

Searching for a mitochondrial root to the decline in muscle function with ageing

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Abstract

Sarcopenia, the age-related loss of muscle mass and strength, is linked to a range of adverse outcomes, such as impaired physical performance, cognitive function, and mortality. Preventing sarcopenia may reduce the burden of functional decline with aging and its impact on physiological and economic well-being in older adults. Mitochondria in muscle cells lose their intrinsic efficiency and capacity to produce energy during aging, and it has been hypothesized that such a decline is the main driver of sarcopenia. Oxidative phosphorylation becomes impaired with aging, affecting muscle performance, and contributing to an age-associated decline in mobility. However, it is unclear whether this deterioration is due to a reduced mitochondria population, decreased mitochondrial energetic efficiency, or a reduced capacity to dynamically transport oxygen and nutrients into the mitochondria, and addressing these questions is an active area of research. Further research in humans will require use of new “omics” technologies, progress in neuroimaging techniques that permit energy production assessment, and visualization of molecules critical for energetic metabolism, as well as proxy biomarkers of muscle perfusion.

Keywords Mitochondria; Aging; Skeletal muscle; Sarcopenia; Branched chain amino acids; Muscle quality

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Ageing, body composition, and muscle quality

Ageing research has substantially advanced by characterizing longitudinal trajectories of ageing phenotypes, although the mechanisms that underlie these changes and the basis for the observed heterogeneity across individuals remain largely unexplored. Longitudinal studies have shown that the ageing process is associated with loss of muscle strength, muscle mass, and aerobic capacity.^{1,2} The loss of lean body mass begins around age 30, which then progressively accelerates in older men and women. However, the decline in muscle strength observed in epidemiological studies is greater than what is expected given the magnitude of changes in lean body mass, suggesting that ageing is associated with impaired muscle biomechanical quality (strength/mass).^{1,2} This

notion is further supported by recent studies, which have observed that low muscle strength is an accurate, independent predictor of gait performance, response to exercise interventions, and mobility disability, while muscle mass is only marginally associated with these outcomes.^{3–5} Interestingly, the Baltimore Longitudinal Study of Aging revealed that visceral obesity, nerve conduction velocity, and performance in cognitive tests relative to executive function were the strongest predictors of muscle quality in older persons.² A subsequent study further demonstrated that the difference between men and women in the rate of muscle quality decline is fully accounted for by differences in percent body fat.⁶ Fat accumulation is a phenotypic manifestation of metabolic impairment, especially when fat accumulates in visceral compartments, such as in the abdomen, liver, or muscle.⁷

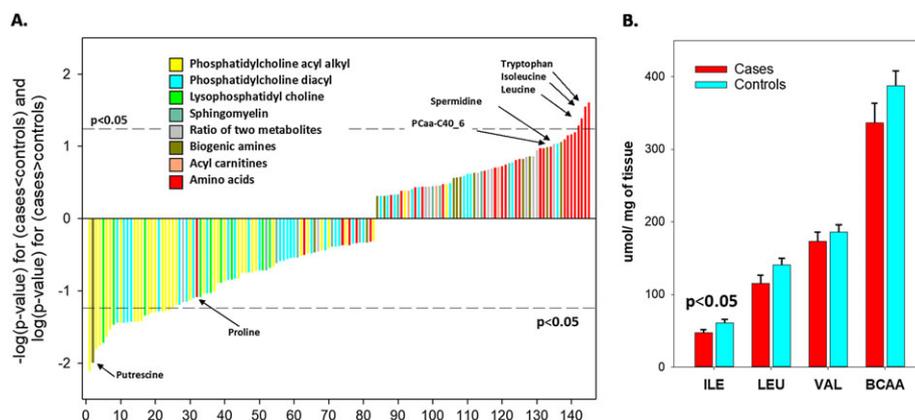
Amino acid uptake, mitochondrial function, and muscle quality

In order to better understand the mechanisms by which muscle quality declines with ageing, a matched (by sex, age, and height) nested case–control study was performed within the Baltimore Longitudinal Study of Aging, characterizing the plasma metabolic profile associated with low vs. high muscle quality in participants that were 60+ years old.⁸ In this study, muscle quality was defined as the ratio of muscle torque (assessed by isokinetic dynamometry) to muscle mass, which was estimated from the muscle cross-sectional area of the mid-thigh acquired by computed tomography. Using a targeted metabolomics approach, participants with low muscle quality presented significantly higher plasma concentrations of isoleucine and leucine, suggesting that low muscle quality is characterized by impaired transport of amino acids, especially branched chain amino acids (BCAAs), across the muscle cell membrane (Figure 1A). Further validating this hypothesis, BCAA concentrations in biopsy specimens collected from the *vastus lateralis* were lower in participants with low muscle quality compared with those with high muscle quality (unpublished results, Figure 1B). Reduced muscle BCAA uptake has serious consequences in regulating protein synthesis, as BCAAs activate mTOR, which is the master regulator for nutrient sensing. When BCAA levels are sufficient, mTOR is activated and causes an increase in p70S6K phosphorylation, subsequently activating the S6 ribosomal protein, thereby inducing protein synthesis.^{9,10} In contrast, at low BCAA concentrations, mTOR recognizes the limited substrate availability for protein synthesis/recycling and inhibits p70S6K phosphorylation, hindering normal protein recycling, and thus allowing ‘wear and tear’ protein damage

to accumulate. Additionally, BCAA supplementation can increase the expression of sirtuin 1 (SIRT1) and other genes involved in oxidative phosphorylation, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), nuclear respiratory factor-1 (NRF1), and mtDNA transcription factor A (TFAM). Cumulatively, these effects result in increased mtDNA content and mitochondrial activity, suggesting a corresponding increase in mitochondrial biogenesis.¹¹ Conversely, low BCAA levels are associated with impaired mitochondrial biogenesis and reduced energy production.^{9,10} Finally, isoleucine and leucine catabolism in muscle causes increased levels of NADH and FADH₂, which are essential cofactors for ATP synthesis.⁸ Incidentally, glycerophospholipids—specifically a subset of phosphatidylcholines—were significantly lower levels in participants with low muscle quality compared with controls.

In summary, these findings demonstrate that impaired transport of BCAAs and other amino acids into muscle negatively affects protein recycling and energy availability, which often corresponds to alterations in phospholipid metabolism. The exact reasons for why amino acid uptake is reduced in older persons with low muscle quality are unknown, and further work is required to identify putative intervention/therapeutic targets. Physiologically, amino acid uptake in muscle cells is regulated by three fundamental mechanisms: insulin signalling, BCAA (primarily leucine) blood concentration, and physical activity.^{12–14} Previous studies have also suggested that these ‘anabolic’ signals cause increased amino acid entry by dynamically enhancing muscle perfusion, and all three signals exhibit a dose–response relationship that is steeper in younger than in older persons.¹⁵ In other words, older persons tend to develop an ‘anabolic resistance’ to the three stimuli. Since muscle

Figure 1 Low muscle quality is characterized by impaired transport of amino acids, especially branched chain amino acids. (A) Metabolomics plasma metabolites are ordered by expression differences between age, sex, and height matched pairs of cases (low muscle quality) and controls (high muscle quality) from the Baltimore Longitudinal Study of Aging. Participants with low muscle quality presented higher plasma concentrations of most amino acids, particularly isoleucine and leucine, compared with the control group (adapted from Moaddel et al.⁸) (N= 79 cases and 79 controls). (B) Concentration of BCAAs in biopsy specimens collected from the *vastus lateralis* was lower in participants with low muscle quality compared with those with high muscle quality (unpublished results) (N= 9 cases and 9 controls). ILE, isoleucine; LEU, leucine; VAL, valine; BCAA, branched chain amino acids.



perfusion adaptation is mediated by endothelial reactivity, which is hampered by a pro-inflammatory state, this hypothesis can also explain why inflammation is such a strong correlate and predictor of age-related sarcopenia.^{16–18} In accordance with this hypothesis, we found that the protein intake that is required to maintain good muscle strength with ageing is increased in the presence of high interleukin-6 levels.¹⁹ Also, consistent with this hypothesis, previous studies have shown that insulin resistance and diabetes are associated with an accelerated decline in muscle strength, mass, and quality with ageing^{20–23} and also that muscle mass affects insulin sensitivity.^{24,25} Surprisingly, in our metabolomics study presented above, blood leucine and isoleucine levels significantly correlated with BMI but not with insulin.

Mitochondrial oxidative capacity and metabolic flexibility

Multiple distinct and pre-packaged adaptive strategies and signalling pathways in muscle allow boosting of energy production when demand increases, such as during prolonged exercise.^{26–28} For example, studies in human and animal models have revealed that the ability for the mitochondrial electron transport system to respond to increased demand declines with ageing, and this may account for some of the observed decrease in fitness in older persons.^{29–31} We have recently reported that when permeabilized muscle fibres are exposed to incremental concentrations of ADP, the ability of the mitochondria *in vitro* to produce energy as ATP increases linearly in samples from younger individuals, while these levels tend to remain stable above a certain threshold in samples from older individuals.³¹

These findings suggest that during ageing, mitochondria lose the ability to produce energy during maximal efforts but not when the energetic demand is lower. The loss in metabolic flexibility in skeletal muscle also occurs in the early stages of type 2 diabetes in nondiabetic adults.³² This impaired mitochondrial function could be due to inadequate perfusion or reduced muscle blood flow, resulting in lower oxygen delivery in skeletal muscle and diminished aerobic capacity.³³ This hypothesis is interesting because it connects both energetic and anabolic deficits to the same mechanism. In accordance with this hypothesis, we have recently shown that muscle mitochondrial capacity is a strong independent correlate of lower extremity performance, especially in tasks that require walking at fast speeds or for long distances, and that long-term exposure to some adiposity-related factors may adversely affect mitochondrial function in humans.³⁴ We have also found that muscle strength significantly mediates the relationship of mitochondrial function with walking performance.²⁸ Overall, these results indicate that oxidative phosphorylation is progressively impaired with ageing; it is

unclear whether this is because the number of mitochondria per muscle volume is diminished, the intrinsic capacity of mitochondria to generate ATP is impaired, or the availability of oxygen and nutrients at different levels of effort is compromised. Future studies that rely on protein content assessment, high resolution microscopic examination of muscle specimens, and techniques for dynamically assessing muscle perfusion during contraction are needed to address this question.

Mitochondrial damage, autophagy, and repair

Oxidative stress and defective mitophagy (mitochondrial autophagy) are potentially involved in the decline of muscle quality with ageing and need to be considered. Dysfunctional mitochondria are characterized by reduced oxidative phosphorylation efficiency and excessive production of reactive oxygen species, which oxidize and damage macromolecules.³⁵ Normally, stressed/damaged mitochondria can be repaired by alternating cycles of fission and fusion, but if this mechanism is insufficient, defective mitochondria then can be removed by mitophagy or induced cellular apoptosis.^{36,37}

The hypothesis that oxidative stress causes degenerative changes in tissues that are highly metabolically active, such as the brain and the muscle, has been proposed for many years.^{38,39} Reactive oxygen species can damage macromolecules in mitochondria, such as the oxidation of cardiolipins and mitochondrial DNA or damage of enzymatic and contractile proteins, which further accelerate energetic dysfunction and functional damage.³⁹ However, evidence in support of this theory is limited, and some experiments in animal models have even shown that inducing oxidative stress can improve function.⁴⁰ A recent study demonstrated that, when mitochondrial function of skeletal muscle samples is stimulated by increasing ADP concentration *in vitro*, the compensatory antioxidant capacity is hindered in muscle tissue from older adults compared with younger persons and may negatively affect respiration at high ADP levels.²⁹ Oxidative stress may also affect satellite cells or muscle stem cell pools in skeletal muscle. In mice, satellite cells are able to reprogram their daily rhythmic functions to adapt to stress, suggesting that these cells might be evolutionarily prepared to reprogram their functions in response to metabolic changes, such as caloric restriction or exercise.⁴¹

There is still limited data suggesting that defective autophagy has a role in sarcopenia with ageing. In mice, defective autophagy has been related to neuromuscular dysfunction and the mechanisms of cyclic denervation and reinnervation that occurs with ageing,⁴² but this hypothesis has never been confirmed in humans. Reestablishment of autophagy has been shown to restore regenerative functions in old satellite cells in mice.⁴³ Therefore, it seems that autophagy is essential to

maintain the quiescent state in satellite cells to support muscle regeneration in sarcopenia. Overall, the role of oxidative stress and mitophagy in the decline of muscle quality remains largely unexplored and there is robust, ongoing research in this area.

Mitochondrial function and defects in the neuromuscular junction.

Defective mitochondrial function has been studied in regard to the neuromuscular junction (NMJ) remodelling that occurs with ageing, producing cycles of denervation–innervation that lead to motor unit loss, specifically in type II fibres, as well as muscle fibre atrophy.⁴⁴ However, it is not clear whether these changes in the NMJ precede or follow the observed decline in muscle mass and strength that is observed with ageing. Some studies have reported altered mitochondria morphology in the plaque region of the NMJ that produce increased levels of oxidative stress, decreased enzymatic activity and ATP production, and impaired calcium buffering.⁴⁵ The combination of these biological changes may have a strong negative impact on excitation–contraction coupling and eventually lead to the loss of motor units. In sarcopenic rats with altered NMJ integrity, the expression of genes and proteins implicated in mitochondrial energy metabolism is downregulated.⁴⁶ Recent studies have suggested that overexpression of PGC-1 α , a transcription factor that promotes mitochondrial biogenesis, could help to maintain NMJ integrity during ageing.^{47,48} Mice lacking autophagy and ubiquitin proteasome proteins, such as the ubiquitin ligases muscle atrophy f-box (Atrogin1/MAFbx) and muscle ring finger-1 (MuRF1), are resistant to atrophy induced by denervation.^{49,50} Interventions, such as caloric restriction and exercise, seem to preserve the morphology and integrity of the NMJ with ageing in mice.^{51,52}

Overall, low muscle quality seems to be associated with (i) metabolic impairments that lead to reduced incorporation of the three major BCAAs, which are used by muscle as energy sources and are associated with muscle strength and endurance; (ii) fat accumulation in muscle tissue that ultimately leads to architectural disruption and loss of function; and (iii) high concentration of lipid species that are associated with impaired mitochondrial function and unrecycled mitochondrial proteins, potentially due to defective mitophagy or proteostasis. The extent and complexity to which these mechanisms are interconnected is unknown and should be

examined in future studies. In addition, other factors that impact ageing muscle could also modulate mitochondrial function, such as (i) defects in the NMJ that leads to myofiber denervation—due to reduced capacity in motor neurons to reinnervate muscle fibres—consequently causing fibres to become atrophied; (ii) the age-associated decline in the satellite cell pool, reducing muscle regeneration after injury; and (iii) ‘inflammaging’, the chronic low-grade inflammation observed in older persons.

Conclusions

An exciting component of gerontology research relates to understanding the changes in energetics that occur as individuals age, especially regarding the biological underpinnings of ageing, such as the role of nutritional intake and the multifaceted relationships to mitochondrial function. The biological pathways that lead to accelerated decline in muscle quality and muscle strength with ageing are actively being investigated and explored, with many questions unanswered. Altered concentrations of specific metabolites could have important translational implications for early identification of subjects that are at high risk for developing sarcopenia, as well as identifying targets for new preventive strategies and treatments.

Acknowledgements

The authors certify that they comply with the ethical guidelines for authorship and publication in the *Journal of Cachexia, Sarcopenia and Muscle*.⁵³ This work was supported by the National Institutes of Health (NIH) and the Intramural Research Program (IRP), National Institute on Aging (NIA). The authors wish to thank Adam Cornish for editing the manuscript and for his suggestions that substantially improved the quality and readability of this work.

Conflict of interest

Marta Gonzalez-Freire, Fatemeh Adelnia, Ruin Moaddel, and Luigi Ferrucci declare that there are no conflicts of interest regarding this work.

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