The effect of training on the expression of mitochondrial biogenesis- and apoptosis-related proteins in skeletal muscle of patients with mtDNA defects


MMPs, training induced a selective reduction of OGG-1, an increase in MnSOD, and a reduction in aconitase activity. Thus, before training, MMPs exhibited an adaptive response of nuclear proteins indicative of a compensatory increase in mitochondrial content. Following training, several parallel adaptations occurred in MMPs and HCs, which may contribute to previously observed functional improvements of exercise in MMPs. However, our results indicate that muscle from MMPs may be exposed to greater levels of oxidative stress during the course of training. Further investigation is required to evaluate the long-term benefits of endurance training as a therapeutic intervention for mitochondrial myopathy patients.

Mitochondria are composed of gene products originating from both nuclear and mitochondrial DNA (nDNA and mtDNA, respectively). Thus mutations in the genes of either genome can cause mitochondrial disease. The most common characteristic of mitochondrial disease is impaired oxidative phosphorylation leading to compromised ATP provision. Thus mitochondrial disease primarily affects energetically demanding tissues such as brain, heart, and muscle. However, mutations within mtDNA occur at significantly higher frequency than in nDNA because mtDNA lacks a protective histone sheath, has minimal DNA repair activity, and is located in close proximity to reactive oxygen species (ROS) production via the electron transport chain (1, 5). Although mtDNA encodes <1% of the total number of mitochondrial proteins, these gene products are indispensable components required for oxidative phosphorylation.

Mitochondria contain multiple copies of their own DNA and patients with mtDNA defects exhibit a unique condition termed heteroplasmacy, in which cells possess a variable combination of wild-type and mutant mtDNA. Thus the extent of the mutant phenotype can fluctuate among cells of the same tissues or among different tissues. However, the ratio of mutant to wild-type mtDNA, within a given tissue, appears to determine the severity of symptoms in patients (38). Patients with mtDNA defects who clearly exhibit muscle dysfunction as one of the primary clinical manifestations are classified as mitochondrial myopathy patients (MMPs). Muscle from MMPs often exhibits an exaggerated proliferation of subsarcolemmal mitochondria, producing a "ragged red fiber" phenotype with histochemical staining (18, 29, 38). This localized mitochondrial proliferation is likely an adaptive compensatory response that attempts to reconcile the deficit in ATP production. Various signaling pathways have been implicated in mediating adaptive increases in mitochondrial content, including reduced ATP/ADP ratios, altered Ca2+ homeostasis, and/or dysregulated ROS production (4, 28, 47, 49). These initial signaling pathways ultimately activate a number of transcription factors to increase the expression of nuclear-encoded mitochondrial genes (21). One of the most important is the nuclear-encoded mitochondrial transcription factor A (TFam) which is responsible for mtDNA replication and gene expression (12, 14). Another is the transcriptional activator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), which can dramatically induce both nuclear and mitochondrial gene expression (25, 50). Once nascent nuclear-encoded mitochondrial proteins are synthesized, they must be translocated into a preexisting mitochondrial reticulum by protein import machinery components, and some of these import components are upregulated during enhanced mitochondrial biogenesis (14, 23, 30, 43). Thus, although some studies have indicated that there is an overproliferation of mitochondrial content in MMPs, the mitochondrial biogenesis regulatory proteins that produce this adaptation, as described above, have not been investigated in MMPs.

Muscle from MMPs exhibits an increase in biochemical markers indicative of elevated ROS production (9, 24, 34). This increase in ROS production is likely an adaptive compensatory response that attempts to reconcile the deficit in ATP production. Various signaling pathways have been implicated in mediating adaptive increases in mitochondrial content, including reduced ATP/ADP ratios, altered Ca2+ homeostasis, and/or dysregulated ROS production (4, 28, 47, 49). These initial signaling pathways ultimately activate a number of transcription factors to increase the expression of nuclear-encoded mitochondrial genes (21). One of the most important is the nuclear-encoded mitochondrial transcription factor A (TFam) which is responsible for mtDNA replication and gene expression (12, 14). Another is the transcriptional activator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), which can dramatically induce both nuclear and mitochondrial gene expression (25, 50). Once nascent nuclear-encoded mitochondrial proteins are synthesized, they must be translocated into a preexisting mitochondrial reticulum by protein import machinery components, and some of these import components are upregulated during enhanced mitochondrial biogenesis (14, 23, 30, 43). Thus, although some studies have indicated that there is an overproliferation of mitochondrial content in MMPs, the mitochondrial biogenesis regulatory proteins that produce this adaptation, as described above, have not been investigated in MMPs.

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ROS are detoxified by antioxidant enzymes within the mitochondrion, such as manganese superoxide dismutase (MnSOD), and oxidative-induced lesions to mtDNA can be repaired by the DNA repair enzyme 8-oxoguanine DNA glycosylase-1 (OGG-1) (16, 19, 37). Elevated ROS levels can induce mtDNA damage, and could increase the susceptibility of muscle to apoptosis, because ROS promote the release of apoptosis-inducing factor (AIF) and cytochrome c by inducing mitochondrial permeability transition pore (mtPTP) opening. The conformation of the mtPTP is regulated by the Bcl-2 family of proteins consisting of both pro- (i.e., Bax) and antiapoptotic members (i.e., Bcl-2) in the outer membrane of the mitochondrion (36). Therefore, it is likely that enhanced mitochondrial ROS generation is an important factor in mitochondrial disease pathogenesis.

MMPs demonstrate reduced work capacity and exercise intolerance, which may negatively impact the quality of life (40). It has previously been shown that endurance training can increase the levels of functional respiratory enzymes in MMPs, leading to an improved whole muscle oxidative capacity (41). Therefore, the purposes of this study were to examine the effect of endurance training on the expression of proteins involved in 1) mitochondrial biogenesis and protein import, 2) oxidative stress levels and/or damage, and 3) apoptotic susceptibility between healthy control (HC) subjects and MMPs. We hypothesized that markers of mitochondrial biogenesis, oxidative stress and apoptosis would be upregulated in MMPs compared with HC subjects. We further hypothesized that the training-induced mitochondrial biogenesis would be blunted in MMPs, as a result of high pretraining expression levels compared with the HC subjects, and that indicators of apoptosis and oxidative stress would be reduced by the endurance training regimen, consistent with a protective effect of exercise.

METHODS

MMPs and endurance training. Patients consisted of 5 men and 5 women (37.6 ± 3.2 yr, mean ± SE) with heterogeneous clinical, biochemical, and molecular features indicative of mitochondrial myopathy (Table 1). HC subjects were age (37.2 ± 3.2 yr, n = 10) and gender matched to MMPs. Criteria utilized for the clinical diagnosis, symptom manifestation, and the underlying mtDNA defects of these patients have been described in detail previously (41, 42). Briefly, patients were diagnosed using histological and biochemical criteria to provide evidence of a mitochondrial myopathy. Each subject was informed of the nature and associated risks of the study and provided written consent before commencing the study. A subgroup of the MMPs (n = 8) and HC subjects (n = 7) performed heart rate-monitored cycle ergometry training for a total of 50 sessions in 14 wk (3–4 times/wk) at an intensity of 70–80% of maximal heart rate. The muscle needle biopsy was obtained ~1–2 h following a graded maximal exercise test, performed to determine training intensity, both before and after 14 wk of endurance training. Samples were immediately frozen and stored in liquid nitrogen for subsequent analysis.

This study was approved by the institutional review boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas. Each patient was informed of the nature and risks of the study and gave their written consent to participate.

Western blot analyses. Pre- and posttraining tissue samples were added equally to each lane of a 15% SDS-polyacrylamide gel (SDS-PAGE) and electrophoresed for 1.5 h. Gels were electroblotted for 2 h onto a nitrocellulose membrane and probed with polyclonal antibodies directed toward cytochrome c (1:750), TfnA (1:500), Bcl-2 (1:2,000), AIF (1:200), outer membrane receptor (TOM20; 1:750), mitochondrial 70-kDa heat shock protein (mtHSP70; 1:500), OGG-1 (1:750), MnSOD (1:1,100), Bax (1:500), and PGC-1α (1:250). Antibodies were obtained from Santa Cruz Biotechnology (Bax, sc-526; Bcl-2, sc-492; AIF, sc-9416), Upstate Cell Signaling Solutions (MnSOD, 06-984), Stressgen (mtHSP70, SPA-825), NOVUS Biologicals (OGG-1, NB001-106A8), or used as described previously (21, 23). Equal loading was verified using the Ponceau red stain, done with every blot.

Membranes were washed in 0.05% Tween-PBS buffer and incubated with horseradish peroxidase-conjugated secondary antibody (1:1,000). Signals were detected using the enhanced chemiluminescence detection system and exposed to film.

Aconitase enzyme activity. The mitochondrial matrix enzyme aconitase has been used as a highly sensitive indicator of ROS-induced damage and inactivation (13). Therefore, we measured aconitase activity in muscle samples to assess ROS-induced damage in MMPs. Whole muscle extracts were prepared from frozen human vastus lateralis biopsies as described previously (26). Muscle samples were pulverized in liquid N₂ and diluted 40-fold (25 mg net weight/ml) in enzyme extraction buffer. Extracts were added to a final reaction mixture containing 0.5 mM sodium citrate, 0.125 mM NADP⁺, and 0.5 mM/mg (4.85 U/ml) isocitrate dehydrogenase in 50 mM Tris·HCl (pH 7.4) reaction buffer. Aconitase converts citrate to isocitrate, which is subsequently converted to α-ketoglutarate by isocitrate dehydrogenase, a reaction that also reduces NADP⁺ to NADPH. Under nonlimiting conditions, the rate of NADPH production measured by an increase in absorbance at 340 nm is equivalent to aconitase activity. Samples were preincubated for 15 min at 37°C, and aconitase activity was measured for 5 min. Aconitase activity was expressed in two ways: 1) per gram of muscle and 2) per unit of mitochondrial content. The latter was calculated by correcting the aconitase rate (U/g muscle) for our estimate of mitochondrial content. This was done for aconitase activity in the basal, pretraining state and additionally following training.

Detection of oxidized proteins. Oxidative modification of proteins alters the side chains of proteins by introducing carbonyl groups in a site-specific manner. This protein carbonylation was detected by immunoblot analyses of carbonyl groups using the Oxyblot Protein Oxidation Kit (S7150, Chemicon International). Extract preparation and immunoblotting were performed according to the recommendations provided by the manufacturer. Briefly, total protein extracts (30 μg) were derivatized to 2,4-dinitrophenyhydrazone (DNPH-hydrazone) by reaction with 2,4-dinitrophenyhydrazone (DNPH). The DNPH-derivatized protein samples were then separated by 12% SDS-PAGE for 2 h. Gels were electroblotted for 2 h onto a nitrocellulose membrane and probed with polyclonal antibodies directed toward DNPH (1:150). Membranes were washed in 0.05% Tween-PBS buffer.
and incubated with a horseradish peroxidase-conjugated secondary antibody (1:300). Signals were detected using the enhanced chemiluminescence detection system and exposed to film.

**Relationship of heteroplasmy to adaptive changes in nuclear-encoded proteins.** The percentage of mtDNA heteroplasmy was available for seven patients obtained from a previous study involving these subjects (41, 42). To evaluate the relationship between the percentage of mtDNA heteroplasmy and nuclear-encoded gene adaptations, we calculated the average protein adaptation depicted in Fig. 1 for each protein, within each patient. The average value for each patient was plotted individually as a function of the percent mtDNA heteroplasmy.

**Statistical analyses.** Data are expressed as means ± SE. Student’s t-tests were used to compare raw data between HC subjects

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**Fig. 1.** Basal expression of selected proteins and aconitase activity from untrained healthy control (HC) subjects and untrained mitochondrial myopathy patients (MMPs). **A:** protein expression is graphically represented as a fold difference of MMPs/HC subjects (*P < 0.05, MMPs vs. HC subjects; n = 10 per group). *Inset:* representative immunoblots. Proteins were selected based on their involvement in mitochondrial biogenesis [cytochrome c (Cyto-c), mitochondrial transcription factor A (Tfam), peroxisome proliferator-activated receptor-coactivator-1α (PGC-1α), outer membrane receptor (TOM20), and mitochondrial 70-kDa heat shock protein (mtHSP70)], oxidative stress [8-oxoguanine DNA glycolase-1 (OGG-1) and manganese superoxide dismutase (MnSOD)] and apoptosis [Bcl-2, Bax, and apoptosis-inducing factor (AIF)]. **B:** whole muscle aconitase activity in HC subjects (open bars, n = 10) compared with a subset of MMPs (solid bars, n = 6) expressed per gram of muscle tissue. Whole muscle aconitase activity was corrected for mitochondrial content by normalizing the mitochondrial content (based on cytochrome c expression) in MMPs to HC subjects (i.e., as a percentage of HC mitochondrial content). Thus the HC aconitase activity (open bar) can also be considered to be expressed per unit (U) of mitochondrial content in HC subjects. Because MMPs have greater mitochondrial content, the corrected aconitase activity per unit of mitochondrial content was significantly reduced in MMPs (hatched bar) compared with HC subjects (*P < 0.05, MMPs vs. HC subjects per U of mitochondrial content). **C:** representative blot of protein oxidation in 1 HC subject and 3 MMPs using the Oxyblot system. MMPs tended to show a greater extent of oxidatively modified proteins as indicated by the greater number of oxidized proteins detected, as well as by a higher intensity banding pattern of oxidized proteins in MMPs compared with HC subjects. **D:** average fold difference for all nuclear-encoded proteins for each MMP compared with the average expression of HC for each protein and expressed as a function of the percentage heteroplasmy for individual MMP (n = 7 patients are included in this analysis).
and MMPs, and paired Student’s t-tests were used for pre- vs. posttraining comparisons. Statistical differences were considered significant if $P \leq 0.05$.

RESULTS

Basal, pretraining levels of mitochondrial biogenesis and import proteins in MMP and HC subjects. The mitochondrial electron transport chain marker, cytochrome $c$, was 2.2-fold greater in muscle samples obtained from MMPs compared with HC subjects ($P < 0.05$; Fig. 1A). To determine whether mitochondrial biogenesis might be regulated differently in MMPs compared with healthy control (HC) subjects, we assessed two important regulators of mitochondrial biogenesis, Tfam and PGC-1$\alpha$. Expression of Tfam was not different between MMPs and HC subjects ($P < 0.05$; Fig. 1A). However, PGC-1$\alpha$ was elevated by 1.4-fold in MMPs compared with HC subjects ($P < 0.05$; Fig. 1A). Because elevated biogenesis in MMPs would depend on enhanced import of nuclear-encoded mitochondrial gene products, we investigated whether two mitochondrial import components, TOM20 and mtHSP70, were differentially expressed in MMPs and HC subjects. MMPs exhibited a 79% greater expression of TOM20 ($P = 0.05$), whereas mtHSP70 was elevated by 44% compared with HC subjects (Fig. 1A).

Oxidative stress markers in MMP and HC subjects. To assess the extent of oxidative stress within patient and control muscle, we evaluated aconitase enzyme activity, a ROS-sensitive mitochondrial matrix marker of oxidative damage (13). No differences existed when comparing the whole muscle aconitase activity between MMPs and HC subjects (Fig. 1B). Assuming that whole muscle cytochrome $c$ levels are generally representative of mitochondrial content, the calculated aconitase activity was reduced by 52% ($P < 0.05$) in MMPs when corrected for mitochondrial content (Fig. 1B). A similar calculated reduction would be obtained using the matrix enzyme citrate synthase, which is elevated by 1.7-fold in MMPs (41). Thus it appears that aconitase activity is specifically reduced in MMPs compared with HC subjects. Our study confirmed that aconitase activity was 48% lower ($P < 0.05$; Fig. 1A) in MMPs than in HC subjects ($P < 0.05$). AIF expression was unaltered following training in both MMPs and HC subjects (Fig. 1A) for each HC subject or in MMPs, but it did evoke a 1.4-fold increase in mtHSP70 in both HC subjects and MMPs ($P < 0.05$; Fig. 2).

Effect of training on mitochondrial biogenesis proteins in MMPs and HC subjects. To determine whether the extent of oxidative exercise on apoptotic susceptibility, we measured Bax and Bel-2 following exercise training in MMPs and HC subjects. Endurance training did not alter Bel-2 expression in HC subjects or in MMPs, but it induced significant 32 and 34% increases in Bax expression in HC subjects and MMPs, respectively ($P < 0.05$; Fig. 2).

Effect of mitochondrial apoptosis proteins in MMP and HC subjects. To assess indexes of oxidative stress following training, we measured aconitase activity, the DNA repair enzyme OGG-1, and the antioxidant enzyme MnSOD. Endurance training led to a modest 23% ($P = 0.07$) elevation of MnSOD in HC subjects, but it produced a marked twofold increase in MMPs ($P < 0.05$; Fig. 2). In contrast to the response of numerous other proteins to the training regimen (Fig. 2), aconitase activity did not increase when expressed per gram of muscle in either MMPs or HC subjects (Fig. 3A). Thus, when aconitase activity was expressed per unit of mitochondria, using cytochrome $c$ as an indicator, it was decreased by 45% ($P < 0.05$) in MMPs, but unaltered in HC subjects following training in a subset of the subjects involved in the study (Fig. 3B). In addition, endurance training induced a 22% suppression of OGG-1 in HC subjects ($P = 0.06$), however, a much greater 48% reduction ($P < 0.05$) in OGG-1 was observed in the MMPs (Fig. 2).

DISCUSSION

It has been suggested that mitochondrial biogenesis is induced within skeletal muscle that contains mtDNA defects as a compensatory adaptation for compromised bioenergetics due to impaired oxidative phosphorylation. However, no studies have investigated the mitochondrial biogenesis regulatory proteins in muscle of individuals afflicted with such defects, such as MMPs. Thus we specifically addressed whether key proteins involved in mitochondrial biogenesis were differentially regulated in MMPs compared with HC subjects. Our study con-
firmed that mitochondrial content was enhanced in MMPs and that this coincided with the elevated expression of the important transcriptional coactivator PGC-1α. Although Tfam levels have previously been shown to be upregulated in conjunction with PGC-1α during organelle biogenesis (20), we did not detect a difference in Tfam between HC subjects and MMPs. Thus Tfam levels may be sufficiently high in MMPs to support mitochondrial biogenesis in the presence of elevated concentrations of PGC-1α. Although biogenesis was elevated in MMPs, this upregulation may consist of mitochondria with both functional and dysfunctional properties. Although this will require verification using isolated mitochondria, the proliferation of defective mitochondria may exacerbate the expected dysfunction present in muscle with mutated mtDNA.

We hypothesized that elevated mitochondrial biogenesis would require an upregulation of mitochondrial import machinery components to accommodate the incorporation of newly synthesized nuclear-encoded mitochondrial proteins that are essential for organelle biosynthesis. Indeed, increased mitochondrial biogenesis in MMPs was associated with a modest elevation in the expression of two important proteins involved in mitochondrial import, TOM20 and mtHSP70. TOM20 is an import receptor that recognizes cytosolic preproteins and mtHSP70 is an intramitochondrial chaperone that ensures that precursor proteins are drawn into the matrix (15, 31, 35). A similar compensatory increase of TOM20 and mtHSP70 has been shown in fibroblasts obtained from patients with mitochondrial disease (23, 33).

Heteroplasmy within MMPs dictates that a certain proportion of mitochondria are defective in oxidative phosphorylation (10, 11, 38). We theorized that this dysfunctional pool of mitochondria within MMPs would exhibit indications of greater ROS production compared with functional mitochondria. Because ROS are capable of inducing mtPTP opening, dysfunctional mitochondria might also exhibit an increase in their susceptibility to apoptosis. Studies have shown that muscle from MMPs exhibit elevated levels of proapoptotic markers, a greater incidence of apoptosis and significantly higher antioxidant levels (9, 27, 34, 45). Indeed, the activity of the oxidative stress-sensitive enzyme, aconitase, and also the extent of oxidatively modified proteins revealed that MMPs are subjected to elevated oxidative stress compared with HC subjects. In addition, MMPs tended toward a higher muscle expression of the antioxidant enzyme MnSOD, which might be a compensatory response to quench elevated levels of ROS. MnSOD transcription and expression is known to increase in response to ROS signaling (39). In addition, because increased ROS can also induce oxidative damage to DNA, we measured the levels of OGG-1, an enzyme responsible for the repair of the most common ROS-induced nDNA and mtDNA lesion, 8-hydroxyguanosine (16, 19, 37). Although this lesion occurs in both the nuclear and mitochondrial genomes, it accumulates at a higher
rate in mtDNA. OGG-1 null animals exhibit a 20-fold higher level of mtDNA lesions compared with wild-type animals (8). OGG-1 expression was dramatically elevated (3.4-fold) in MMPs, suggesting that this increase represents a compensatory protective response to reduce mtDNA and/or nDNA damage.

We hypothesized that the potential elevation in ROS levels in MMPs may make the mtPTP more susceptible to opening, and thus increase the vulnerability of muscle from MMPs to apoptotic cell death. The pore is also regulated by proapoptotic Bax and anti-apoptotic Bcl-2, and the Bax-to-Bcl-2 ratio is often utilized as a marker of apoptotic susceptibility (36). MMPs had inherently higher levels of Bcl-2 than HC subjects, an adaptation that significantly lowers the Bax-to-Bcl-2 ratio, suggesting a protective attempt to reduce mitochondrial apoptotic susceptibility. A similar adaptive response in antiapoptotic protein expression has been reported in heart tissue from a transgenic mouse model with a mtDNA mutation (51). The basal levels of cytochrome c and AIF could also potentially influence the extent of apoptosis once triggered by a death stimulus. MMPs had greater levels of cytochrome c and AIF compared with HC subjects, indicating a greater potential for mitochondrially driven apoptosis if released from the organelle. However, the elevated cytochrome c levels in MMPs also represent a beneficial adaptation within the electron transport chain to restore an energetic deficit brought about by the mtDNA defect. Recent evidence has also suggested that AIF may be a dual-function protein that serves an antioxidant role within the mitochondrion (46). Thus the cellular environment within muscle of MMPs appears to be one in which a precarious balance exists between increases apoptotic susceptibility, and energetic adaptations favoring ATP production.

It has been suggested that clinical manifestations and severity of mitochondrial disease is dependent on the extent of heteroplasmy within a given tissue (11). Indeed, our data suggest that the magnitude of the compensatory response indicated by our nuclear-encoded protein expression is dependent on the extent of heteroplasmy, beyond ~75%. It has been previously shown that this threshold for compensatory adaptations and pathophysiological phenotypes ranges between 60 and 85% (6, 7, 17, 18, 22), and our data corroborate these findings and are the first to show that nuclear-compensatory gene expression in MMPs is dependent on the extent of heteroplasmy within skeletal muscle.

Muscle oxidative phosphorylation in MMPs may be so impaired that common daily functions become difficult and infringe on the quality of life. Endurance training represents a potential therapeutic intervention to increase the number of functional mitochondria and enhance oxidative capacity (41). Our results provide information on the underlying basis for mitochondrial adaptations to training in MMPs. The data indicate that after 14 wk of training, several mitochondrial components were increased in parallel in both MMPs and HC subjects. These included cytochrome c, mtHSP70, and Bax. Thus common adaptive responses were observed. The magnitude of these changes should be considered in light of the cellular environment which existed prior to training (Fig. 1A), in which MMPs already demonstrated adaptive increases in multiple proteins related to biogenesis and apoptosis. Despite the evidence for a training-induced increase in mitochondrial content, our results indicate that this occurred in the absence of a corresponding increase in expression of either PGC-1α or Tfam, two important regulatory proteins of mitochondrial biogenesis. There is evidence for rapid and transient adaptations of PGC-1α in response to exercise (2, 32), as well as a training-induced increase in Tfam in humans (3). Thus it is possible that these changes also occurred in both MMPs and HC subjects at early time points in our training study to initiate the mitochondrial biogenesis evident after 14 wk. Alternatively, factors other than changes in expression (e.g., localization in the nucleus, or activation) may also play a role in transcription factor or coactivator activity leading to organelle biogenesis, as recently suggested (48). We also believe that the exercise training-induced adaptations are likely to be different from those that lead to a chronically elevated PGC-1α level in the muscle of MMPs (Fig. 1A), in the absence of training. These latter adaptations appear to be the result of lifetime-long compensatory mechanisms that produce an elevation in PGC-1α, and consequently mitochondrial content, in the absence of exercise. Despite this apparent elevation in mitochondrial content, the work capacity of MMPs in the absence of training remains limited, perhaps because the increase in organelle volume is not fully functional in energy production. Thus it is clear that endurance training elicits a number of beneficial physiological and molecular adaptations, including further increases in mitochondrial proteins (Fig. 2), which can help to improve exercise capacity (41). However, the nature of the adaptive compensatory mechanisms which occur over a
lifetime, compared with those that are initiated by exercise training, remain to be resolved.

Our results also show that training significantly induced the expression of mtHSP70, but it resulted in no change in TOM20 for both MMPs and HC subjects. Previous results from our laboratory have shown that chronic contractile activity of rat skeletal muscle results in a significant elevation in both TOM20 and mtHSP70 (43). This suggests mtHSP70 induction is required to accommodate a greater mitochondrial import, but that basal TOM20 expression is sufficient to support training-induced mitochondrial biogenesis in human muscle.

In addition to the common adaptive responses to training in the HC subjects and the MMPs, a number of divergent changes also occurred. Notable among these are the decline in the DNA repair enzyme OGG-1, the much greater increase in MnSOD, and the reduction in aconitase activity observed in the MMPs, compared with the HC subjects. The reduction in OGG-1 with training essentially nullifies the adaptive increase observed prior to training, thus reducing the capacity for DNA repair. In addition, in the HC subjects the unchanged aconitase activity following training suggests that the antioxidant capacity of muscle has undergone an adaptive process to effectively offset any exercise-associated elevations in ROS production. This does not appear to be the case in the MMPs, despite the large increase in MnSOD expression, because the reduction in aconitase activity implies that the level of oxidative damage exceeds the anti-oxidant capacity in these patients. Although this remains to be established experimentally, our data suggest that exercise training induces a greater oxidative stress in MMPs, compared with HC subjects.

A summary of the differences between MMPs and HC subjects and their response to endurance training is depicted in Fig. 4. Our data illustrate that enhanced mitochondrial content in MMPs appears to be at least partially mediated by higher levels of PGC-1α as well as protein import machinery components. Although the overall mitochondrial content may be enhanced due to these compensatory adaptations, the inherent mtDNA mutations within MMPs produce a proportion of dysfunctional mitochondria. Thus ATP provision is compromised due to defective mitochondrial function, and this can lead to common clinical manifestations, such as exercise intolerance (Fig. 4). In addition, these dysfunctional mitochondria may increase ROS production to cause enhanced apoptotic susceptibility and/or macromolecular damage. However, our data suggest that there are compensatory increases in both antioxidant (MnSOD) and DNA repair capacity (OGG-1) to combat these processes. Additionally, MMPs have elevated Bcl-2 levels, which effectively lowers the Bax-to-Bcl-2 ratio. This may serve to provide a protective mechanism to reduce the rate of release of proapoptotic proteins. Although increases in both cytochrome c and AIF in MMPs may be an attempt to reconcile energetic deficits by enhancing oxidative phosphorylation, these are both proteins that can induce apoptosis and may increase the apoptotic susceptibility with a death stimulus. Additionally, our data demonstrate that the extent of the nuclear compensatory response in MMPs appears to be dependent on a threshold level of heteroplasmy (~75%), and the magnitude of the compensatory response above this critical threshold tends to rise steeply. Endurance training in MMPs and HC subjects resulted in an increase in mitochondrial content, which was likely facilitated by a greater level of the mitochondrial import protein mtHSP70. Additionally, training also caused an upregulation of the antioxidant enzyme MnSOD (Fig. 4). Given the parallel exercise-induced adaptive responses of MMPs and HC subjects, and the fact that endurance training in MMPs has previously been shown to improve energy metabolism and work capacity (41), these molecular adaptations probably contribute to the documented functional benefits of endurance training in MMPs. However, endurance training also reduced the expression of DNA repair machinery and also seemed to increase oxidative damage within MMPs, and that HC subjects and MMPs exhibited similar adaptations, it is likely that these molecular responses, contribute to the functional benefit of endurance exercise in MMPs. + Stimulate; −inhibit.

Fig. 4. Summary of the alterations evoked by mitochondrial DNA (mtDNA) defects in skeletal muscle and the effect of endurance training in MMPs and HC subjects. Basal differences: mtDNA defects cause mitochondrial dysfunction that can potentially initiate retrograde signaling pathways [Ca²⁺, ATP/ADP, reactive oxygen species (ROS)] that can alter nuclear-encoded genes. Our results indicate a number of compensatory nuclear gene responses leading to enhanced mitochondrial content in MMPs. Because a portion of mtDNA is defective, biogenesis will necessarily result in the production of dysfunctional mitochondria leading to lower ATP production. Depending on the extent of the mutant phenotype (i.e., %heteroplasmy), this may result in a feed-forward loop that exacerbates retrograde signaling pathways to further increase the compensatory nuclear response. The abnormal mitochondrial phenotype exhibited in MMPs may elevate ROS production to increase apoptotic susceptibility and/or cause cellular damage. Our study has shown that MMPs exhibit compensatory increases in the expression of MnSOD, Bcl-2, and OGG-1. MMPs also exhibit an elevated expression of the proapoptotic proteins cytochrome c and AIF, likely an attempt to support mitochondrial biogenesis and restore energy production for the cell. However, their upregulation in MMP muscle may also increase the apoptotic susceptibility with a death stimulus. Training effects: endurance training (dashed lines leading to underlined proteins) induced similar adaptive responses in both HC subjects and MMPs. Endurance training may positively influence muscle function in MMPs by increasing the expression of the antioxidant enzyme MnSOD, the mitochondrial import protein mtHSP70, and cytochrome c, which may facilitate electron transport. Given that endurance exercise has been shown to improve muscle function within MMPs, and that HC subjects and MMPs exhibited similar adaptations, it is likely that these molecular responses, contribute to the functional benefit of endurance exercise in MMPs. + Stimulate; −inhibit.
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direct investigations of mitochondrial bioenergetic functions, protein release and mtDNA replication are required to establish whether exercise training is an ideal therapeutic intervention for MIMPs over the long term.

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REFERENCES

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