Aerobic training is safe and improves exercise capacity in patients with mitochondrial myopathy

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Exercise intolerance is a prominent symptom in patients with mitochondrial myopathy (MM), but it is still unsettled whether exercise training is safe and beneficial for patients with MM. To address this, we studied the effect of 12 weeks cycle training on exercise capacity, quality of life and underlying molecular and cellular events in five patients with single large-scale deletions, one with a microdeletion and 14 with point mutations of mitochondrial DNA (mtDNA), and 13 healthy subjects. Each training session lasted 30 min, and was performed at an intensity of 70% of VO2max (maximal oxygen uptake). Each subject performed 50 training sessions in 12 weeks. All subjects were evaluated before and after training, and 13 MM patients were studied after 8 weeks of deconditioning. Evaluation included VO2max and mutation load and mtDNA quantity, mitochondrial enzymatic activity, and number of centrally nucleated, apoptotic, ragged red and cytochrome oxidase (COX)-negative fibres in muscle biopsies from the quadriceps muscle. After 12 weeks of training, VO2max and muscle citrate synthase increased in MM (26 and 67%) and healthy (17 and 65%) subjects, while mtDNA quantity in muscle only increased in the MM patients (81%). In the MM patients, training did not change mtDNA mutation load in muscle, mitochondrial enzyme complex activities, muscle morphology and plasma creatine kinase. After deconditioning, VO2max and citrate synthase activity returned to values before training, while muscle mtDNA mutation load decreased. These findings show that aerobic training efficiently improves oxidative capacity in MM patients. Based on unchanged levels of mutant load in muscle, morphological findings on muscle biopsy and plasma creatine kinase levels during training, the treatment appears to be safe. Regular, supervised aerobic exercise is therefore recommended in MM patients with the studied mutations.

Keywords: mitochondrial myopathy; training; mtDNA; mutation load

Abbreviations: CK = creatine kinase; HR = heart rate; MM = mitochondrial myopathy; mtDNA = mitochondrial DNA; RRF = ragged red fibres; VO2max = maximal oxygen uptake; Wmax = maximal workload


Introduction

Mitochondrial disease caused by mutations in mitochondrial DNA (mtDNA) is recognized as one of the most common causes of inherited neurological disease (DiMauro et al., 2001). Most mtDNA mutations co-exist with wild-type mtDNA in cells; a condition called heteroplasmy. At high mutant loads, the functional consequence of mtDNA mutations almost invariably involves impaired energy production. The mutation load in skeletal muscle is typically high, and since muscle is the most oxidative tissue in the organism, exercise intolerance and mitochondrial myopathy (MM) is a very common symptom in persons with mitochondrial disease. The exercise intolerance promotes a sedentary lifestyle, and patients have often been advised to avoid physical exertion.

With the prominent muscular symptoms and current lack of treatment for mitochondrial disease, aerobic training has been considered as a treatment for these conditions (Cejudo et al., 2005; Siciliano et al., 2000; Taivassalo et al., 1996, 1998, 1999a, 1999b, 2001). The studies on training have all been small, including <10 mitochondrial patients each. Although all studies have found a training-induced improvement of oxidative capacity and quality of life, the only study in which
molecular changes were investigated found that the mutant load in muscle increased with training in six out of nine studied subjects (Taivassalo et al., 2001). Since oxidative capacity during exercise is inversely correlated with muscle mtDNA mutation load in patients with MM (Jeppesen et al., 2003), the possibility exists that the mutant load in muscle increases with training, which in the long run will produce adverse effects.

In the present study, we studied the effect of aerobic training in 20 patients with well-defined molecular defects of mtDNA, not only to see if exercise capacity and quality of life improves but also to study the events underlying the training response on the molecular and cellular levels, which may predict the long-term effects of training.

Material and methods

Subjects

Twenty persons (10 men and 10 women) with four different mtDNA mutation types (Table 1) were included. Patients were 41 ± 12 years old (range 26–57 years), weighed 64 ± 12 kg and were 170 ± 11 cm tall. To study the effect of training on low levels of mutant loads in muscle, eight relatives of probands with the 3243 A→G point mutation of mtDNA were studied (subject nos 13–20 in Table 1). Only four of these relatives were clinically unaffected. Thirteen healthy, sedentary subjects (eight men and five women completed the same training programme as the patients. The demographic data of the healthy subjects were age 40 ± 11 years (range 24–60 years), weight 77 ± 17 kg and height 173 ± 13 cm. Four other persons with mtDNA mutations, and six healthy subjects had originally been included in the programme, but were excluded for the following reasons: one patient with a 4409 T→C mutation of mtDNA stopped training due to lower back-pain, one healthy subject got pregnant during training and three persons with the 3243 A→G mtDNA mutation and five healthy subjects completed <50% of the training sessions due to low motivation.

Five of the persons with 3243 A→G mutation in muscle had diabetes mellitus and were treated with either insulin or restricted diet. One person had myoclonic epilepsy and was treated with anti-epileptic medication. No other subject took medication.

The study was approved by the Scientific-ethical committee of Copenhagen (No. KF 01-205/00). The subjects were all informed about the nature and risks of the study, and gave written consent to participate.

Training programme

The training programme lasted 12 weeks and consisted of 50 sessions of cycling in the subject’s home. Subjects were supervised by phone weekly. Each session lasted 30 min, preceded by 5 min of warm-up. Subjects exercised at a target heart rate (HR)

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Defects in mtDNA</th>
<th>Gender/age</th>
<th>Muscle mutation load (%)</th>
<th>Clinical symptoms and signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6694–12988 deletion</td>
<td>M/29</td>
<td>29</td>
<td>CPEO</td>
</tr>
<tr>
<td>2</td>
<td>7177–13767 deletion</td>
<td>M/36</td>
<td>40</td>
<td>CPEO, EI</td>
</tr>
<tr>
<td>3</td>
<td>9110–14605 deletion</td>
<td>F/53</td>
<td>29</td>
<td>CPEO, EI</td>
</tr>
<tr>
<td>4</td>
<td>8580–11538 deletion</td>
<td>F/63</td>
<td>60</td>
<td>CPEO, EI</td>
</tr>
<tr>
<td>5</td>
<td>≈1500 bp deletion</td>
<td>F/47</td>
<td>46</td>
<td>CPEO, EI</td>
</tr>
<tr>
<td>6</td>
<td>ND2 gene microdeletion*</td>
<td>M/27</td>
<td>89</td>
<td>EI</td>
</tr>
<tr>
<td>7</td>
<td>8344 A→G</td>
<td>M/30</td>
<td>86</td>
<td>MERRF, encephalopathy, ataxia, EI</td>
</tr>
<tr>
<td>8</td>
<td>3243 A→G</td>
<td>M/46</td>
<td>97</td>
<td>MELAS, migraine, growth retardation, hearing impairment, EI</td>
</tr>
<tr>
<td>9</td>
<td>3243 A→G</td>
<td>F/40</td>
<td>84</td>
<td>MIDD, EI</td>
</tr>
<tr>
<td>10</td>
<td>3243 A→G</td>
<td>M/40</td>
<td>60</td>
<td>MIDD, EI</td>
</tr>
<tr>
<td>11</td>
<td>3243 A→G</td>
<td>M/36</td>
<td>69</td>
<td>MIDD</td>
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<tr>
<td>12</td>
<td>3243 A→G</td>
<td>F/40</td>
<td>73</td>
<td>MIDD, encephalopathy, growth retardation, EI</td>
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<tr>
<td>13</td>
<td>3243 A→G</td>
<td>F/42</td>
<td>84</td>
<td>MIDD, encephalopathy, EI</td>
</tr>
<tr>
<td>14</td>
<td>3243 A→G</td>
<td>M/34</td>
<td>75</td>
<td>MIDD, encephalopathy, EI</td>
</tr>
<tr>
<td>15</td>
<td>3243 A→G</td>
<td>M/57</td>
<td>53</td>
<td>MIDD, EI</td>
</tr>
<tr>
<td>16</td>
<td>3243 A→G</td>
<td>M/36</td>
<td>27</td>
<td>CU</td>
</tr>
<tr>
<td>17</td>
<td>3243 A→G</td>
<td>F/40</td>
<td>2</td>
<td>CU</td>
</tr>
<tr>
<td>18</td>
<td>3243 A→G</td>
<td>F/42</td>
<td>2</td>
<td>CU</td>
</tr>
<tr>
<td>19</td>
<td>3243 A→G</td>
<td>F/36</td>
<td>37</td>
<td>CU</td>
</tr>
<tr>
<td>20</td>
<td>3243 A→G</td>
<td>F/53</td>
<td>76</td>
<td>Hearing impairment, EI</td>
</tr>
</tbody>
</table>

EI = exercise intolerance; CU = clinically unaffected; MIDD = maternally inherited deafness and diabetes mellitus; MERRF = myoclonic epilepsy and ragged red fibres; MELAS = mitochondrial, encephalopathy, lactic acidosis and stroke-like episodes.

In subject nos 1–4 the numbers in row 2 show the deletion sites (base pair number) of mtDNA. Deletion sites were not determined in subject no. 5.

*This mtDNA mutation has been described in Schwartz et al. (2002).
corresponding to 65–75% of maximal oxygen uptake (VO$_{2\text{max}}$). To establish HR zones for training, all subjects carried out an incremental cycle test to exhaustion at our laboratory, to determine VO$_{2\text{max}}$ and maximal workload (W$_{\text{max}}$). The subjects rested for 1 h, and then performed a 15 min cycle test at 70% of VO$_{2\text{max}}$ to identify the target HR for training. The subjects used a pulse watch (Accurex, Polar, Finland) during training sessions. The watch was programmed with the individual’s HR zones and provided both visual and auditory feedback to the subjects, and also recorded date, duration and HR throughout exercise. Plasma creatine kinase (CK) levels and pulse watch data were controlled at the time points shown in Fig. 1.

**Deconditioning phase**

The 13 persons with mtDNA mutations recruited last for the study (subject nos 1, 7, 9–19 in Table 1) were asked to stop any kind of physical training for 8 weeks at the end of the 12 week training period.

**Evaluations**

The following evaluations were assessed at the time points shown in Fig. 1:

- **VO$_{2\text{max}}$ and plasma lactate and CK**: Determination of VO$_{2\text{max}}$ and peak exercise-induced plasma lactate levels was carried out as described previously (Jeppesen et al., 2003).

- **Muscle histology**: Needle biopsies were performed in the vastus lateralis muscle as described previously (Jeppesen et al., 2003). The number of ragged red fibres (RRF), cytochrome oxidase (COX)-negative fibres, centrally nucleated fibres, apoptotic nuclei and the muscle fibre size and -type and capillary density were assessed using 10 µm frozen serial sections stained with trichrome, cytochrome oxidase, haematoxylin and eosin, ATPase at pH 4.3 and 10.6, and ULEX. Myofibre nuclei were considered apoptotic if they stained positive in 4′,6-diamidino-2-phenylindole-dihydrochloride (DAPI), terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) and poly (ADP-ribose) polymerase (PARP) triple staining. Entire sections (500–3500 fibres) were evaluated for all variables, except muscle fibre size and capillary density analyses (140–220 fibres) that required a perfect cross-section orientation of the biopsy. A computer imaging analysis software was used for the assessment of muscle fibre size and type and capillary density analyses (TEMA, CheckVision, Denmark). A single investigator, blinded to the training regimen, performed all analyses.

- **Histological analysis**: Could not be performed in one patient due to too small sample size, and in 7 out of the 13 healthy subjects, due to freeze artefacts, because the muscle biopsies by error were frozen directly in liquid nitrogen.

- **Quantification of mutated mtDNA**: For deletions, the mutation levels were determined by Southern blot analysis after digestion by BamHI and using PCR-amplified total mtDNA as a probe. The blots were autoradiographed and the relative intensity of the bands corresponding to normal size mtDNA and mtDNA with deletion was determined using the Gelbase/Gelblot software from UVP, Cambridge, UK. Intra-assay variability was <10%.

For point mutations, the levels were determined by solid-phase minisequencing (Suomalainen et al., 1998). Using known proportions of cloned mt3243A and mt3243G we determined the sensitivity of the method to 1%. The standard error was 10%.

- **mtDNA quantification**: mtDNA quantification was performed using real-time quantitative PCR (SDS7000, Applied Biosystems) amplifying short segments of 28S rDNA and ND1 mtDNA (from 3485 to 3553) separately using the SYBR TaqMan master mix. The two real-time PCRs (mtDNA and nDNA) were initially shown to have similar amplification efficiency within the range of expected DNA concentration, allowing the use of the ΔΔCT method for calculating the relative amounts (Applied Biosystems, User Bulletin 2, primer sequence and conditions are available on request). Each target sequence was analysed in triplicate, at least three times for each sample. The intra-assay measurements varied only 10–15%, whereas inter-assay measurements varied up to 2-fold. Relative mtDNA content between the individuals varied up to 3-fold. Measurements were performed in all persons with mtDNA mutation in skeletal muscle and in 11 out of the 13 healthy subjects pre- and post-training. Due to small sample size, the analyses were not possible in two of the healthy subjects. In the persons who had carried out the deconditioning programme, mtDNA was also quantified after deconditioning.

- **Biochemical investigations**: Mitochondrial enzyme activities of citrate synthase and complexes I–IV were measured in post-nuclear supernatants of 30 µg frozen muscle as described previously (Wibrand et al., 2001). Analyses were performed in all persons with mtDNA mutations and in 11 out of the 13 healthy subjects, due to small sample size in 2.

**Self-reported changes in daily activities**

After the training period, all 20 persons with mutations of mtDNA were asked to fill out a questionnaire where they were asked to grade changes with training as worse, unchanged or improved for the statements shown in Fig. 2.

**Statistical analysis**

Differences among groups were evaluated by an unpaired Student’s t-test, and training-induced changes were evaluated by a paired Student’s t-test. P < 0.05 was considered statistically significant. All values are expressed as mean ± SD.
Results

Training and deconditioning: compliance and effect on VO$_{2\text{max}}$, maximal workload, plasma lactate, heart rate and indicators of daily living

Compliance to training was based on recorded data from the pulse watches. The 20 persons with mtDNA mutations and 13 healthy subjects who completed the study had no adverse effect during training, and performed 93 and 89% of the 50 training sessions.

Training improved VO$_{2\text{max}}$ and W$_{\text{max}}$ by 26 and 29% in the persons carrying mtDNA mutation ($P < 0.001$; Fig. 3), while peak exercise increases in plasma lactate and HR were similar (Table 2). The group of healthy subjects had an improvement in VO$_{2\text{max}}$ and W$_{\text{max}}$ of 17 and 18%, which was lower than the improvement found in the patients ($P < 0.05$; Fig. 3). The 13 persons with mtDNA mutations, who completed 8 weeks of deconditioning following training, had a training-induced improvement of VO$_{2\text{max}}$ and W$_{\text{max}}$ of 31 and 35%. After 8 weeks of deconditioning, VO$_{2\text{max}}$ and W$_{\text{max}}$ decreased by 24 and 26%, and thus returned to pre-training values.

All persons with mtDNA mutations in muscle reported improvements of one or more daily activities (Fig. 2), while none reported worsening in any of the items.

Training and deconditioning: effect on mtDNA mutation load in skeletal muscle

The pre-training percentage of mtDNA mutation load in skeletal muscle was 55 ± 28% (Fig. 4A). There was no significant change in the mtDNA mutation load in skeletal muscle after 12 weeks of training (change = −1 ± 4%). In the 13 persons participating in the deconditioning programme, mtDNA mutation load in muscle did not change with training, but the mtDNA mutation load after deconditioning decreased by 3 ± 4% ($P < 0.01$; Fig. 4B).

There was a training-induced increase in the muscle mtDNA quantity in persons with mtDNA mutations (change = 81 ± 124%; $P < 0.05$; Fig. 5A and B), whereas mtDNA copy number was unchanged in healthy subjects (change = −6 ± 49%; Fig. 5C and D). After 8 weeks of deconditioning, mtDNA content returned to pre-training values in the persons with a training-induced increase in mtDNA (Fig. 5A and B). The magnitude of the training-induced change in mtDNA copy number did not correlate with the pre-training levels of mtDNA or training-induced changes in VO$_{2\text{max}}$ in all studied subjects.

Fig. 2 Training-induced changes in walking distance, muscle strength, endurance, level of physical activity and fatigue in the 20 persons with mtDNA mutations. The numbers written in the black and white bar represent the number of persons who had no change (black bar) or improvement (white bar) with training. No person reported worsening with training and the persons who reported no change were equally distributed between non-symptomatic and symptomatic persons.

Training and deconditioning: effect on mitochondrial enzyme activities in skeletal muscle

Before training, citrate synthase activity, complexes I–IV or citrate synthase-corrected complexes I–IV activities in muscle did not differ between the group of persons with mtDNA mutations and healthy subjects, but citrate synthase-corrected complexes I–IV activities were lower in the group with single, large-scale deletions of mtDNA compared to healthy subjects ($P < 0.01$; data not shown). There was a training-induced increase in the citrate synthase activity in the persons with mtDNA mutations (change = 115 ± 76 mU/mg protein) and healthy subjects (change = 120 ± 57 mU/mg protein) (Table 3 and Fig. 6). Absolute activities of complexes I–IV were increased by training in both persons with mtDNA mutation and healthy subjects (data not shown), but when corrected for citrate synthase, complexes I and IV activities only tended to increase in the group of persons with mtDNA mutations. After 8 weeks of deconditioning, citrate synthase activity and complexes I–IV activities returned to pre-training values. There was no correlation between the change in citrate synthase or citrate synthase-corrected complex activities and the training-induced change in mtDNA copy number did not correlate with the pre-training levels of mtDNA or training-induced changes in VO$_{2\text{max}}$ in all studied subjects.

Training and deconditioning: effect on muscle morphology and plasma CK levels

Fibre type composition and size did not change significantly with training or deconditioning in any of the subjects, although there was a trend towards an increase in fibre size with training in all groups (Table 4). Number of capillaries per fibre and the capillary density remained unchanged in healthy subjects, but decreased with training in the 20 persons with mtDNA mutations and increased to pre-training values with deconditioning. Before training, 13 persons (subject nos 1–13; Table 1) had central nuclei.
Fig. 3 Maximal workload (A) and oxygen uptake (B) before and after 12 weeks of training and training-induced increases in VO$_{2\text{max}}$ (C) in 20 persons with mutations of mtDNA and in 13 healthy subjects. Each symbol in C represents a person: closed triangles upwards = persons with the mtDNA 3243 A→G mutation; closed triangle downwards = person with the mtDNA 8344 A→G mutation; closed circle = person with microdeletion of the mtDNA ND2 gene; closed square = persons with single large-scale deletions of mtDNA; and open square = healthy subjects; closed (black) symbols represents patients with one or more clinical signs of mitochondrial disease; open (white) symbols represent clinically unaffected persons with or without mutation of mtDNA. * Denotes change with training ($P < 0.05$). § Denotes difference in VO$_{2\text{max}}$ responses to training between persons with mtDNA mutations and healthy subjects ($P < 0.05$).

Table 2 Peak exercise-induced plasma lactate and peak exercise HR

<table>
<thead>
<tr>
<th></th>
<th>Persons with mitochondrial DNA mutations ($n = 20$)</th>
<th>Healthy subjects ($n = 13$)</th>
<th>Persons with mitochondrial DNA mutations ($n = 13$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before training After training</td>
<td>Before training After training</td>
<td>Before training After training</td>
</tr>
<tr>
<td>Peak exercise plasma lactate (mmol/l)</td>
<td>8.7 ± 3.2 9.0 ± 3.0</td>
<td>8.1 ± 2.8 10.4 ± 2.8</td>
<td>8.3 ± 3.2 8.9 ± 3.2</td>
</tr>
<tr>
<td>Peak exercise heart rate (beats/min)</td>
<td>166 ± 18 165 ± 18</td>
<td>180 ± 13 177 ± 13</td>
<td>171 ± 17 171 ± 18</td>
</tr>
</tbody>
</table>

The values were measured in 20 persons with mtDNA mutations in muscle and in 13 healthy subjects before and after 3 months of training and in 13 of the persons with mtDNA mutations who also performed 8 weeks of deconditioning following training.
Fig. 4 (A) Mutation load in muscle of persons carrying mutations of mtDNA, before and after 12 weeks of training (n = 20) and after 8 weeks of deconditioning (n = 13). (B) Percentage change in mtDNA mutation load with training and deconditioning in the same subjects. Each symbol in B represents a person; closed triangles upwards = persons with the mtDNA 3243 A→G mutation; closed triangle downwards = person with the mtDNA 8344 A→G mutation; closed circle = person with microdeletion of the mtDNA ND2 gene; closed square = persons with single large-scale deletions of mtDNA; and open square = healthy subjects; closed (black) symbols represent patients with one or more clinical signs of mitochondrial disease; open (white) symbols represent clinically unaffected persons with or without mutation of mtDNA. * Denotes significant change from pre-training (P < 0.05). The drop in mutation load after deconditioning was significantly different from both pre-training and immediately post-training values (P < 0.05).

Fig. 5 Graphs A and C show the ratio between mitochondrial DNA and nuclear DNA (mtDNA/nDNA) and graphs B and D show the percentage change in mtDNA/nDNA from pre-training in skeletal muscle in persons carrying mutations of mtDNA (A, B) and healthy subjects (C, D). Pre-training and post-training values are based on 20 persons with mtDNA mutations and 11 healthy subjects, who trained for 12 weeks, and values after 8 weeks of deconditioning are based on 13 persons with mtDNA mutations. Each symbol represents a person; closed triangles upwards = persons with the mtDNA 3243 A→G mutation; closed triangle downwards = person with the mtDNA 8344 A→G mutation; closed circle = person with microdeletion of the mtDNA ND2 gene; closed square = persons with single large-scale deletions of mtDNA; and open square = healthy subjects; closed (black) symbols represent patients with one or more clinical signs of mitochondrial disease; open (white) symbols represent clinically unaffected persons with or without mutation of mtDNA. * Denotes significant change from pre-training (P < 0.05).
and ragged red and/or COX-negative fibres on muscle biopsy (Fig. 7), but the numbers did not change with training or deconditioning.

Eight persons (subject nos 1–8; Table 1) had apoptotic nuclei in myocytes before training. The number of apoptotic nuclei did not change with training or deconditioning.

Plasma CK levels were increased in three patients before start of training. There were no training-induced increases in plasma CK levels in the 20 persons carrying mtDNA mutation (before training $= 245 \pm 270$; training at 8 weeks $= 239 \pm 251$; training at 12 weeks $= 299 \pm 345$).

**Discussion**

Exercise intolerance is a very common symptom in patients with mtDNA defects, which seriously interferes with the performance of normal daily activities such as walking stairs and cycling. In healthy subjects, VO$_{2\text{max}}$ is primarily limited...
### Table 4 Pre- and post-training histological findings in skeletal muscle

<table>
<thead>
<tr>
<th></th>
<th>Persons with mtDNA mutations</th>
<th>Healthy subjects</th>
<th>Persons with mtDNA mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before training</td>
<td>After training</td>
<td>n</td>
</tr>
<tr>
<td>Ragged red fibres (%)</td>
<td>5.7 ± 7.9</td>
<td>4.1 ± 5.4</td>
<td>19</td>
</tr>
<tr>
<td>COX-negative fibres (%)</td>
<td>3.8 ± 6.1</td>
<td>3.5 ± 5.9</td>
<td>19</td>
</tr>
<tr>
<td>Fibre type I (%)</td>
<td>55 ± 19</td>
<td>50 ± 15</td>
<td>19</td>
</tr>
<tr>
<td>Fibre type II (%)</td>
<td>45 ± 19</td>
<td>50 ± 15</td>
<td>19</td>
</tr>
<tr>
<td>Fibre size type I (µm²)</td>
<td>4431 ± 1406</td>
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</tr>
<tr>
<td>Fibre size type II (µm²)</td>
<td>4287 ± 2107</td>
<td>4857 ± 2475</td>
<td>19</td>
</tr>
<tr>
<td>Capillary density (per fibre)</td>
<td>1.9 ± 0.9</td>
<td>1.6 ± 0.5*</td>
<td>19</td>
</tr>
<tr>
<td>Capillary density (mm²)</td>
<td>384 ± 144</td>
<td>298 ± 85*</td>
<td>19</td>
</tr>
<tr>
<td>Central nuclei (%)</td>
<td>4.2 ± 7.9</td>
<td>7.3 ± 13.3</td>
<td>19</td>
</tr>
<tr>
<td>Apoptotic nuclei (%)</td>
<td>0.5 ± 0.3</td>
<td>1.1 ± 1.4</td>
<td>19</td>
</tr>
</tbody>
</table>

Findings in skeletal muscle of 20 persons with mtDNA mutations in muscle, in 13 healthy subjects and in 13 of the persons with mtDNA mutations who also performed 8 weeks of deconditioning following training.

*n* = the number of persons studied.

* Denotes a difference between pre- and post-training.

**Fig. 7** Skeletal muscle biopsy obtained from a patient with a single, large-scale deletion of mtDNA after 12 weeks of training and stained with (1) a. Merosin, b. TUNEL, c. PARP and d. merged pictures of a–c; (2) haematoxylin and eosin; (3) trichrome; and (4) combined cytochrome oxidase and succinate dehydrogenase staining. The patient has numerous apoptotic nuclei [picture (1) a–d], central nuclei (2), ragged red (3) and COX-negative fibres (4).
by cardiopulmonary capacity (Rowell et al., 1986), but in patients with MM, exercise capacity is primarily limited by the muscle’s capacity to extract and use oxygen. It is well known that regular exercise training of healthy muscle dramatically improves mitochondrial enzyme capacity (Gollnick et al., 1982). It can therefore be speculated that widening of the biochemical bottleneck in MMs by regular exercise training, as also evidenced by the biochemical findings of the present study, could help to alleviate exercise intolerance in these conditions.

In the present study, aerobic training for 12 weeks significantly improved maximal oxidative capacity in 20 persons with four different mtDNA mutation types and a variety of mutant loads. While this improvement may not have a significant impact on activities of daily living in asymptomatic carriers of mtDNA mutations, the improvement in VO_{2max} translates into clinically meaningful effects in patients with severe oxidative defects, as also reflected in the questionnaire (Fig. 2). The improved maximal work rates and VO_{2max} were not caused by the investigator or the subjects pushing the max-test harder after the training programme. This is so, because the level of physical exertion is directly reflected by the peak heart rate and plasma lactate response to exercise, and all subjects reached similar heart rates and lactate levels during max-tests before and after the training programme. The mtDNA mutation load in muscle, plasma CK levels and the level of muscle regeneration and apoptosis did not change with training. This indicates that 3 months of endurance training efficiently improves oxidative capacity in patients with MM and that short-term training appears to be safe in these patients.

Using aerobic training as therapy for mitochondrial myopathies has only been studied in small studies with no control groups for comparison, and only one study assessed the molecular genetic consequences of training (Taivassalo et al., 2001). In that study, subjects carried out a training programme similar to the one used in the present study. The finding of an increase in the mutant load in muscle by 9% raised serious concerns as to whether aerobic training should be recommended as therapy for MM. The increase in mutant load, however, was to a great extent driven by a large increase in just one patient. Without this patient, the average increase in five out of eight patients was only 5%. Considering that a number of the patients included had single, large-scale deletions, associated with an error of mutant load detection of no better than 10%, it is uncertain if the reported increase in mutation load reflects a true change. In contrast, we found no training-induced increase in the mutation load in muscle irrespective of mutation type, even though our study was designed to favour an increase by the inclusion of persons with low levels of mutant loads in muscle. In this way, a potential ceiling-off effect in persons with high mutation loads in muscle was avoided.

Studies investigating the effect of endurance training have found that a training programme of 10–14 weeks of moderate intensive exercise, similar to the programme used in the present study, is sufficient to obtain significant cardiovascular and morphological, molecular and biochemical changes in the trained muscles (Fischer et al., 2004; Hoppeler et al., 1973; Orngreen et al., 2005; Olsen et al., 2005; Rodriguez et al., 2002; Turner et al., 1997). When investigating muscle biopsies with EM after a 10–14 week training programme, mitochondrial volume increases by 40–70% in healthy skeletal muscle (Hoppeler et al., 1973; Rodriguez et al., 2002; Turner et al., 1997). It is unclear whether this adaptation is coupled to an increase in mtDNA copies.

The influence of exercise on mtDNA copy numbers has only been studied once with findings of a temporary decrease of mtDNA molecules in muscle of healthy subjects after acute, very intense exercise (Marcuello et al., 2005). In the present study, training did not change the quantity of muscle mtDNA in healthy subjects and half of the persons with mtDNA mutations, although mitochondrial enzyme activity increased almost 2-fold. The increases in mitochondrial enzyme activity in subjects with unchanged mtDNA levels could be a result of a training-induced increase in mtDNA transcription or mitochondrial mRNA translation. A similar training-induced increase in protein expression in the face of unchanged DNA levels is well known for nuclear genes (Fischer et al., 2004). The mechanism underlying the significant decrease in skeletal muscle mutation load with deconditioning is uncertain. The decrease in mutation load did not correlate with changes in mtDNA copy number.

Changes in mtDNA molecule number could facilitate shifts in mutant load, if the mutation was associated with a replicative advantage, which has been suggested as a mechanism for expansion of mutant over wild-type mtDNA (Chinnery and Samuels, 1999; Larsson et al., 1990; Weber et al., 1997). The persons with training-induced increases in mtDNA copies, however, did not have higher levels of mutant load in muscle versus the persons with no change, and all had similar training-induced responses in mutant load and respiratory chain enzyme activities compared to persons with stable mtDNA content in muscle. Thus, it appears that mutant mtDNA in persons with the common 3243 A→G point mutation and single, large-scale deletion of mtDNA does not have a replicative advantage over wild-type mtDNA if the overall mtDNA pool is increased with training.

Even sedentary persons are exposed to repeated exertional episodes of skeletal muscle over time. Thus, if mutation load should increase with exercise in muscle of MM patients, the level of heteroplasmy should increase with age. No prospective study has looked at this, but a cross-sectional study of a large cohort of patients with the common 3243 A→G mutation indicates that mutation load in muscle does not change with time (Frederiksen et al., 2006). This study supports the findings of the present study that mtDNA mutation load is not increased by training or deconditioning in patients with the common 3243 A→G mutation.

Mitochondrial myopathies are generally not considered as muscular dystrophies, even though muscle weakness,
atrophy and dystrophic changes on muscle biopsy can be seen in these patients (Olsen et al., 2003). The ongoing destruction of muscle in muscular dystrophies enhances muscle regeneration, which eventually may exhaust the pool of regenerative stem cells, the satellite cells, and result in accelerated loss of musculature (Ansved et al., 2001). Since training may increase muscle regeneration, we assessed the number of centrally nucleated fibres in muscle, and monitored plasma CK levels, which both are indices of muscle damage (Jones et al., 1986). In the present study, all patients with deletions and seven patients carrying point mutation of mtDNA had centrally nucleated fibres on muscle biopsy pre-training. Twelve weeks of training did not increase the number of centrally nucleated fibres or plasma CK levels in these patients, indicating that there was no induction of muscle damage in any of the patients recruited in the study.

It is known that sporadic cases of MM caused by mtDNA mutations generally have no mutation in the satellite cells (Shoubridge et al., 1997; Taivassalo et al., 1999b). Induction of muscle injury has therefore been suggested as a potential therapy form to lower the mutant load of skeletal muscle, even though this potentially may exhaust the satellite cell pool (Shoubridge et al., 1997; Taivassalo et al., 1999b). Since we found no change with training in centrally nucleated fibres or in mutant load in the sporadic cases of mitochondrial disease (subject nos 1–6 in Table 1), lowering of mutant loads in muscle of this subgroup is therefore not feasible with endurance training, but may be achieved by eccentric exercise or heavy strength training. For the same reason, the interesting finding of a small but significant decrease in muscle mutant load after 2 months of deconditioning cannot be explained by recruitment of satellite cells with lower or absent levels of the mtDNA mutation. Although a positive result, we are uncertain about the importance of the drop in mutant load with deconditioning which warrants further studies.

We have previously shown that 12 weeks of aerobic training does not change the capillary density in patients with different neuromuscular disorders or in healthy subjects (Olsen et al., 2005; Orngreen et al., 2005). In the present study, the persons with mtDNA mutation in skeletal muscle had a training-induced decrease in capillary density. The increase in fibre size in these persons partly explains this decrease, but capillaries per fibre also decreased. The drop in capillary density with training did not correlate with the level of mtDNA mutation in skeletal muscle, the specific mtDNA mutation type or the changes in VO2max and citrate synthase activity. The mechanism underlying the drop in capillary density with training in persons carrying mtDNA mutations is unknown, but could be linked to angiopathy as previously suggested in mitochondrial disorders (Iizuka et al., 2005).

Dysfunction of mitochondrial metabolism has been linked to programmed cell death and, apoptosis, and studies have found a direct association between COX-deficiency and apoptotic fibres (Giovanni et al., 2001; Mirabella et al., 2000; Umaki et al., 2002), which was also found in this study. During training, there is an increased energy demand, which theoretically could increase the oxidative stress and free radical production leading to increased apoptosis in patients with mitochondrial disease. However, in this study, muscle apoptosis did not increase with endurance training.

The majority of persons with mtDNA mutations included in our study had the 3243 A→G point mutation or single large-scale deletions of mtDNA. Only two patients had mtDNA mutations different from these common mtDNA mutations. While there was no increase in mtDNA mutation load in skeletal muscle in the persons with the 3243 A→G mutation or deletions of mtDNA, the patient with the 8344 A→G mutation had an increase of 4% after deconditioning. It is uncertain whether this increase reflects variation in training/deconditioning responses among patients with different mtDNA mutations.

This study has documented clinically meaningful effects of aerobic training in patients with MM that are coupled to a wide range of clinical, morphological, biochemical and molecular genetic safety markers that indicate no adverse effects of training. The findings suggest that aerobic training should be encouraged as a therapy for patients with the 3243 A→G mutation and single large-scale deletions of mtDNA. Further studies are warranted to assess the validity of aerobic training in other mitochondrial disorders, and to assess the validity of long-term aerobic training.

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