Effect of denervation on mitochondrially mediated apoptosis in skeletal muscle

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Adhihetty PJ, O’Leary MF, Chabi B, Wicks KL, Hood DA. Effect of denervation on mitochondrially mediated apoptosis in skeletal muscle. J Appl Physiol 102: 1143–1151, 2007. First published November 22, 2006; doi:10.1152/japplphysiol.00768.2006.—Chronic muscle disuse induced by denervation reduces mitochondrial content and produces muscle atrophy. To investigate the molecular mechanisms responsible for these adaptations, we assessed 1) mitochondrial biogenesis- and apoptosis-related proteins and 2) apoptotic susceptibility and cell death following denervation. Rats were subjected to 5, 7, 14, 21, or 42 days of unilateral denervation of the sciatic or peroneal nerve. Muscle mass and mitochondrial content were reduced by 40–65% after 21 and 42 days of denervation. Denervation-induced decrements in mitochondrial content occurred along with 60% and 70% reductions in transcription factor A (TFam) and peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α, respectively. After 42 days of denervation, Bax was elevated by 115% and Bcl-2 was decreased by 89%, producing a 16-fold increase in the Bax-to-Bcl-2 ratio. Mitochondrial reactive oxygen species production was markedly elevated by 5- to 7.5-fold in subsarcolemmal mitochondria after 7, 14, and 21 days of denervation, whereas reactive oxygen species production in intermyofibrillar (IMF) mitochondria was reduced by 40–50%. Subsarcolemmal and IMF mitochondrial levels of MnSOD were also reduced by 40–50% after 14–21 days of denervation. The maximal rate of IMF mitochondrial pore opening (∇ψmax) was elevated by 25–35%, and time to ∇ψmax was reduced by 20–25% after 14 and 21 days, indicating increased apoptotic susceptibility. Myonuclear decay, assessed by DNA fragmentation, was elevated at 7–21 days of denervation. Our data indicate that PGC-1α and TFam are important factors that likely contribute to the reduced mitochondrial content after chronic disuse. In addition, our results illustrate that, despite the reduced mitochondrial content, denervated muscle has greater mitochondrial apoptotic susceptibility, which coincided with elevated apoptosis, and these processes may contribute to denervation-induced muscle atrophy.

mitochondrial biogenesis; muscle disuse; reactive oxygen species; peroxisome proliferator-activated receptor-γ coactivator-1α

SKELETAL MUSCLE IS A MALLEABLE tissue capable of considerable adaptation, depending on the imposed physiological demands. Chronic muscle disuse due to hindlimb suspension, microgravity, immobilization, and denervation has been shown to induce muscle atrophy (3, 4, 15, 42). Muscle disuse accelerates the rate of protein degradation and, simultaneously, reduces the protein synthesis rate, resulting in a reduction in overall fiber size (16, 24, 31, 46). Recently, programmed cell death (apoptosis) has emerged as an important contributor to muscle disuse-induced atrophy (39, 41). In addition, apoptosis has been shown to contribute to the age-induced decrease in muscle mass (i.e., sarcopenia) and to muscle degeneration in pathological states such as muscular dystrophies, burn injury, ischemia-reperfusion, and mitochondrial myopathies (1, 13, 34). Apoptosis is an organized dismantling of the cell, with defining morphological features that include plasma membrane blebbing, nuclear breakdown, and DNA fragmentation. However, skeletal muscle is unique: fibers are multinucleated, and, rather than undergoing wholesale cellular degradation, there is a reduction in the number of myonuclei per fiber, termed nuclear apoptosis (3, 36). A reduction of the number of myonuclei would result in a decrease in the synthesis of nuclear gene products per unit of muscle fiber area, contributing to the atrophic response.

Mitochondria are involved in apoptosis, because they contain proapoptotic molecules, which can be released to initiate cell death, and they are the primary cellular producers of reactive oxygen species (ROS) (1). Skeletal muscle mitochondria exist as a reticulum that projects from the sarcosomal membrane [subsarcolemmal (SS) mitochondria] and extend to intermingle between the myofibrils [intermyofibrillar (IFM) mitochondria]. Cytochrome c and apoptosis-inducing factor (AIF) are proapoptotic factors that can be released from these mitochondria through a specialized channel termed the mitochondrial permeability transition pore (mtPTP), leading to DNA fragmentation (8, 12, 25, 28, 44, 48). The mtPTP is regulated by Bcl-2 family members, including proapoptotic Bax, which facilitates pore opening, and antiapoptotic Bcl-2, which inhibits pore opening (37). Although the release of cytochrome c and AIF can potentially induce cell death, a number of protective cytosolic proteins, including 70-kDa heat shock protein (HSP70), have been shown to inhibit AIF- and cytochrome c-mediated apoptotic pathways (6, 35).

ROS production in muscle can occur via extracellular reactions (33) and as a by-product of normal mitochondrial oxygen consumption (∇O2) (9). ROS are important apoptotic molecules: they 1) promote mtPTP opening, 2) provoke cytochrome c detachment from the inner mitochondrial membrane, and 3) can activate redox-sensitive transcription factors involved in muscle remodeling and apoptotic gene expression (7, 18, 20, 27, 32). Although ROS are capable of inducing a broad spectrum of cellular damage, several antioxidants, such as manganese superoxide dismutase (MnSOD), scavenge and neutralize ROS. Chronic muscle disuse not only reduces overall fiber size; it also dramatically decreases mitochondrial content (14, 49). Although a number of signaling pathways and transcription factors have been implicated in evoking contractile muscle mass, mitochondrial respiration; peroxisome proliferator-activated receptor-γ coactivator-1α...
activity-induced mitochondrial biogenesis, little is known about the activity of these pathways during chronic muscle inactivity. Given the prominent role of mitochondria in induction of apoptosis, an activity-induced reduction in mitochondrial content might suggest a decreased apoptotic vulnerability within muscle. However, this is inconsistent with evidence suggesting a higher incidence of apoptosis during muscle disuse (39, 41). Thus the overall purpose of this study was to evaluate the relation between mitochondrial content and function, apoptotic susceptibility, and muscle fiber atrophy during denervation-induced muscle disuse. We hypothesized that mitochondrial apoptotic activity would increase, despite an overall decrease in mitochondrial content within the denervated muscle.

**METHODS**

*Animal surgery.* Male Sprague-Dawley rats (n = 60, 350–400 g body wt; Charles River, St. Constant, PQ, Canada) were housed in pairs and given food and water ad libitum. The rats were anesthetized with pentobarbital sodium (60 mg/kg ip). Denervation was induced by surgical removal of a 0.5-cm segment of the common peroneal nerve, which innervates the tibialis anterior (TA)-extensor digitorum longus (EDL) muscle group, or the sciatic nerve, which innervates the gastrocnemius-plantaris muscle group, as previously described (14, 49). Two groups of experimental animals were used. The first group of animals was subjected to 5, 7, 21, or 42 days of unilateral sciatic nerve section. Gastrocnemius muscles from these animals were frozen in clamps at the appropriate time and used for biochemical analyses. The second group of animals was subjected to 7, 14, or 21 days of common peroneal nerve section. The shorter denervation period in the second group reflected the finding that the most significant adaptations occurred within 21 days. The common peroneal nerve section used in the second study is less invasive, the animals recuperate sooner, and the animals are able to move more easily than those subjected to sciatic nerve section. TA muscles were removed and used for mitochondrial respiration and functional studies, and the EDL muscles were frozen and used for biochemical analyses. In each of the experimental paradigms, the contralateral control limb was used as an internal control. Our previous experiments showed that the muscles of the contralateral limb are unaffected by sciatic or peroneal denervation and can serve as a valid intra-animal control (14, 48). Since both experimental paradigms induced similar decrements in muscle mass (Fig. 1A) and cytochrome c oxidase (COX) activity (data not shown for all muscles) and the affected muscles are known to be of similar fast-twitch fiber type composition, we illustrate both sets of data.

*Mitochondrial isolation.* TA muscles were quickly excised and immediately placed in ice-cold buffer, briefly minced, and homogenized. Differential centrifugation was used to fractionate SS and IMF mitochondria, as described previously (11, 30, 45). Mitochondria were resuspended in medium (100 mM KCl, 10 mM MOPS, and 0.2% BSA) and subsequently used for mitochondrial respiration, ROS production, and mtPTP opening.

*Assessment of mtPTP opening.* mtPTP opening was measured by monitoring the decrease in light scattering associated with mitochondrial swelling at 540 nm, as described previously (2). Briefly, mtPTP opening is facilitated under conditions of elevated Ca²⁺ concentration and oxidative stress, causing massive mitochondrial swelling, outer membrane rupture, and proapoptotic release (19, 21, 22, 47). Briefly, isolated IMF mitochondria were resuspended (to obtain a final con-
Mitochondria (50 SS and IMF mitochondria, as previously described (2). Briefly, Tris with a Clark oxygen electrode (Yellow Springs Instruments, Yellow glutamate (state 4 respiration) or 0.4 mM ADP (state 3 respiration) (natoms O2 mM sucrose, 3 mM HEPES, and 5 mM succinate (pH 7.4)) with continuous stirring at 30°C in a water-encased respiratory compartment. VO2 (natoms O2·min⁻¹·mg⁻¹) was assessed in the presence of 11 mM glutamate (state 4 respiration) or 0.4 mM ADP (state 3 respiration) with a Clark oxygen electrode (Yellow Springs Instruments, Yellow Springs, OH), as described previously (2, 11, 30).

ROS assay and mitochondrial respiration. ROS were measured in SS and IMF mitochondria, as previously described (2). Briefly, mitochondria (50 μg) were incubated with 50 μM dichlorodihydroflurorescein diacetate and VO2 buffer at 37°C, and ROS production was monitored over time in a 96-well plate. ROS production was directly proportional to the fluorescence emission (480–520 nm) measured with a multidetection microplate reader (Synergy HT, Biotek Instruments). Microplate data were compiled and analyzed with KC4 (version 3.0) software. ROS production was assessed during state 4 respiration by addition of 10 mM glutamate to the mitochondria immediately before addition of dichlorodihydrofluorescein diacetate.

Immunoblotting. Frozen rat muscles were pulverized to a fine powder in a stainless steel mortar cooled with liquid nitrogen. Powdered tissues were diluted 1:20 (wt/vol) in 100 mM Na-K-phosphate extraction buffer (pH 7.2) containing 2 mM EDTA in the absence of protease or phosphatase inhibitors and processed as previously described (30). Whole muscle protein extracts were separated by 12% SDS-PAGE and subsequently transferred to nitrocellulose membranes. Membranes were blocked (1 h) with a 5% skim milk-1/2TBST [Tris-buffered saline-Tween 20: 25 mM Tris·HCl (pH 7.5), 1 mM NaCl, and 0.1% Tween 20] solution. Blots were then incubated with antibody directed against AIF (1:1,500 dilution), cytochrome c (1:750 dilution), MnSOD (1:2,000 dilution), Bax (1:500 dilution), Bcl-2 (1:1,000 dilution), HSP70 (1:500 dilution), transcription factor A (Tfam; 1:500 dilution), or peroxisome proliferator receptor-γ co-activator (PGC-1α) (1:500 dilution) overnight at 4°C. Antibodies were obtained from Santa Cruz Biotechnology [catalog nos. sc-9416 (AIF), sc-526 (Bax), and sc-492 (Bcl-2)], Stressgen [catalog no. SPA-810 (HSP70)], and Upstate Cell Signaling Solutions [catalog no. 06-984 (MnSOD)]. Tfam and cytochrome c antibodies were produced in our laboratory (43). After primary antibody incubation, blots were washed three times in TBST for 5 min each, incubated with the appropriate secondary antibody at room temperature (45 min), and washed again three times in TBST for 5 min each. Bound antibody detection was revealed using the enhanced chemiluminescence method. Films were scanned and analyzed using SigmaScan Pro (version 5) software (Jandel Scientific, San Rafael, CA).

COX enzyme activity. Whole muscle powdered tissues were diluted in a buffer [0.1 M KH2PO4 and 2 mM EDTA (pH 7.2)] and sonicated three times on ice for 5 s each, as described previously (11). The enzyme activity was determined by the maximal rate of oxidation of COX enzyme activity (units/mg) in a buffer [0.1 M KH2PO4 and 2 mM EDTA (pH 7.2)].

RESULTS

Effect of denervation on muscle mass and mitochondrial content. Chronic denervation of the sciatic nerve or the common peroneal nerve resulted in rapid and significant reductions of 40, 49, and 39% of gastrocnemius, EDL, and TA muscle mass, respectively, by 21 days (Fig. 1A). These results indicate that our peroneal and sciatic denervation paradigms were equally effective at inducing a loss in muscle mass. Cytochrome c expression (units/μg protein) and COX activity (units/g muscle mass), two typical markers of mitochondrial content, decreased by 30 and 70%, respectively, after 42 days of denervation (Fig. 1B). These data confirm that denervation dramatically reduces mitochondrial content. We then assessed the levels of PGC-1α and Tfam to evaluate whether the reduction in mitochondria coincided with decrements in the expression of these regulatory proteins. Chronic denervation for 42 days caused 60 and 70% reductions in Tfam and PGC-1α expression, respectively, compared with control (Fig. 1C). The correlation between the decrease in COX activity and PGC-1α over the course of denervation was 0.71 (P < 0.05).

Denervation-induced adaptations in expression of pro- and antiapoptotic proteins. To assess the effect of denervation on the susceptibility of muscle to mitochondrially mediated apoptosis, we evaluated the whole muscle expression of proapoptotic Bax and antiapoptotic Bcl-2, two important proteins that regulate mtPTP opening. Denervation for 21 and 42 days resulted in 88 and 115% increases, respectively, in Bax expression, while coincident decreases of 79 and 89% occurred in Bcl-2 expression (Fig. 2A). This resulted in a large 16-fold increase in the Bax-to-Bcl-2 ratio, an important indicator of the vulnerability of cells to apoptosis (Fig. 2A). Approximately twofold increases (P < 0.05) were found in the whole muscle expression of proapoptotic AIF at 7–42 days of denervation, further suggesting a greater susceptibility to apoptotic cell death (Fig. 2B). In contrast, the antiapoptotic protein HSP70 was increased (P < 0.05) two- to threefold between 7 and 42 days of denervation (Fig. 2C).
SS and IMF mitochondrial respiration and ROS production. Given the important role of ROS in mitochondrially mediated apoptotic cell death, we measured the respiration rate and ROS production after denervation. SS mitochondrial state 3 respiration rate was reduced by ~40% (P < 0.05) after 7 days of denervation and remained suppressed after 14 and 21 days of denervation (Fig. 3A). IMF mitochondrial state 3 respiration rates were not altered by 7 or 14 days of denervation but, rather, tended to decline (by 20%) at 21 days (Fig. 3A). Rates of state 4 respiration were unaffected by denervation in SS mitochondria but were elevated by 35% (P < 0.05) only after 14 days of denervation in IMF mitochondria (Fig. 3B).

Mitochondrial ROS production was measured and expressed per nanoatom of oxygen consumed by the mitochondrial subfractions. In SS mitochondria, ROS production was markedly elevated by 5-, 6.5-, and 7.5-fold after 7, 14, and 21 days of denervation, respectively (Fig. 3C). In contrast, ROS production in IMF mitochondria was suppressed by ~40–50% after denervation (Fig. 3C). These data demonstrate that SS mitochondria contribute to a greater level of ROS and that SS and IMF mitochondria differentially adapt to periods of denervation.

Effect of denervation on MnSOD expression and permeability transition pore function. We assessed MnSOD levels to determine whether the differential ROS production in SS and IMF mitochondria could be explained by the expression of this antioxidant protein. SS and IMF mitochondrial expression of MnSOD were reduced by 40–50% after 14 and 21 days of denervation (Fig. 4A). To assess mitochondrial apoptotic susceptibility, we measured the maximal rate of pore opening (\(V_{\text{max}}\)) and the time to \(V_{\text{max}}\) in IMF mitochondria. \(V_{\text{max}}\) was elevated by 25–35% and the time to \(V_{\text{max}}\) was reduced by 20–25% after 14 and 21 days of denervation (Fig. 4, B and C). SS mitochondrial pore kinetics were not measured because of an insufficient mitochondrial yield from denervated muscle.

TUNEL assessment of apoptotic nuclei after denervation. To specifically address the extent of apoptosis in denervated muscle, we used TUNEL to measure DNA fragmentation. A section of denervated EDL muscle in Fig. 5A shows the location and number of myonuclei. As a positive control, the section was treated with DNase to artificially induce DNA cleavage; then it was subjected to TUNEL. This TUNEL-positive image was overlapped with the nuclei image to confirm that DNA fragmentation was colocalized with the nuclei of the fibers. There was no evidence of TUNEL-positive staining in any of the control muscle tissue samples. However, TUNEL-positive staining was evident after 7, 14, 21 days of denervation at a frequency of 1.5–2.5 per 100 nuclei. This TUNEL-positive staining was colocalized with the PI stain for nuclei, as illustrated with the positive control (Fig. 5A) and was always located within the basal lamina, as shown by laminin.
staining (data not shown). In a limited number of samples, we also used PAX-7 immunofluorescence to evaluate whether these TUNEL-positive nuclei could originate from satellite cells activated during the denervation process. Under no circumstances was the PAX-7 staining colocalized with TUNEL-positive nuclei (not shown). This suggests that our TUNEL-

Fig. 3. Isolated mitochondrial respiration rates and reactive oxygen species (ROS) production after denervation of TA. A: denervation significantly suppressed subsarcolemmal (SS) mitochondrial state 3 respiration, but intermyofibrillar (IMF) state 3 respiration was unaltered. B: after 14 days of denervation, IMF mitochondrial state 4 respiration was significantly elevated compared with control. SS mitochondrial state 4 respiration was unchanged after denervation. C: ROS production per nanoatom of oxygen consumed in SS and IMF mitochondria expressed as fold change vs. control. Values are means ± SE (n = 6–8 experiments). *P < 0.05 vs. control.

Fig. 4. SS and IMF expression of MnSOD and IMF mitochondrial permeability transition pore kinetics in TA muscle after denervation. A: MnSOD in SS and IMF mitochondrial subfractions was significantly decreased after denervation. Top: typical Western blots for SS and IMF mitochondria. Bottom: means ± SE (n = 4–6 animals per time point). Den, denervated; Cont, control. B and C: within IMF mitochondria, maximal rate of pore opening (Vmax) was increased and time to Vmax was reduced after 14 and 21 days of denervation. Values are means ± SE (n = 4–8 experiments). *P < 0.05 vs. control.
positive nuclei are not within satellite cells. Although we cannot completely eliminate the possibility that TUNEL-positive nuclei may originate from endothelial cells and/or fibroblasts, the localization of the TUNEL-positive nuclei within the basal lamina suggests that these myonuclei are undergoing apoptosis. Thus the increase in TUNEL-positive staining seems to reflect an increase in myonuclear apoptosis that is evident as early as 7 days after denervation (Fig. 5, B and C).

**DISCUSSION**

Denervation represents a muscle disuse paradigm that causes a dramatic reduction in muscle mass (14, 49). Muscle disuse, in the presence or absence of the nerve, has been shown to activate numerous well-known proteolytic pathways (i.e., ubiquitin-proteasome, lysosomal, and calpain) to increase the rate of protein degradation. A concurrent reduction in the rate of protein synthesis leads to a marked reduction in fiber size (16, 24, 31, 46). In addition to these pathways, apoptosis has recently emerged as a significant contributor to the atrophic response associated with chronic muscle disuse, sarcopenia, and pathological muscular diseases (1, 13, 34, 40). Muscle is unique, in that it contains multiple myonuclei, and apoptosis within muscle serves to reduce the number of myonuclei per fiber, rather than leading to wholesale destruction of the cell. This reduction in myonuclear number will necessarily reduce the transcription of nuclear gene products, lowering the overall rate of protein synthesis and contributing to the muscle-disuse atrophic response (41). The most obvious qualitative feature of muscle disuse-induced atrophy is the reduction in fiber size; however, there is also a decline in mitochondrial content per gram of muscle mass, as shown in the present study. This decrease might be suggestive of a reduced capacity for mitochondrially mediated apoptosis. However, this is inconsistent with existing evidence supporting the importance of mitochondrially mediated apoptotic signaling pathways during muscle wasting (1, 3, 34, 36, 39). This suggests that chronic inactivity could potentially induce adaptations to mitochondria that might make them more susceptible to the release of apoptotic proteins. Thus the primary purposes of our study were to determine the effect of denervation-induced muscle inactivity on the relation between mitochondrial content and mitochondrially driven apoptosis.

It is well established that mitochondrial biogenesis is dependent on the transcriptional coactivator PGC-1α and the mitochondrial transcription factor Tfam (5, 17, 23, 29). However, our data are the first to illustrate that decrements in mitochondrial content induced by chronic muscle disuse coincide with reductions in these important mitochondrial regulators. We cannot delineate whether these inactivity-induced reductions are the result of a lower myonuclear number, reduced activation of relevant signaling pathways, or enhanced protein degradation. However, these data suggest that PGC-1α and Tfam are likely important factors that play a role in decreasing mitochondrial content during muscle inactivity.

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**Fig. 5.** TdT-mediated dUTP nick-end labeling (TUNEL) of denervated EDL muscles. A: sections of control muscle treated with DNase to induce DNA fragmentation represent a positive control for TUNEL staining. Magnification ×60. Top left, unstained section showing individual muscle fibers; bottom left, DNA fragmentation indicated by TUNEL-positive green fluorescent staining; top right, location of nuclei indicated by nuclear-specific propidium iodide (PI) stain (red fluorescence); bottom right, TUNEL (green) and PI (red) staining overlap to produce yellow coloration, indicating localization of TUNEL staining specifically to nuclei of muscle fibers. B: representative TUNEL-stained sections of control and 7-day denervated muscle samples. Magnification ×20. Top left and right, no evidence of TUNEL-positive nuclei in control muscle. Bottom left and right, TUNEL-positive staining in denervated muscle. White arrows, TUNEL-positive nuclei. All TUNEL-positive nuclei in denervated muscle colocalized with PI staining (not shown), as shown for positive control in A. C: number of TUNEL-positive nuclei per 100 nuclei in control (C; n = 4) and denervated (7, 14, and 21 days; n = 4 each) muscle fibers.
To assess the effect of denervation on whole muscle apoptotic susceptibility, we measured the expression of Bax and Bcl-2, which are involved in regulation of the mitochondrial permeability transition pore (37). The Bax-to-Bcl-2 ratio is commonly used as an index to assess the vulnerability to mitochondrionally mediated cell death (1). Indeed, denervation for up to 42 days caused a profound increase in the Bax-to-Bcl-2 ratio, up to 16-fold above nondenerverated muscle. Increases in the Bax-to-Bcl-2 ratio have been reported during muscle denervation (41), although the magnitude of the increase in the Bax-to-Bcl-2 ratio reported by Siu and Alway (41) was not as marked as that in the present study. This was likely a result of the differences in Bcl-2 expression in these two studies. Although we cannot explain the reason for this discrepancy, it is noteworthy that the Bax-to-Bcl-2 ratio increased in both studies. An elevated Bax-to-Bcl-2 ratio suggests that the mitochondrial permeability transition pore (mtPTP) might be more susceptible to opening, but the apoptotic consequences are also dependent on the proapoptotic protein levels (AIF and cytochrome c) within mitochondria. Our data indicate that whole muscle levels of AIF were significantly elevated, whereas cytochrome c was suppressed, after denervation. The distribution of these proteins is also likely to be affected by the denervation paradigm. Indeed, greater levels of AIF and cytochrome c have recently been shown within the cytosolic fraction after 14 days of muscle denervation (41). On the basis of our measured differences in total cytochrome c and AIF expression, we speculate that denervation-induced apoptotic cell death might be preferentially mediated by AIF, as opposed to cytochrome c, since an overall decrease in cytochrome c expression was observed. However, this has yet to be experimentally proven. In either case, the elevation in HSP70 after denervation may be a compensatory adaptation to reduce the propagation of the cytochrome c- and AIF-mediated death pathways (6, 35). Similar compensatory elevations in HSP70 have been observed in aged skeletal muscle and in denervation-induced muscle disuse (10, 41).

Given the important role of ATP supply and ROS production in mediating apoptosis, we next assessed ROS production in relation to rates of state 3 and 4 respiration in isolated SS and IMF mitochondria after denervation. We related ROS production to the rate of respiration in each mitochondrial subfraction, since it has been shown that ROS production is inversely related to V̇O₂ (1, 38). State 3 (ADP-stimulated) respiration rates in SS mitochondria were significantly suppressed as early as 7 days after denervation. IMF mitochondrial state 3 respiration rates were unaffected but tended to decline by 21 days of denervation (75% of control). This impairment in oxidative phosphorylation was not evident during basal, state 4 rates of respiration. Thus these data suggest that the capacity for respiration and ATP production is reduced, mainly in SS mitochondria. In concert with this, the markedly increased rates of ROS production, found uniquely in SS mitochondria, suggest that this organelle subfraction is primarily affected by the muscle denervation paradigm. This dysfunction could be due to an impaired expression and assembly of electron transport chain components, similar to that in patients with mitochondrial myopathy and ragged red fibers (26). We speculate that the enhanced ROS production is more likely to be a result of altered electron transport chain complex assembly than reduced antioxidant enzyme levels, since expression of the antioxidant marker MnSOD decreased similarly in both mitochondrial subfractions, yet this did not result in increased ROS production in IMF mitochondria. However, this does not preclude the possibility that other antioxidant enzymes (i.e., catalase and glutathione peroxidase) may be differentially altered in SS and IMF mitochondria in response to denervation. This warrants further investigation. However, the markedly higher ROS production observed in SS mitochondria likely has its greatest effect on peripheral myonuclei that are in close proximity to the SS subfraction. This could provoke further damage to cellular macromolecules (e.g., mtDNA) and initiate signaling events to modify local gene expression.

Since our data suggest that factors associated with promoting mtPTP opening (i.e., elevated ROS and increased Bax-to-Bcl-2 ratio) were induced with denervation, we specifically addressed mitochondrial apoptotic susceptibility by evaluating mtPTP kinetics. In the present study, this information could only be obtained from IMF mitochondria because of a limited pool of SS mitochondria after denervation. IMF mitochondria exhibited less time to V̇O₂ max and a greater velocity of pore opening than IMF mitochondria from nondenervated muscle. This could provoke further damage to cellular macromolecules (e.g., mtDNA) and initiate signaling events to modify local gene expression.
opening after 14 and 21 days of chronic denervation, both of which indicate enhanced mitochondrial apoptotic susceptibility. These data suggest that IMF mitochondria from denervated muscle are more prone to proapoptotic protein release on receipt of a triggering stimulus. This likely occurred earlier in SS mitochondria, given the marked increase in ROS production by 7 days in this subfraction, which likely contributed to the greater incidence of DNA fragmentation by 7 days, as assessed by TUNEL. Thus we propose that SS mitochondria contribute substantially to the apoptotic signaling program at the early stages of the denervation paradigm and a subsequent increase in the contribution from IMF mitochondria with increasing duration of denervation.

In conclusion, denervation induces a reduction in mitochondrial biogenesis that is likely related to the decrease in the mitochondrial regulators Tfam and PGC-1α. Despite this lower total mitochondrial content, mitochondrially driven apoptosis signaling is increased. This is due to an increased Bax-to-Bcl-2 ratio, an elevated susceptibility to pore opening, a greater ROS production, and a reduced antioxidant enzyme capacity. In addition, energy provision is impaired, likely a consequence of attenuated nuclear and mitochondrial gene expression, leading to faulty assembly of protein complexes. These findings are summarized in Fig. 6. Apoptosis has now been shown to contribute to muscle degeneration in the normal aging process, with chronic muscle disuse and a variety of specific muscular pathologies. Continuing to elucidate the underlying apoptotic mechanisms mediating the atrophic response is important in establishing potential therapeutic interventions that could prevent and/or reduce skeletal muscle wasting and preserve physiological function.

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