Resistance training in patients with single, large-scale deletions of mitochondrial DNA

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Dramatic tissue variation in mitochondrial heteroplasmy has been found to exist in patients with sporadic mitochondrial DNA (mtDNA) mutations. Despite high abundance in mature skeletal muscle, levels of the causative mutation are low or undetectable in satellite cells. The activation of these typically quiescent mitotic cells and subsequent shifting of wild-type mtDNA templates to mature muscle have been proposed as a means of restoring a more normal mitochondrial genotype and function in these patients. Because resistance exercise is known to serve as a stimulus for satellite cell induction within active skeletal muscle, this study sought to assess the therapeutic potential of resistance training in eight patients with single, large-scale mtDNA deletions by assessing: physiological determinants of peak muscle strength and oxidative capacity and muscle biopsy-derived measures of damage, mtDNA mutation load, level of oxidative impairment and satellite cell numbers. Our results show that 12 weeks of progressive overload leg resistance training led to: (i) increased muscle strength; (ii) myofibre damage and regeneration; (iii) increased proportion of neural cell adhesion molecule (NCAM)-positive satellite cells; (iv) improved muscle oxidative capacity. Taken together, we believe these findings support the hypothesis of resistance exercise-induced mitochondrial gene-shifting in muscle containing satellite cells which have low or absent levels of deleted mtDNA. Further investigation is warranted to refine parameters of the exercise training protocol in order to maximize the training effect on mitochondrial genotype and treatment potential for patients with selected, sporadic mutations of mtDNA in skeletal muscle.

Keywords: mitochondrial myopathy; single, large-scale mitochondrial DNA deletions; satellite cells; resistance exercise; treatment approach

Abbreviations: CK = creatine kinase; COX = cytochrome c oxidase; mtDNA = mitochondrial DNA; 1 RM = one-repetition maximum; SDH = succinate dehydrogenase

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Introduction

Exercise intolerance in patients with heteroplasmic mitochondrial DNA (mtDNA) mutations is due to high proportions of mutated relative to wild-type genomes within skeletal muscle, impairing cellular capacity to increase oxidative energy production in relation to increased energy demands during physical activity. The potential therapeutic role of exercise training to improve muscle oxidative capacity and decrease mutation load in patients with heteroplasmic mtDNA mutations has been suggested (Taivassalo et al., 1998, 2001; Taivassalo and Haller, 2004) and more recently, the safety and efficacy of endurance training has been reinforced (Jeppesen et al., 2006; Taivassalo et al., 2006). Another form of exercise, resistance training, may provide an alternative, unique approach for therapy for a selected group of mitochondrial myopathy patients, those with single, large-scale mtDNA deletions and sporadic mtDNA point mutations.

In these patients, dramatic tissue variation in mutation levels has been established where, despite high abundance of mutated mtDNA in mature muscle, levels of the causative mutation are low or undetectable in myogenic progenitor cells satellite cells (Moraes et al., 1989; Fu et al., 1996; Weber et al., 1997). This surprising finding led to the
notion that activation of these typically quiescent mitotic cells in adult muscle could result in the shifting of normal mitochondrial templates to mature muscle and restoration of a more normal mitochondrial genotype and biochemical function (Fu et al., 1996; Clark et al., 1997). Induction of satellite cells is known to occur in response to muscle fibre injury, involving replacement of damaged myofibres through regeneration of new fibres from the satellite cell population (Hawke and Garry, 2001). Models of muscle injury (myotoxin injection, Clark et al., 1997 or biopsy trauma, Shoubridge et al., 1997) have been tested as a means to activate satellite cells in two different patients harbouring sporadic tRNA mutations of mtDNA, and in both cases, lower levels of mutation as well as normal biochemical function were detected in the focus of regenerating muscle.

Resistance exercise has been shown to provide a stimulus for satellite cell induction in healthy and aged human skeletal muscle (Hikida et al., 2000; Kadi and Thornell, 2000; Roth et al., 2001) and would conceivably offer a more physiological treatment strategy and means of promoting satellite cell activation in patients with sporadic mtDNA mutations. Resistance training involves the repetition of muscle contractions against an opposing load and is well established to increase strength in healthy individuals (Kraemer et al., 1988). Dependent on the muscle contraction type and intensity, resistance exercise can be associated with muscle overload or injury, where in mature post-mitotic muscle, the resultant myofibre hypertrophy or repair processes depend on these mitotic, myogenic cells. Activated satellite cells reportedly provide additional nuclei to enlarging muscle, thereby maintaining the nuclear:cytoplasmic ratio at a constant level, or they fuse together to form a regenerating myofibre (Schultz et al., 1985; Darr and Schultz, 1987; Schultz and McCormick, 1994). Concomitant with these processes is the incorporation of satellite cell-derived mitochondria and mtDNA.

In a previous report, we described the effects of heavy resistance exercise on decreasing the level of mutation in a single patient with a sporadic tRNA point mutation (Taivassalo et al., 1999); however, measures of muscle strength and molecular markers of muscle damage or satellite cells were not assessed. In this study, we have assessed the effects of resistance training on physiological parameters as well as muscle regeneration and biochemical and molecular genetic changes in biopsy samples in eight patients with single, large-scale mtDNA deletions, and reported the value of resistance exercise training as a treatment strategy.

### Methods

#### Patient description

Eight female patients (39± 9 years) with molecular evidence of a sporadic, heteroplasmic, single, large-scale deletion of mtDNA were enrolled in this study (Table 1). Muscle weakness and exercise intolerance were experienced by all patients to a varying degree (mild to severe). The study was approved by the institutional review boards of Newcastle University, 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.

#### Outcome measures

All patients underwent physiological exercise testing and muscle needle biopsies were obtained before and after 12 weeks of resistance training for determination of the following:

#### Exercise physiology

**Maximal muscle strength.** The one-repetition maximum (1RM), defined as the maximal weight capable of lifting through the full range of motion one time, was determined for leg extension and leg press exercises on weight-stack devices similar to the ones used by the patients during their training. Two consecutive failures to reach full extension signalled the end of the test. The greatest weight successfully lifted was counted as the 1RM, index of maximal muscle strength.

**Peak aerobic exercise capacity.** To determine whether changes at the muscle cellular level were translated into changes in whole-body peak aerobic capacity, an incremental exercise test to exhaustion was performed on a stationary bicycle in each patient as previously described (Taivassalo et al., 2006) to obtain peak work (watts), capacity for oxygen utilization (VO₂) and rate of systemic oxygen delivery (cardiac output, Q). From these measures, peak arterio-venous oxygen difference (a-v O₂ diff) was calculated using the Fick equation (VO₂ = Q x a-v O₂ diff), to serve as an index of peak capacity for oxygen extraction within

### Table 1 Clinical and genetic characteristics of eight female patients with single, large-scale mtDNA deletions who have undergone resistance training

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Clinical presentation</th>
<th>Size of mtDNA deletion (Kb)</th>
<th>Level of deletion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>CPEO severe exercise intolerance, muscle weakness</td>
<td>4.2</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>CPEO, severe exercise intolerance</td>
<td>5.0</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>CPEO, exercise intolerance</td>
<td>5.0</td>
<td>83</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>CPEO, exercise intolerance</td>
<td>3.4</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>CPEO, exercise intolerance</td>
<td>4.2</td>
<td>79</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>CPEO, exercise intolerance</td>
<td>4.5</td>
<td>42</td>
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<tr>
<td>7</td>
<td>25</td>
<td>CPEO, hearing impairment, retinitis pigmentosa, exercise intolerance</td>
<td>3.0</td>
<td>64</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>CPEO, exercise intolerance, muscle weakness</td>
<td>4.2</td>
<td>32</td>
</tr>
</tbody>
</table>
skeletal muscle and reflecting mitochondrial capacity for oxidative phosphorylation (Taivassalo et al., 2003).

**Muscle damage.** Serum creatine kinase (CK, enzymatic marker of muscle damage) levels were determined before and after muscle strength evaluation, as well as periodically throughout the training.

**Quality of life**

The effect of resistance training on health, quality of life and physical functioning was obtained by the Short Form Health Survey Questionnaire (SF-36) by the Medical Outcomes Study (Ware and Sherbourne, 1992). This reliable and validated survey was completed by each patient. As described in our previous studies, scores of eight different components of the SF-36 were aggregated and tabulated to give a single physical component summary score, used to assess the self-determined improvement with exercise training per patient. As a point of reference, the score for the general healthy US population, standardized for age and gender, is 50 ± 10.

**Muscle needle biopsy**

A needle biopsy of the mid-portion of vastus lateralis muscle was performed to obtain on average a 200-mg sample as previously described (Taivassalo et al., 2006). These samples were immediately divided into three parts: frozen and stored in liquid nitrogen for molecular genetic determinations, or frozen in isopentane cooled by liquid nitrogen for histological and histochemical analysis. Samples for each patient from each time point (baseline and 12 weeks) were taken from the same leg and were extremely careful to biopsy at least 2 cm away from the site of the previous biopsy to ensure that we were not biopsying the site of likely regeneration as result of the previous trauma.

**Histochemistry.** Cryostat sections (10 μm) were cut from transversely orientated muscle blocks and subjected to histochemical staining (Taylor et al., 2004). The number of fibres with central nuclei was determined. To determine the proportion of oxidative impairment in muscle containing single, large-scale mtDNA deletions, sections were reacted for both cytochrome c oxidase (COX) and succinate dehydrogenase (SDH) activities. The SDH section was used to determine the number of fibres exhibiting increased levels of enzyme activity in the subsarcolemmal region, so-called ‘ragged blue’ fibres. Sequential assay of COX and SDH activities was used to identify COX-deficient fibres (Taylor et al., 2004). Over 200 fibres were counted on each biopsy.

**Fibre diameter and typing (ATPase staining).** Cryostat sections (12 μm) were air-dried for 30 min. To determine fibre type, sections were stained for ATPase activity (Brooke and Kaiser, 1970), all fibres in the biopsies were assessed and the fibre type proportions determined as a percentage of total fibres counted. Fibre diameters were determined by measuring the smallest diameter in >20 randomly sampled fibres (H&E staining) and 15–20 type 1 fibres (ATPase staining) in each biopsy (pre- and post-training) for all eight patients.

**Immunohistochemistry.** To determine if there was a change in muscle following training, we searched for evidence of changes in neural cell adhesion molecules (NCAM)-positive satellite cells and regeneration by determining expression of neonatal myosin. All NCAM-positive satellite cells and muscle following training, we searched for evidence of changes in

**Mitochondrial DNA studies.** We used two different techniques to determine if there was a change in the percentage of deleted mtDNA in the muscle biopsies—Southern blotting and quantitative real-time PCR. Southern blotting is the accepted ‘gold standard’ method for quantifying levels of mtDNA rearrangements, but requires large amounts of DNA, whereas real-time PCR can be performed on small amounts of DNA extracted from muscle sections or groups of individual muscle fibres.

**Biochemistry.** The activities of the individual respiratory chain complexes I, II and IV, and the matrix marker enzyme citrate synthase were measured in a post 600g av muscle supernatant prepared from frozen muscle specimens (Kirby et al., 2007).

**Mitochondrial DNA studies.** Two different methods were used to obtain the percentage of deleted mtDNA and mtDNA copy number. Fresh frozen muscle sections (20 μm) were mounted on polyethylene naphthalate (PEN) slides (Leica Microsystems, Milton Keynes, UK) and subjected to dual COX/SDH histochemistry as described above, and air-dried after dehydration. Groups of muscle fibres (200,000 μm²), representing between 12 and 25 fibres depending on the size of the fibres, were cut at random through the section using a Leica Laser Microdissection (AS-LMD) System. Following centrifugation (7000 × g for 10 min), the cells were lysed in 10 μl of cell lysis buffer (50 mM Tris–HCl, pH 8.5, 1 mM EDTA, 0.5% Tween-20, 200 ng/ml protease K) at 55°C for 2 h and then 95°C for 10 min to denature the protease K. PCR primers and fluorogenic probes [monoclonal mouse anti-human NCAM (DAKO)], diluted 1 in 10 in 1% BSA was applied to each section. Sections were incubated for 1 h, washed in PBS followed by incubation with the secondary antibody (biotinylated anti-mouse diluted 1:200 in 1% BSA) for 45 min. Following a further PBS wash, sections were incubated with StreptABCComplex/AP for 30 min and the slides washed in PBS. The slides were then incubated in Liquid permanent red solution for 20 min, washed in distilled water, counterstained in Meyer’s haematoxylin, dehydrated and mounted in DPX.
(Applied Biosystems) for regions of MTND1 (forward primer, L3485-3504; reverse primer, H3553-3532; probe, L3506-3529) and MTND4 (forward primer, L12087-12109; reverse primer, H12170-12140; probe, L12111-12138) were synthesized, and 5 μl of DNA lysate was amplified with each of the MTND1 and MTND4 primers and probes as previously described (He et al., 2002; Bender et al., 2006; Krishnan et al., 2007). For determination of mtDNA copy number, areas of the biopsy containing both COX-positive and COX-deficient fibres were included and three samples were analysed in triplicate and the mean value determined.

**Resistance training protocol**
All patients were given individualized exercise guidelines and underwent a supervised programme of high intensity resistance training of both legs, three times per week for 12 weeks. Bilateral leg extension and leg press exercises were performed with the focus of activating the quadriceps muscles on standard weight-stack equipment available in a training facility. Patients had been familiarized on proper technique which emphasized both the concentric (muscle contraction during shortening) and eccentric (muscle contraction during lengthening) phases. For each exercise session, patients were instructed to first do a general warm-up, consisting of stationary cycle riding at a very light workload for 5–10 min followed by general stretching. Thereafter, each patient performed three sets (with time for full recovery between sets) of six to eight repetitions of both leg extension and leg press at an intensity of 80–85% of 1RM. According to the progressive overload concept, the 1RM was determined at baseline and re-assessed every 3 weeks to ensure maintenance of training intensity relative to progressions in muscle strength. The general training protocol was derived from basic guidelines for resistance exercise prescription as put forth by the American College of Sports Medicine Position Stand 2002, and was based on a training protocol shown to elicit significant increases in leg strength in healthy humans (Roth et al., 2001).

**Statistical analysis**
To evaluate the effects of training in all eight patients, a paired t-test was performed for comparison at baseline and 12 weeks. Non-parametric statistics were applied to detect differences in quality of life scores before and after training.

**Results**

**Training compliance**
Patients completed 26.6 ± 2.4 of the total possible 36 exercise sessions (75%) with no adverse reports. CK levels were normal before training and there were no increases in serum CK after strength evaluation or at the end of the 12-week training programme (pre 187 ± 115; post 166 ± 159 U/l), although all patients showed transient rises in CK during the exercise protocol that were likely attributable to the progressive increases in resistance lifted.

**Exercise physiology**

**Maximal muscle strength**
Quantitative muscle testing revealed improvements in maximal quadriceps strength after 12 weeks of resistance training where 1RM on leg extension increased 15% (110 ± 45 to 127 ± 52 lbs, P < 0.02) and leg press 25% (188 ± 51 to 236 ± 49 lbs, P < 0.01).

**Aerobic exercise capacity**
Resistance training had no effect on peak work capacity (85 ± 19 to 90 ± 18.5 W) or cardiac output (12.4 ± 1.9 to 12.3 ± 1.9 l/min) during cycling exercise. However, a trend for increase in peak aerobic capacity (VO2 1.26 ± 0.22 to 1.33 ± 0.26 ml/kg/min; P = 0.06) was detected and attributable to an improved ability for muscle to extract available oxygen during exercise (peak a-vO2 difference 10.3 ± 1.8 to 11.1 ± 2.0 ml/dl, P < 0.05).

**Quality of life**
Resistance training did not lead to patient-reported improvements in quality of life as assessed by the SF36 scores (pre 35.4 ± 6.6; post 37.3 ± 6.8).

**Muscle biopsy analysis**

**Fibre diameter**
There were no significant changes in the average muscle fibre diameter (pre-training levels 53.9 ± 13.6 μm, post-training 58.2 ± 16.3 μm; P = 0.52). Type 1-specific fibre diameter (64.3 ± 10.7 μm pre-training to 68.3 ± 16.5 μm post-training; P = 0.276), or COX-positive muscle fibre diameter (66.4 ± 19.5 μm pre-training, 68.9 ± 22.1 μm post-training, P = 0.790).

**Markers of muscle damage and regeneration**
The resistance exercise protocol damaged the quadriceps muscle fibres with significant increases in the percentage of central nuclei as determined from the H&E sections (pre-training = 12.0 ± 7.5%, post-training = 28.4 ± 16.5, P = 0.004). The percentage of regenerating fibres increased as determined by neonatal myosin staining (pre-exercise 1.0 ± 1.0 to 3.0 ± 2.4 post-exercise, P = 0.04). The number of satellite cells as determined by NCAM immunoreactivity and using the calculation: Number of satellite cells/(number of satellite cells + the number of myonuclei) × 100 (Kadi et al., 1999) increased from 18.9 ± 13.2% pre-training to 42.3 ± 12.3% post-training (P = 0.003) (Fig. 1).

**Muscle histochemistry**
All biopsies showed an extensive number of COX-deficient muscle fibres as shown by the blue nitroetrazolium reaction product, in contrast to the brown dianibenzidine reaction product in the COX-positive fibres (Fig. 2A). However, in many of the biopsies this analysis is complicated by the presence of many fibres which have intermediate staining (Fig. 2A). These intermediate fibres are a mixture of light blue and light brown colours which were difficult to categorise as either truly COX-positive or COX-deficient. We subsequently characterized these fibres...
further by using densitometry to study the enzyme activity in each fibre (Andrews et al., 2006). The COX diamino-benzidine reaction product in the intermediate fibres was less than in the positive fibres and more than in the deficient fibres, with SDH activity similar between positive and intermediate, but less than COX-deficient. The densitometry scale is an inverse linear scale ranging from 0 (black) to 255 (white). The ratio of COX/SDH is very different between COX-positive, COX-deficient and the COX-intermediate fibres ($P<0.001$) (Fig. 2B). We analysed the differences in the three groups of fibres before and after exercise training (Fig. 2C). There was no significant change in the percentage of COX-positive fibres ($54.1 \pm 24.1$) pre-exercise to ($54.7 \pm 20.2$) post-exercise ($P = 0.907$). The levels of COX-deficient fibres decreased after strength training ($21.9 \pm 13.1$ pre-exercise to $14.2 \pm 6.9$ post-exercise ($P = 0.05$). There was a corresponding increase in the number of intermediate fibres ($23.9 \pm 15.0$ pre-exercise to $31.0 \pm 16.6$ post-exercise), but this was not significant ($P = 0.078$). These changes in the COX/SDH histochemistry were not associated with any change in fibre type as determined by ATPase staining.

**Biochemistry**

Compared to the pre-training values, as expected there was no change in mitochondrial volume as reflected by CS activity after 12 weeks of training. There was little change in nuclear-encoded respiratory chain complex II activity (Fig. 3), although a trend towards increased Complex IV activity (nearly double to that of baseline, $P = 0.087$) suggests resistance training may have increased activity of mtDNA encoded proteins.

**Mitochondrial DNA studies**

The copy number per unit area in the samples did not significantly change after resistance training when compared to baseline values (2.4 \pm 1.5 copies per $\mu m^2$ baseline, 2.2 \pm 1.7 copies post-training). The Southern blot detected a 4.3% fall in overall level of deleted mtDNA in the patient group from $63.3 \pm 21.5\%$ at baseline, to $59.0 \pm 23.7$ post-training, but this change was not statistically significant ($P=0.188$). Real time analysis of mixed muscle fibres also showed a non-significant ($P=0.300$) fall (4.7%) of deleted mtDNA from $78.4 \pm 13.7\%$ to $73.6 \pm 16.1\%$ within the patient group.

**Discussion**

This study evaluated the effects of 12 weeks of resistance exercise strength training in a group of mitochondrial myopathy patients harbouring a single, large-scale deletion of mtDNA in skeletal muscle. The major findings were that within the trained muscle: (i) maximal strength improved; (ii) the level of resistance exercise-induced notable damage and regeneration; (iii) numbers of NCAM-positive satellite cells were increased; and (iv) oxidative capacity improved, as reflected by both an increased ability to extract oxygen as well as corresponding changes in the percentage of COX deficient (decreased) and intermediate staining (increased) fibres. Taken together, we believe the above findings support our hypothesis of resistance exercise-induced mitochondrial gene-shifting in muscle-containing satellite cells which have low or absent levels of deleted mtDNA.
Resistance training is normally expected to increase muscle strength through interplay of neurological adaptation and increase in muscle cross-sectional area (hypertrophy), without improvements in muscle oxidative or aerobic exercise capacity. However, the rationale underlying the use of resistance exercise for mitochondrial myopathy patients in this study is different and unique: resistance training is expected to improve mitochondrial phenotype and genotype through successful activation and incorporation of muscle satellite cells containing predominantly wild-type mtDNA, thereby lowering mutation load and improving muscle oxidative capacity. Furthermore, sufficient change at the cellular level may be expected to translate into improvements during aerobic exercise.

In our group of eight patients, exercise testing revealed the expected increase in leg muscle strength following resistance training. However, in addition, we found evidence of enhanced muscle oxidative capacity. The improvement in
peak arterio-venous oxygen difference detected during cycling exercise indicates an increased ability of muscle to extract and utilize available oxygen during aerobic exercise. This adaptation is suggestive of improved mitochondrial function within skeletal muscle (Taivassalo et al., 2001, 2003, 2006). This finding after resistance training in our patients supports an improved capacity for mitochondrial oxidative phosphorylation that may result from the satellite cell-derived shift in mitochondrial genotype. Muscle biopsy findings revealing a decrease in COX-deficient cells and trend for increased COX activity after resistance training also support this notion. The greater proportion of NCAM-positive cells suggest increased satellite cell numbers within trained muscle, and is consistent with the interpretation that they are the source of wild-type templates.

There is evidence of muscle regeneration in the post-training biopsies of the patients, with 2- to 3-fold increases from baseline in the percentage of fibres with central nuclei and neonatal myosin stained fibres. These findings suggest that muscle damage incurred from resistance exercise was the primary stimulus for the 2.3-fold increase in NCAM-positive satellite cell numbers. Although there was a trend, we did not find significant increases in either average fibre diameter or specifically in Type 1 or COX-positive fibre diameter, which may reflect the high regenerative activity in the post-training biopsies.

We anticipated that the evidence for increased muscle regeneration would be accompanied by a decrease in COX-deficient fibres and a decrease in the percentage of mutated mtDNA. We did show a decrease in the number of COX-deficient fibres and interestingly, a proportional increase in intermediate staining muscle fibres. This is an encouraging result, further suggesting that we have altered the biochemical phenotype of the muscle. What we did not show was an overall decrease in the level of mutated mtDNA in the trained muscle. Whilst there was a trend on both Southern blotting and real-time PCR, the observed decreases were not significant. Each muscle biopsy was performed using a needle from different areas in the vastus lateralis. However, previous studies from our group have shown that even in the same muscle there is substantial variation both in the number of COX-deficient muscle fibres and in the percentage of mutated mtDNA (Barron et al., 2005). The inherent variability seen within the muscle is, therefore, likely to be a major complication in trying to interpret relatively small changes in the level of deleted mtDNA.

A somewhat unexpected outcome of our study was that the shift of mitochondrial genotype is much less in these patients when compared to a previous study of muscle regeneration after resistance training (Taivassalo et al., 1999). In a case study of a single patient with a sporadic mt12315G>A MTTL2 gene mutation, marked changes in mutation load and increased muscle fibre diameter were detected, without significant evidence of increased NCAM staining. There are key differences between the two studies. In the current study, patients harboured single, large-scale deletions rather than an mtDNA point mutation and this may affect the degree of muscle necrosis since some patients with mtDNA point mutations may develop rhabdomyolysis episodes (McFarland et al., 2004), an event which would be very unusual in patients with single, large-scale mtDNA deletions. The current exercise protocol was also longer in duration, elicited lower intensity, involved the lower rather than upper limbs, and combined both concentric (muscle shortening) and eccentric (muscle lengthening) contractions per exercise movement (i.e. leg extension or press) rather than focussing on separate concentric or eccentric-only muscle contractions.

In light of our current results, there are aspects of the resistance training paradigm and timing of the biopsy after training that are worthy of discussion. The protocol was designed to include dynamic repetitions combining both concentric and eccentric contractions, with the aim of providing both overload and injury stimuli for satellite cell induction during an exercise paradigm that was feasible and practical to perform by the patient. In doing so, the eccentric component was not as intense as in the previous study. We chose an intensity (80–85% 1RM) that we deemed achievable for patients over 12 weeks of training but still considered intense enough to induce adaptation or injury. Based on the biopsies, this was sufficient to induce muscle damage. However, as we have previously proposed (Taivassalo et al., 1999), maximal gene-shifting would be anticipated from exercise that results in more complete muscle regeneration via the fusion of satellite cells containing normal mtDNA. Supramaximal (~120% 1RM) eccentric contractions have reportedly been used by healthy individuals to maximize gains in muscle strength as well as hypertrophy (Deschenes and Kraemer, 2002) and therefore would be more likely to induce greater muscle damage, satellite cell activation and lead to a greater shift in
Resistance training in mtDNA defects

Mitochondrial genotype in this patient population. The effects of such intense exercise in quadriceps muscle of patients with single, large-scale mtDNA deletions are not known. Although less practical, an even greater shift in mitochondrial genotype may be obtained through electrically evoked eccentric muscle contraction, an idea consistent with recent data showing greater satellite cell activation and more complete muscle regeneration after an acute bout of electrical eccentric stimulation compared with voluntary eccentric exercise in healthy human lower limb (Crameri et al., 2007).

In addition to exercise intensity, patient compliance with the progressive overload training protocol may account for the less pronounced changes in genotype. Healthy persons that are initially untrained can expect to achieve muscle strength gains up to the range of 40% (Deschenes and Kraemer, 2002). Whether the lower magnitude of improvement in muscle strength (15–20%) observed in our study is related to the training protocol, patient compliance or to the muscle damage in the patients is not clear at present. Muscle strength is directly proportional to the size of myofibres, given the higher proportion of regenerating fibres in the post-training biopsy, the smaller increase in strength is not surprising.

Finally, the post-training biopsy was taken immediately following the training programme, which was based on the concept of progressive overload, systematically increasing the workload over the 12-week period to ensure that adaptive processes within muscle will continue to respond. Given that increases in the weight lifted near the end of the training programme may have induced new damage, it is difficult to differentiate more recent from ongoing regeneration within the trained muscle. Furthermore, given the current biopsy findings demonstrating marked increases in central nuclei and neonatal positive fibres, perhaps allowing time for fibres to complete the regeneration process would have allowed significant detection of a lower muscle mutation load, or further increases in peak or submaximal endurance exercise capacity. We also believe that the physical effort and muscle soreness associated with this continued high-intensity exercise until the post-training evaluation may have masked any reported improvements in quality of life.

In conclusion, this study shows that resistance training can lead to increased muscle strength in patients with mitochondrial myopathies attributable to single, large-scale mtDNA deletions and warrant further investigation as a potential treatment for patients with sporadic mtDNA disease. Another exciting possibility is to combine the effects of strength and endurance training, since they have different effects on mitochondrial biogenesis and genotype. Thus, a preliminary period of strength training to induce satellite cell activation and transfer of normal mitochondrial fibres to existing muscle (decreasing number of COX-deficient fibres and increasing level of wild type relative to mutant mtDNA), followed by endurance training-induced mitochondrial biogenesis to expand newly incorporated wild-type genomes could be the more effective treatment approach.

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