Mechanisms Underlying Changes in Function of Aging Skeletal Muscle

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Muscle wasting is a hallmark of the aging process in many species, including humans.[1] The functional consequences of this loss of muscle mass include weakness, decreased physical function, and decreased ability to perform activities of daily living (ie, loss of independence). The ability to sustain muscle activity for a prolonged period (muscle endurance or fatigue-resistance) is a less well-recognized, but equally important characteristic of muscle that is critical for independent living. Muscle fatigability is largely determined by intrinsic muscle properties such as intramuscular energy metabolism.[2]

Skeletal muscle requires chemical energy in the form of adenosine triphosphate (ATP) to satisfy the energetic demands of various ATPases. Although the storage capacity for ATP in the muscle is fairly low (<10 mM), the concentration of ATP is rarely depleted even during high-intensity muscle contractions during which ATP consumption increases by 100-fold. Mitochondria play a primary role in sustaining adequate ATP supply to the working muscle and help prevent muscle fatigue by preserving metabolic homeostasis. The importance of mitochondria to muscle function has spawned much interest in understanding how these organelles are altered by aging and finding potential strategies to preserve mitochondrial function across the lifespan.

The common dogma is that mitochondrial function is impaired with old age, evidenced by declining mitochondrial content and function measured by electron microscopy, enzyme activities, ATP synthesis rates in isolated mitochondria, and magnetic resonance spectroscopy.[3-6] The underlying mechanisms are complex and occur at several distinct molecular and cellular levels. The free-radical theory of aging suggests that oxidative stress contributes to age-related mitochondrial dysfunction.[7] Indeed, several groups have shown that oxidative damage to DNA increases with aging.[6,8] Aging is also associated with decreased mitochondrial DNA copy number,[4,6] mRNA transcripts for mitochondrial genes,[6,9] mitochondrial protein synthesis,[10] and mitochondrial protein expression,[4,6] all of which are likely to contribute to impaired muscle oxidative capacity.

A lingering question is whether the aforementioned dysfunctions are due to aging per se, or are secondary to environmental factors such as physical activity, diet, and medications in older adults. Several studies that have matched young and older subjects for physical activity or used physical activity as a covariate have been unable to detect decreases in muscle oxidative capacity with aging.[11-14] Kent-Braun and colleagues have focused much attention on this issue by using phosphorous magnetic resonance spectroscopy (^{31}P-MRS) to assess skeletal muscle oxidative capacity in vivo.[12-14] The kinetics of phosphocreatine (PCr) recovery following depletion during a muscle contraction provides a convenient, robust method for assessing mitochondrial function.[15] An advantage of this approach is the ability to test the capacity of mitochondria for ATP synthesis when all circulatory and regulatory systems are intact; the absence of intact systems is a major limitation of many in vitro techniques. Numerous studies in the tibialis anterior muscle have shown that

oxidative capacity in vivo is similar in young and older adults who have been carefully matched for habitual physical activity levels, measured by accelerometry.[12-14] It is important to note that not all muscles are affected the same way by the aging process. It is imperative that additional studies be performed in a variety of functionally distinct muscle groups.

Physical (in)activity may be an important determinant of many age-related detriments that were previously ascribed to chronological age. Exercise has long been recognized as a potent stimulus for mitochondrial biogenesis and function, and beneficial adaptations to exercise training are evident across the lifespan. However, the extent to which endurance exercise can prevent, reverse, or delay age-related mitochondrial dysfunction is unclear.

A recent study published by Nair's group provided some insight to this issue by studying young and older adults who were either sedentary (less than 20 minutes of structured exercise, twice per week) or highly endurance trained (at least 1 hour of vigorous cycling or running 5 days a week or more for the last 4 years or longer).[4] Maximal rates of ATP synthesis in isolated mitochondria were significantly decreased with old age in the sedentary group. Trained individuals exhibited higher ATP synthesis rates, but, importantly, the age-difference was no longer apparent. A similar pattern was evident for citrate synthase activity. Mitochondrial DNA copy number (NADH dehydrogenase subunits 1 and 2) were significantly lower with age in sedentary adults. Although mtDNA was increased in trained individuals, there was a persisting age-related decrement. Since proteins are the ultimate functional molecules expressed by genes, we used mass

spectrometry to measure the relative abundance of numerous proteins involved in muscle energy metabolism. A comparison of sedentary young and older adults revealed 27 proteins involved in oxidative and glycolytic ATP synthesis that were expressed at significantly lower levels in older adults. Although endurance training normalized the majority of these age effects, the expression of several proteins remained substantially lower in older compared to young endurance athletes. Altogether, these data suggest that regular endurance exercise is a viable approach to help maintain mitochondrial function with old age; however, some inevitable changes seem to occur even in masters level athletes.

In addition to its role in locomotion and physical function, skeletal muscle also plays a major role in whole-body glucose homeostasis. By virtue of its mass, skeletal muscle represents the most important site for peripheral glucose disposal in response to insulin and exercise. Insulin resistance is broadly defined as reduced peripheral glucose rate of disappearance in response to insulin and decreased suppression of hepatic glucose production. Insulin sensitivity is shown to decline with aging, although some emerging evidence indicates that changes in body composition are better predictors of age-related insulin resistance than chronological age.[16-19] It is also well-established that exercise effectively enhances both peripheral and hepatic insulin sensitivity. The link between mitochondrial function and insulin sensitivity is tenuous. Many insulin-resistant populations exhibit decreased mitochondrial function,[19-21] prompting some investigators to posit that mitochondrial dysfunction causes insulin resistance, although in numerous cases the two are dissociated. It is likely that mitochondrial dysfunction and insulin

resistance with aging are paraphenomena that are linked by a common determinant—physical inactivity. We measured peripheral and hepatic insulin sensitivity by euglycemic hyperinsulinemic clamp and found that young and older adults exhibited similar insulin sensitivity.[4] Furthermore, insulin action was enhanced by endurance exercise training to a similar extent in young and old. These data reinforce findings that insulin resistance may be related to adiposity rather than age, and exercise exhibits important beneficial effects in a manner that is independent of age.

The vast majority of studies of aging and muscle metabolism have focused on mitochondria. However, glycolytic ATP synthesis represents an important energy-producing pathway in skeletal muscle, particularly under conditions in which oxygen delivery may limit the capacity for mitochondrial respiration. One can appreciate how decreased cardiac output, impaired blood flow, reduced capillarity, or peripheral vascular disease could render skeletal muscle exercise-intolerant if it were to rely solely on mitochondria for ATP supply. The majority of studies to date have examined the effects of age on glycolytic function by measuring enzyme activities. The results have been mixed. We again turned to phosphorous magnetic resonance spectroscopy to measure the metabolic flux through anaerobic glycolysis in contracting skeletal muscle.[22] The intramuscular pH can be calculated from the position of the inorganic phosphate peak relative to phosphocreatine. During exercise, the resonance frequency of the Pi peak shifts in a pH-dependent manner. This relationship can be exploited to determine the muscle pH and the flux through anaerobic glycolysis. These calculations require estimates of proton buffering capacity of the muscle, the proton stoichiometry of the

creatine kinase reaction, and proton efflux. We found that glycolytic flux was lower in older compared to young adults during a series of maximal isometric contractions performed with blood flow intact to the muscle.[13,14] However, when blood flow was occluded by suprasystolic cuff pressure, glycolytic flux increased in older adults to match the level of the young.[14] These data suggest that similar to oxidative capacity, the capacity for non-oxidative ATP synthesis is not impaired by old age.

In summary, there is overwhelming evidence that muscle energy metabolism is impaired with old age. Much of this impairment occurs secondary to physical activity and adiposity. Although exercise is an effective strategy to delay the onset of many age-related changes at the level of skeletal muscle bioenergetics, a component of aging remains inevitable. It is clear that we need to study the muscle-group specificity of these age effects and also distinguish between those who are "older" (ie, >65 years) and those who are "senescent" (ie, centenarians).

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Q&A

Q: Some researchers believe that older people have uncoupled mitochondria in their skeletal muscle. Some of your experiments on the adenosine triphosphate (ATP) production with luciferase technique indicate this is not true. That is a different defect from oxidative capacity in which the state-3 rates are down. Can you comment on that?

Dr Lanza: The research you are referring to showed uncoupling with age. I think the method for measuring ATP synthesis would not pick up changes in coupling because oxygen consumption increases. ATP synthesis is tightly coupled to metabolic demand, so I think you would have to measure oxygen consumption in the mitochondria to pick up any changes in coupling.

Q: It depends how you do the assay. Certainly some of your work does not exclude the possibility that uncoupling is occurring. Do you think it is possible? There are ways that you could have looked for that. Some researchers might argue that that is an important

physiologic muscle defect that would limit exercise, because even if a person has adequate state-3 rates, if he or she is uncoupled when exercising, and oxygen flux limits performance, that person would be affected. Is that potentially a mitochondrially derived aging problem?

Dr Lanza: Yes, but the challenge is measuring it. Some research groups have done this, I think using a combination of phosphorous spectroscopy and near infrared, which measures oxygen consumption. But to my knowledge, most of this research has not been done in vivo.

Q: This can be done easily in vitro with muscle biopsies. But this is a potentially large aging-induced problem and I do not think we understand why it occurs. Some researchers think it is adaptive, that it turns off oxidation. I have noticed induction of super oxide dismutase (SOD) in the elderly, so I theorize that we generate more reactive oxygen species (ROS) when we get old, and through adaptive increase in uncoupling, oxidative stress is reduced. However, stress reduction is at the expense of energy performance.

Some research shows that uncoupling protein levels are related to exercise; with exercise, levels of uncoupling protein go down. This is seen in diabetes and some other models, but I do not know whether it has been shown in the elderly. Still, some people think mitochondrial dysfunction in aging is an important issue, but your data tell us that it is not. Can you comment?

Dr Lanza: I would not say it is an unimportant issue. I would say that it is important to account for environmental factors before blaming chronological age on some of these effects. This does not mean that elderly people do not have mitochondrial dysfunction or insulin resistance. It just means that exercise may be a good tool to help combat these effects.