existing mitochondrial network. The most important regulator of mitochondrial biogenesis is peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-alpha (PGC-1α). PGC-1α is responsible for coordination and interaction with many co-translational factors, including peroxisome proliferator-activated receptors activated by peroxisome proliferation, myocyte enhancing factors (MEF) and cAMP response element-binding protein (CREB) causing transcription of nuclear genes encoding mitochondrial proteins. A critical role of PGC-1α is activation of nuclear respiratory factors 1 and 2 (Nrf-1, Nrf-2) on promoters. The effect of this is the transcription of an even more significant group of mitochondrial proteins coded by the nucleus, including mitochondrial transcription factor (Tfam). Tfam is responsible for regulating the rate of transcription and

the content of mtDNA and the organisation of mtDNA into nucleoid structures. These processes are considered to be a factor that maintains the integrity of mtDNA. The lowering of the PGC-1α level may explain the slowdown in mitochondrial biogenesis. This thesis was confirmed by a study in which it was found that the overexpression of PGC-1α in MS in old mice improved the oxidation ability, and at the same time decreased the degree of mitochondrial degradation and ultimately prevented muscle atrophy (73). Interestingly, the increased expression of PGC-1α accompanied the attenuation of age-related increases in the number of inflammatory cytokines and the prevention of age-related insulin resistance (73). However, differences in measurements of PGC-1α levels in ageing skeletal muscles have been demonstrated, and some studies do not confirm any decline in PGC-1α expression (23), while other studies confirmed its decline with age (58). The results of the analysis of the degree of gene and protein expression of both Nrf-1 and Tfam are also ambiguous. However, preliminary evidence suggests that Nrf-1 binding to the Tfam promoter appears to increase in the elderly (14, 38).

The balance between fission and mitochondrial fusion has a significant effect on their dynamics. Both processes are necessary for the transfer of mitochondria between cell divisions and to cover the increased demand for ATP. These processes allow the detachment of dysfunctional mitochondria from the network and their removal by autophagy, thus playing a pivotal role in maintaining the quality of mitochondria and the integrity of mtDNA. Mitochondria that are removed by fission often have lower membrane potential and become the target of autophagy (71). In one study, it was proved that the activation of the mitochondrial fission mechanism is enough to induce muscle atrophy (59), while an other study contradicted it (62). It has been shown in yeast that increased cell division leads to shortened viability and higher sensitivity to apoptosis induced by ROS (62). The ability to mix mitochondrial components through mitochondrial fusion is an essential factor in preventing mtDNA mutations which can consequently lead to disturbances in the respiratory function. As a result, it is also possible to accumulate mutant mtDNA material, which can then be removed efficiently in the fission process and autophagy. This is sufficient proof that fusion has a determining function in the regulation of mtDNA integrity and respiratory function (32). Human fusion is controlled by the optic atrophy1 (Opa1) gene and the two GTPases mitofusin1 and 2 (Mfn1 and Mfn2 isoforms). Mfn1 and Mfn2 are in the outer mitochondrial membrane (OMM), where they organise binding and synthesis. Opa1, meanwhile, is a factor facilitating the fusion of localisation on the internal mitochondrial membrane (IMM). Additionally, Opa1 helps in controlling degradation processes affecting apoptosis and maintaining a stable structure of internal mitochondrial cristae, which prevents the release of apoptotive cytochrome c (10). In one study, it was shown that the expression of the Mfn2 gene was lower in skeletal muscles of older people (12). Noteworthy is the study of knockout mice Mfn1 and Mfn2, where increased mitochondrial proliferation and an increased number of mutations correlating with the decrease in mtDNA have been demonstrated. Interestingly, these changes occurred in parallel with accelerated muscle atrophy (15). Moreover, it has been shown that mutation in another key Opa1 fusion protein leads to reduced oxidative phosphorylation capacity and ATP production in skeletal muscle in humans (42). The results of the research discussed above show that the age-correlated dynamics of fission, remodelling and fusion processes affect the respiratory function, the production of ROS, the integrity of mtDNA, and thus the ageing of cells.

The processes of mitochondrial turnover are as important as the processes of fission and fusion. This is mainly provided by the autophagy-lysosome system, a cellular system that degrades the mitochondria as well as other cellular components. The turnover is performed mainly through the autophagy-lysosome system, which is designed to degrade mitochondria and other cellular components. Elimination of mitochondria in the autophagy-lysosome system is referred to as "mitophagy" and is a process regulated and controlled by a set of autophagy gene (Atg) products. Research on yeast has proved that the recognition process is managed by Atg32, a specific autophagy receptor on the OMM and by the Atg8 receptor and its Atg7 activator. However, when it comes to mammals, mitophagy is not adequately described. In mammals, Nix and light chain 3 (LC3) are considered to be homologous to Atg32 and Atg8. It also appears that mammals have an additional pathway for mitophagy through the ubiquitination of OMM proteins, followed by recognition with the LC3 complex (52).

It is known that mitophagy selectively removes damaged mitochondria that are depolarised or exhibit excessive production of ROS (71). This thesis is confirmed by the fact that inhibition of autophagy causes a decrease in oxygen consumption, increased ROS production, and an increase in the number of mtDNA mutations. Studies in old rats have shown that over the years there is a decrease in the effectiveness and dynamics of autophagy, both systemically (13) and in skeletal muscle. The slowdown in mitophagy may have a negative effect on ageing muscles, and mitophagy has been negatively correlated with oxidative damage and apoptosis (75). Autophagy can extend lifespan, which is confirmed by studies conducted in several species of animals. The pathological forms of mitochondrial proteins can be removed by the ubiquitin-proteasome system, which is an essential element of mitochondrial quality control and protein degradation (70). There is evidence from studies in mammals where the age-related decline in ubiquitin-proteasome activity in skeletal muscle has contributed to increased muscle atrophy (43). Ageing can have a mixed effect on the components of

the ubiquitin-protease system (1). A specific group of these proteins decreases, while others remain stable. Interestingly, the nature of changes in proteasomal activity may be specific for a given type of muscle fibre (46, 51).

Increased muscle cell apoptosis dependent on mitochondria. Another mechanism by which mitochondria affect changes and cell viability is apoptosis, known as programmed cell death. The factor activating apoptosis may be the impairment of oxidative phosphorylation or redox potential, although other specific proapoptotic signals have also been discovered. There are two pathways by which mitochondria can induce apoptosis: one is dependent on and the other is independent of caspase. The dynamism of apoptosis greatly increases with age and probably contributes to sarcopenia and other age-related dysfunctions. Many studies have shown a correlation between apoptosis and sarcopenia or other skeletal muscle dysfunction; however, it has not been proved conclusively (45). In older people, the number of apoptotic cells increases, mainly in type II fibres Interestingly, it has been shown that both in humans and in animals, with age, increased activity of the caspase-independent pathway occurs, whereas the caspase-dependent pathway activity does not increase (45). In a study conducted in ageing people, researchers observed an increase in transcripts of the apoptosis-inducing factor (AIF) gene in skeletal muscles, but not in Bax, Bcl-2 or caspase-3 expression (53). One human study showed no change in caspase-3 or -7 (74). This is confirmed by animal studies, which discovered that the mitochondrial permeability transition pore (mPTP) is more vulnerable to being opened (14), and that the activities of caspase-independent AIF and apoptotic mitochondrial endonuclease G (EndoG) increase (14, 41) during ageing. Discrepancies in research results are at least partly explained by a study conducted in rats where apoptosis and apoptosis markers were shown to be specific for both age and type of fibre, particularly with respect to the caspase-dependent pathway (14). One study showed that inactivity is accompanied by increased caspase-3 activity in young rats, but not in older specimens, and by significantly elevated EndoG levels in older rats, but not in young ones (41). It appears that old and young skeletal muscles in rats respond to apoptotic stimuli using different signalling pathways (41), and the explanation of these mechanisms will require further research.

Factors mitigating ageing. It is well known that physical training has a positive effect on reducing the effects of ageing and may even slow it down. Positive effects of exercises include induction of mitochondrial biogenesis and protein synthesis, increased skeletal muscle gene expression, and increased skeletal muscle oxidation. Unfortunately, the decline in physical activity in older people and animals is widespread (28). Therefore, the question should be asked whether the malfunctioning of the mitochondria is the main symptom of ageing or is simply a consequence of the resting habits of the elderly. These doubts have been analysed in many studies, but there is great difficulty in the objective assessment of the level of physical activity (55). There is clear evidence that a properly selected training exercise plan can significantly improve the function of skeletal muscle mitochondria in older people (46). In addition, training has an effect on slowing down the processes of apoptosis, which, as already mentioned, intensify with age. It was shown that after four months of aerobic exercise in older people there was an increase in protein synthesis and increased activity of some mitochondrial enzymes such as citrate synthase and cytochrome c oxidase. In addition, the level of gene expression associated with mitochondria and biogenesis improved, and the degree of these changes was similar to those observed in younger people (65). Exercise also increases the activity of antioxidant enzymes and heat shock proteins, which in turn reduces the level of ROS and reduces the likelihood of oxidative damage in the mitochondria during the ageing process. Regular endurance training will not completely restore cellular metabolism to the levels of young people. Therefore, it was assumed that there is a so-called age effect independent of external factors (27). This was confirmed by studies conducted with an omics technique, which showed that after six months of training, the transcriptional signature of ageing was substantially, but not completely reversed to a young adult transcriptome (46). In summary, exercise is an important factor in mitigating the effects of ageing, including mitochondrial changes, but it is not able to completely inhibit these changes.

Another important aspect affecting the rate of ageing is the reduction of calories in the daily diet. This restriction requires the consumption of about 20%–40% fewer calories than usual, which slows weight loss and skeletal muscle strength decline. Caloric restriction (CR) is considered to be the most effective method that delays both primary ageing (natural ageing) and secondary ageing (accelerated ageing due to disease and inappropriate lifestyle). Most rodent studies show that CR extends the maximum lifespan by up to 50% and reduces the incidence of many age-related diseases, including cancer and metabolic diseases (68).

The data obtained until now show that the benefits attributed to CR are mainly related to the reduction of oxidative stress (68). It has been shown that ten years of CR in primates resulted in a significant reduction in oxidative damage to lipids and proteins (77), and in old rats CR also reduced production of ROS (17). Compared to animals fed *ad libitum*, ageing animals with CR show fewer mitochondrial mutations of mtDNA and nuclear DNA, and less oxidative damage to skeletal muscle mitochondria (6). It seems that dietary restrictions can effectively modulate mitochondrial efficiency and their content and function. Limitation of calories in the diet reduces energy expenditure in animals and people through mitochondria, which consume less oxygen,

while maintaining normal levels of ATP production (6, 63). It has been confirmed in rodent studies that this energy adaptation is dependent on the reduced proton leak, and the reduction of proton leak is possible in turn due to the change of environment to one less oxidative (6). Although CR may affect some of the ETS enzymes, it does not affect gene expression, protein levels or citrate synthase activity or other TCA proteins (20). The effect of CR on the dynamics of mitochondria has also been analysed. It appears that CR increases mitochondrial biogenesis compared to the control group, thus slowing down the decrease in PGC-1α gene expression with age in rodent skeletal muscle. As a result, the oxidation ability of old CR animals is probably maintained (20). The significance of caloric restriction and even fasting in regulating aging processes was indicated by Lam and McKeague (36). According to them, it is possible that the unique combination of dietary studies on animals, better DNA damage and mutation mapping, and improvement of analytical methods for quantifying damage burden on the genome might help discover the biochemical mechanisms of mtDNA changes and their association with disease phenotypes.

However, regardless of the mechanisms underlying CR, it should be clearly stated that caloric restriction, as well as physical exercise, may partly inhibit the negative effects of ageing such as decline in muscle strength, number, type and thickness of muscle fibres, and age-related sarcopenia.

Finally, scientists have recently been interested in using so-called mimetics to mitigate age-related mitochondrial dysfunction. The most popular is resveratrol (3,4,5,9-trihydroxystilbene), a phytoalexin present in red wine. Studies revealed that the addition of resveratrol to the rodent diet clearly increases the number of copies and the function of mtDNA and, consequently, increases the processes of mitochondrial synthesis. There was also an increase in exercise capacity and motor function, and a decrease in metabolic dysfunctions in the rodents tested (72).

An analysis at the molecular level showed that resveratrol induces an increase in the expression of PGC-1α, Tfam, and mitochondrial uncoupling protein 3 (UCP3), and also increases adenosine monophosphate-activated protein kinase α (α-SIRT1), 5' adenosine monophosphate-activated protein kinase (AMPK), and PGC-1α activity. How stress-affected mice responded to the administration of resveratrol was also checked. These mice had higher physical strength, maximum contraction force and oxygen consumption, and higher levels of transcripts from the α and ETS PGC-1 genes than those that did not receive mimetics (49). Convergent results were obtained in rats receiving resveratrol, but no decrease in apoptosis was observed. However, the effects of long-term resveratrol supplementations are not that promising. Unfortunately, over an extended period mice did not show such apparent positive effects of supplementation. Although resveratrol administration allowed the maintenance of high type II fibre contraction and reduced oxidative stress, it did not prevent a progressive decline in muscle mass and strength (25).

Summary. The amount of research on the subject of muscle ageing in humans and animals is very significant, and all of it clearly confirms the dominant role of mitochondria in these processes. Mitochondria undergo many morphological, biochemical and genetic changes. The changes include increased DNA mutations, decreased enzyme activity and mitochondrial respiratory chain efficiency, lower expression of some mitochondrial proteins, and a fall in total mitochondrial content with more morphological changes. Mitochondria, as centres producing energy in the form of ATP, can lower the potential of the membrane, reduce the concentration of ATP in the cell, and ultimately signal cell apoptosis through changes in respiratory activity and capacity. If the intensity of apoptotic processes is not balanced by increased protein synthesis, it eventually leads to the disappearance of muscle fibres and age-related sarcopenia. Fortunately, there is indisputable evidence that we can at least partially slow down the occurrence of the effects of old age by using a special diet based on reduced calories, supplementation with mimetics, and, probably the most important factor, maintenance of regular physical activity in old age. Most researchers still believe that the accumulation of oxidative damage caused by long-term ROS overproduction is responsible for the majority of age-related changes. Available studies confirm that due to the decreased production of ROS by the targeted expression of antioxidant enzymes, it is possible to reduce age-related dysfunction and restore mitochondrial functions to the level of young animals. The changes in mitochondrial functioning occurring with age are multifactorial, and the most frequent causes of their dysfunctions are related to both mitochondria and systemic changes, including hormonal disorders (66). Unfortunately, the prescription for eternal youth has still not been discovered. More research is needed in this field. It is essential to determine which age-related changes are universal and which depend on physical activity or lifestyle behaviours. However, more and more studies undermine the dominant role of ROS in cell ageing and emphasise their stimulating role in the mobilisation of some skeletal muscle regeneration processes. The most important for the proper functioning of cells is the balance between ROS production and their removal. It is also necessary to identify which mitochondrial dysfunctions can be slowed down and even inhibited completely by a healthier lifestyle. In our opinion, this will be the goal of further interesting research in the coming years.

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