Mitochondrial protein import in aging muscle: can Tom still do it? Focus on “Biogenesis of the mitochondrial Tom40 channel in skeletal muscle from aged animals and its adaptability to chronic contractile activity”

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Mitochondria are vital to the function of all nucleated mammalian cells and are particularly important in skeletal muscle for provision of the ATP necessary to fuel muscle contraction. The mitochondrial content of skeletal muscle is not static and can respond rapidly to increases (6) and decreases (7) in contractile activity by reducing and increasing mitochondrial biogenesis, respectively, thereby matching the ATP supply to the contractile demand of skeletal muscle. Similarly, mitochondria are regularly renewed, with the half-life of a marker of mitochondrial content (cytochrome c) being ~7 days in skeletal muscle (2), ensuring the fidelity of the mitochondrial pool by regularly replacing mitochondria that are old and damaged. In addition to their physiological roles, mitochondria are also implicated in the pathogenesis of many disease states and in aging in general. The involvement of mitochondria in aging has been an area of very significant study, with many studies focusing on the role of mitochondria in the age-related decline of skeletal muscle mass and function known as sarcopenia. The specific roles in which mitochondria are implicated in sarcopenia with aging are many, and include reductions in ATP production (10), oxidative damage to contractile proteins (19) and other cellular constituents, myofiber atrophy, and myocyte death with aging (23).

One of the hallmark features of sarcopenia is a decline in mitochondrial enzyme activity per gram of skeletal muscle (4), which is an important cause of declining muscle aerobic function with aging (9). Given the aforementioned plasticity of mitochondria in response to skeletal muscle contractile activity, and the well-known decline in habitual physical activity with aging, perhaps the mitochondria in aged muscle have simply been downregulated to match the lower muscle contractile demands. On the other hand, perhaps the response of mitochondrial biogenesis to muscle contractile activity becomes impaired with aging. Recent work showing impaired responsiveness of various components of the mitochondrial biogenesis signaling pathway following acute electrically stimulated muscle contractions (17), pharmacological activation of an upstream regulator of mitochondrial biogenesis, AMP-activated protein kinase (AMPK) (21), and long-term endurance exercise training (1) in aged skeletal muscle is consistent with an uncoupling between muscle contractile activity and mitochondrial biogenesis in aged muscles. To make matters more complicated, however, there is compelling evidence indicating that the reduced mitochondrial enzyme activity per unit of aged muscle is not due to reduced mitochondrial content (18), but rather reduced mitochondrial function (5, 9), and this latter problem is thought to occur secondary to reduced mitochondrial turnover (22). Thus, recent studies have focused not only on trying to understand the potential mechanisms that might reduce mitochondrial content, but also mechanisms that could account for impaired oxidative capacity per mitochondrion in aging muscle. To some degree these mechanisms likely overlap since a reduced rate of mitochondrial biogenesis could be an important contributor to reduced mitochondrial turnover and resulting dysfunction as well as to a decline in mitochondrial content. Therefore, regardless of the view one takes concerning reductions (or not) in muscle mitochondrial content with aging, studies aimed at understanding age-related changes in mitochondrial biogenesis will help further understanding of the basis for the reduced mitochondrial enzyme activity per unit of muscle with aging.

Mitochondria are unique organelles in that they contain both nuclear DNA (nDNA)-encoded and mitochondrial DNA (mtDNA)-encoded proteins. This necessitates a sophisticated mitochondrial protein import machinery to bring in proteins encoded by the nDNA (and which are synthesized in the cytoplasm) into their proper location within the mitochondria. There are two multisubunit complexes that are primarily responsible for the import of mitochondrial proteins into the mitochondrial matrix: the translocases of the outer membrane (TOM complex) and the translocases of the inner membrane (TIM complex) (Fig. 1). Tom40 is a β-barrel protein that forms the protein-conducting channel of the outer membrane or inserted into the mitochondrial outer membrane. [From Rapaport (20).]
TOM complex (20). The steps leading to Tom40 import and assembly into the TOM complex in the mitochondria, and the responsiveness of this pathway to muscle contractile activity, are relatively well characterized in young adult muscle (11). It is the assembly of the Tom40 machinery, and how its responsiveness to muscle contractions might be altered with aging, that is the focus of the article by Joseph and colleagues (14) in this issue of *American Journal of Physiology-Cell Physiology*.

In their study, Joseph and colleagues (14) employed the use of mitochondria isolated from the tibialis anterior muscle of young and aged rats in a model well characterized for the trajectory of sarcopenia, the Fischer 344 × Brown Norway F1-hybrid rat. The ages studied span a period when the tibialis anterior muscle (which is largely fast twitch in the rat) loses ~40% of its mass, and thus sarcopenia is severe in the older animals studied. The authors focused their study on the subsarcolemmal mitochondria because these mitochondria are more responsive to chronic contractile activity. To characterize the capacity for Tom40 protein import under nonstimulated conditions, Joseph and colleagues followed the import of radiolabeled Tom40 precursor protein into isolated mitochondria and examined Tom40 assembly into the outer membrane by blue native-polyacrylamide gel electrophoresis. The authors then characterized the responsiveness of this pathway to chronic muscle contractile activity by examining Tom40 precursor import and assembly following chronic electrically stimulated contractions (7 days of 3 h per day). The chronic contractile activity studied is intended in some respects to mimic the effects of muscle contractions during exercise; however, this level of “exercise” would be unsustainable for the young animals and would be even more challenging for the aged animals. The benefit of this approach, therefore, is that it is independent of the animal’s willingness and/or capacity for exercise (both of which decline markedly at advanced age) and thereby allows the responsiveness of the mitochondrial protein import machinery to be studied under optimal conditions.

The TOM complex imports proteins that are designated for the matrix and are primarily involved in Krebs Cycle and β-oxidation pathways. Interestingly, Joseph and colleagues (14) found that the rate of Tom40 assembly under nonstimulated conditions was elevated in aged animals. The authors argue that because enhanced mitochondrial protein import capacity is also seen in conditions of severely disrupted cellular homeostasis [e.g., cells depleted of mtDNA and in patients with mtDNA disease (15)], the response seen in the aged muscles may represent a compensatory response to chronic mitochondrial insufficiency. While this is an interesting possibility, the burden of mtDNA damage and degree of mitochondrial insufficiency (e.g., as represented by fraction of muscle fibers in a cross section that are negative for complex IV activity) in patients with severe mtDNA disease is many orders of magnitude greater than what is seen in aged muscle (3, 12, 13). As such, whether this lower mtDNA damage burden, or other factors occurring with aging (e.g., the authors also suggest that the elevated Tom22 seen with aging may have increased Tom40 assembly), is responsible for the relatively high Tom40 protein assembly under nonstimulated conditions requires further study.

In contrast to the observations under nonstimulated conditions, Joseph and colleagues (14) found that the increase in import of Tom40 precursor following 7 days of chronic electrically stimulated muscle contractions was reduced in the aged animals. Whether this contributes to the blunted responsiveness of mitochondrial biogenesis with aging following AMPK activation (21), muscle contractile activity (17), and long-term endurance training (1) depends on whether Tom40 precursor protein import is limiting to the assembly of Tom40. Since Tom40 assembly following chronic contractile activity was similar between age groups, it seems unlikely that this is the case. As such, the results of Joseph and colleagues (14) suggest that the subtle age-related changes in Tom40 import and assembly are unlikely to explain the blunted responsiveness of skeletal muscle mitochondrial biogenesis to long-term endurance training in very advanced age (1). It seems more likely that the limitation in skeletal muscle mitochondrial biogenesis seen with exercise training at advanced age relates to either the low intensity of exercise stimulus that can be sustained at advanced stages of sarcopenia (1) and/or a relative uncoupling between the exercise stimulus and signaling events that drive mitochondrial biogenesis (17, 21) in senescent muscle.

In providing further insight to this issue, future studies could employ methods of inducing mitochondrial biogenesis that are not contingent on muscle contraction. One such possibility is to examine mitochondrial biogenesis following acute muscle injury and subsequent myogenesis during regeneration, a setting in which mitochondrial enzyme activities and respiratory capacity have been shown to recover over a period of 2–4 wk following bupivacaine-induced injury in rat muscle (8). If mitochondrial biogenesis in aged muscles were superior in this setting than seen with exercise training, this would support the idea that the blunted capacity for mitochondrial biogenesis in aged muscle with exercise training is in part due to insufficient stimulus.

One final point worthy of consideration in attempting to understand the basis for blunted mitochondrial biogenesis in aged skeletal muscle, and a resulting lower oxidative capacity per unit of muscle, is the role of denervation. Although denervation has long been implicated in aged muscles (16), to date, the role this may play in blunting mitochondrial biogenesis in aged muscle has not been considered. For example, if a significant fraction of muscle fibers are functionally denervated in aged muscle, then it follows that these would not be recruited during daily use or exercise training. Thus, a failure of mitochondrial biogenesis in denervated fibers could dilute what might be a normal response in innervated muscle fibers. In situ immuno- and histochemical labeling methods in cross sections of aged muscles that examine heterogeneity in mitochondrial content between muscle fibers, in combination with markers of denervation, would be helpful in this context. In conclusion, while the results of Joseph and colleagues (14) suggest some modest alteration in the protein import machinery in aged muscles, it is clear that important questions remain in seeking to understand the basis for the reduced mitochondrial enzyme activity per unit of muscle with aging.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author.

**REFERENCES**


