Metabolic and Muscle Adaptation to Aerobic Training in Patients Affected by Chronic Progressive External Ophthalmoplegia

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Abstract

Mitochondrial myopathies with respiratory chain defects are multisystem diseases characterised by mitochondrial DNA (mtDNA) mutations responsible for impairment of aerobic cell metabolism. At the skeletal muscle level involvement of respiratory chain function is the cause of insufficient ATP production and deranged metabolism, a main effect of which is represented by abnormal production of lactate.

Recently, it has been reported beneficial effects of aerobic training on muscle performance in mitochondrial myopathy subjects. Aim of this study was to evaluate in 9 patients affected by chronic progressive external ophthalmoplegia (CPEO) and large-scale mtDNA rearrangements functional adaptation of skeletal muscle to aerobic training, and to relate it to muscle biopsies parameters assessed before the training. To this purpose, CPEO patients underwent an exercise test performed at the anaerobic lactate threshold before and after a supervised 10-week course of aerobic training.

A correlation was found between the training-related decrement in exercise peak lactate and cytochrome c oxidase (COX) enzyme activity (inverse correlation, r=-0.81, p<0.05), and with the number of COX- (r=0.72, p<0.05) and ragged red fibers (r=0.64, p=0.05). On the contrary, no relation was found with the amount of deleted mtDNA in muscle biopsy.

These results indicate that aerobic training can be beneficial also in those CPEO patients more severely affected by mitochondrial dysfunction. The level of COX activity in muscle biopsy rather than the amount of mutated mtDNA seems to be a useful predictor for the effectiveness of aerobic training program, suggesting some gene expression mechanisms in mediating muscle adaptation to training itself in these patients.

Key words: aerobic training, lactate, mitochondrial diseases, muscle biopsy.

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Mutations in mitochondrial DNA (mtDNA) are the most frequent causes of mitochondrial myopathy in adults [4]. In chronic progressive external ophthalmoplegia (CPEO), a distinct form of mitochondrial (encephalo)myopathy, mutant and wild-type mtDNAs co-exist, a condition referred to as mtDNA heteroplasmia; however the relative frequency of each species varies widely in different cells and tissues of a given patient [9]. This condition is one of the cardinal feature of mitochondrial diseases, contributing to explain their clinical heterogeneity. At skeletal muscle level, exercise intolerance revealed by symptoms such as cramps, myalgia, and fatigueability is commonly present in CPEO patients. The failure of the aerobic metabolism is in fact responsible for the precocious activation of anaerobic metabolism, as showed by the anaerobic lactate threshold (LT), a critical point at which metabolic modifications bring about the energy-demand transition form aerobic to anaerobic exercise [2].

Some authors including ourselves have demonstrated that patients with mitochondrial myopathies can benefit from aerobic exercise training. The mechanisms underlying this adaptation of skeletal muscle to training are not understood, even if some events linked to gene shifting have been postulated [18].

In this study, we compared the responses to aerobic training in mitochondrial myopathy patients affected by CPEO with histological, biochemical and mtDNA data from muscle biopsy, with the aim to establish the rela-
tionships between the muscle adaptation to training and basic pathologic alterations in this disease.

Materials and Methods

Patients

The study was performed on a group of 8 female (mean age ± standard deviation: 64.1±7.1 yrs) and 1 male aged 23 yrs affected by CPEO and diagnosed on the grounds of clinical, family-history and muscle biopsy data (Table 1). Seven patients harbored single large-scale mtDNA deletions in muscle, one patient multiple deletions, while in the remaining one the mutation was unknown - mtDNA rearrangement and the most common MELAS and MERRF mtDNA point mutations having been excluded.

The clinical criteria for inclusion of these patients in the study were the following:

i. mild degree of skeletal myopathy, as established by an Activity of Daily Living score ≤ 2 for each item [15], MRC score ≥ 4 [1] and functional testing score ≤ 2 [6]. The patients could therefore manage an autonomous life and were deemed capable of performing the proposed test exercises;

ii. absence of cardiac or respiratory involvement, as assessed by means of ECG and cardiac ultrasound scans, chest X-ray and spirometry tests;

iii. absence of joint or bone deformities;

iv. body weight not exceeding 20% of the theoretical anthropometric value.

Patients were drug-free from at least 4 months before the time of the study. The exercise protocol was approved by our institution’s Committee on Human Experimentation. All subjects gave their informed consent after having been explained the purposes and procedures of the study.

Muscle biopsy

Muscle biopsy specimens, obtained from the left deltoid, were frozen in liquid nitrogen-chilled isopentane for routine histological and histochemical study and mtDNA analysis [5].

For each of five different histochemical fields, serial sections were studied for fiber-type characterization in a number of fibers ranging from 500 to 600 per case. Morphometric study was performed by a micrometric optical method at a magnification of 250x. In order to quantify skeletal muscle involvement, we calculated the following biopsy parameters:

- percentage of type 1 and 2 fibers;
- number of ragged red fibers (RRF) (% of type 1 fibers);
- number of cytochrome c oxidase – negative (COX-) and succinate dehydrogenase-positive fibers (SDH+) (% of type I fibers);
- COX activity spectrophotometrically assessed in isolated muscle mitochondria [10];
- percentage of deleted mtDNA determined by Southern blot and densitometry [19].

Exercise protocol

CPEO patients underwent to testing exercises before and after an aerobic training, performed at a constant, near-LT workload, previously determined [13].

Testing exercises

Assessments were made 3-4 h after a normal mixed-diet meal. The exercise consisted of 7 constant, near LT workload steps, each one lasting 3 minutes, interspaced by a 2-minute rest period.

Consecutive blood samples were collected from an antecubital vein under basal conditions, half-way through each resting period and during recovery at 1, 15 and 30 min after the end of exercise. Venous lactate levels (laboratory reference values < 1.5 mmol/L) were measured.

Table 1 Muscle biopsy and exercise peak lactate data in CPEO patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/Age</th>
<th>RRF</th>
<th>COX-</th>
<th>COX activity</th>
<th>Deleted mtDNA levels</th>
<th>DNA mutation</th>
<th>Peak lactate pre/post training</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/63</td>
<td>6</td>
<td>8</td>
<td>6.23</td>
<td>64</td>
<td>Single deletion</td>
<td>6.2 / 4.6</td>
</tr>
<tr>
<td>2</td>
<td>F/72</td>
<td>9</td>
<td>6</td>
<td>7.43</td>
<td>52</td>
<td>Single deletion</td>
<td>3.7 / 2.7</td>
</tr>
<tr>
<td>3</td>
<td>F/62</td>
<td>4</td>
<td>11</td>
<td>7.11</td>
<td>34</td>
<td>Single deletion</td>
<td>5.9 / 3.7</td>
</tr>
<tr>
<td>4</td>
<td>F/69</td>
<td>5</td>
<td>9</td>
<td>1.75</td>
<td>20</td>
<td>Single deletion</td>
<td>6.0 / 3.9</td>
</tr>
<tr>
<td>5</td>
<td>F/69</td>
<td>2</td>
<td>5</td>
<td>13.72</td>
<td>48</td>
<td>Single deletion</td>
<td>3.8 / 2.6</td>
</tr>
<tr>
<td>6</td>
<td>F/67</td>
<td>6</td>
<td>8</td>
<td>24.09</td>
<td>37</td>
<td>Single deletion</td>
<td>3.5 / 1.8</td>
</tr>
<tr>
<td>7</td>
<td>F/62</td>
<td>3</td>
<td>4</td>
<td>16.01</td>
<td>51</td>
<td>Single deletion</td>
<td>3.2 / 2.5</td>
</tr>
<tr>
<td>8</td>
<td>M/23</td>
<td>2</td>
<td>4</td>
<td>13.02</td>
<td>39</td>
<td>Multiple deletions</td>
<td>8.0 / 6.2</td>
</tr>
<tr>
<td>9</td>
<td>F/49</td>
<td>4</td>
<td>8</td>
<td>8.70</td>
<td>30</td>
<td>No mutation</td>
<td>4.9 / 3.5</td>
</tr>
</tbody>
</table>

1 % of ragged red fibers out of type 1 fibers; 2 % of COX negative type 1 fibers; 3 nanomoles of substrate utilised min-1 mg-1 of noncollagen proteins in isolated muscle mitochondria. Mean control values: 906±203; 4 percentage of deleted over total mtDNA; 5 peak lactate levels expressed in mmol/L.
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assessed spectrophotometrically on an ERIS Analyzer 6170 (Eppendorf Geratebau, Hamburg, Germany).

As the blood samples were drawn, heart rate, ventilation rate and capillary hemoglobin O₂ saturation, pO₂ and pCO₂ were recorded, as previously reported [14].

Aerobic exercise training regime

Patients underwent training in our laboratory. Although they were all familiar with the exercise equipment and operators, they were invited to relax comfortably in the exercise room for at least half an hour before the exercise.

Each patient underwent a 10-week aerobic training program consisting of supervised exercise every other day on an electrically braked pedal-rate bicycle ergometer (Ergocard III, Esaote Biomedica S.p.A.). The training schedule included 30 minutes’ exercise for the first 5 weeks and then 45 minutes’ exercise, interrupted half-way by 5-min rest intervals, for the remaining 5 weeks. As for testing exercises, exercise training was performed at constant near-LT workload, LT having been determined as previously reported [14] and expressed as the percentage of predicted normal maximum power output (pnPOmax). The pedaling rate was maintained constant through visual feedback at a value between 60 and 70 rpm. Serum CK were weekly determined, but it was not significantly increased in the subjects.

Four untrained healthy female (58.9±2.3 yrs) and one 24 yrs old male volunteers performed the same experimental protocol as CPEO patients.

Data analysis

Goodness-of-fit models, in terms of minimal square residuals, were utilized to fit all curves of lactate. After a Kolmogorov-Smirnov test confirmed that the data did not present a Gaussian distribution, non-parametric analysis was selected. In particular, the Wilcoxon test was utilised in order to estimate the differences before and after training, and the Spearman rank correlation test and a regression analysis to test relationships among blood and biopsy parameters. In all tests, we have considered a significance level of 0.5%.

Results

Muscle biopsy

A prevalence of type 1 fibers was found in 5 patients. The percentage of type 1 affected fibers (Table 1) ranged from 2 to 6% for RRF, from 4 to 11% for COX-, and from 5 to 18 for SDH+, respectively.

Testing exercise

While in the control subjects basal lactate values after training were similar to baseline, the patients exhibited a decrease: -14.2±1.7%, p non-significantly vs pre-training levels.

After training, exercise lactate levels was significantly reduced compared to pre-training condition since the first step of the exercise onward, in both CPEO patients and controls. (Fig. 1).

Peak lactate mean values (Fig. 1) was reduced after training: -38.2±2.10% vs. -30.9±1.9% in controls, p being borderline between the two groups.

In CPEO patients, at thirty minutes from the cessation of exercise, the post-training lactate levels decreased by 10.5% with respect to pre-training value (p non-
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significant). In normals, lactate levels reached baseline in both pre- and post-training conditions.

No significant modifications were observed after training as far as heart and respiratory rate, capillary haemoglobin O₂ saturation, pO₂ or pCO₂ were concerned in both patients and controls.

Statistical correlations

No relationships were observed between the post-training reduction in basal lactate and muscle biopsy parameters.

In CPEO patients, an inverse statistical correlation was observed between the post-training decrement in testing exercise peak lactate and the COX activity (r= -0.81, p<0.05) (Fig. 2, on the bottom), while a positive relationship was found with the number of COX- (r=0.72, p<0.05) (Fig. 2, on the top) and RRF fibers (r=0.64, p=0.05).

No association was detected between the lactate peak decrease and the percentage of deleted mtDNA.

Discussion

Chronic progressive external ophthalmoplegia is a distinct form of mitochondrial (encephalo)myopathy caused by a defect in mitochondrial respiratory chain. Patients affected by CPEO characteristically exhibit exercise intolerance, cramps, precocious fatigability and abnormal lactate kinetics during exercise.

To date, no effective pharmacological treatment of mitochondrial diseases has been found. Some researchers [12, 17] have demonstrated that patients with mitochondrial myopathies can benefit from aerobic exercise training. The present data confirm the decrease of lactate after aerobic training in CPEO patients. In fact we found that the decrement in mean lactate after training reached significance correspondingly at the first step of the exercise test and maintained such significance values up to cessation of exercise. Also basal and recovery values appeared reduced in the patients, even if lactate levels at thirty minutes after cessation of exercise were still significantly higher than baseline.

In healthy subjects the improvement of exercise response after aerobic training could be related to an increased anaerobic threshold mediated by several factors such as a better distribution of blood flow in trained muscles, increased oxidative capacity at the cellular level [3], and revertibility of fiber types [7]. In addition, in patients affected by mitochondrial myopathies, we have also to consider possible modifications of the percentage of deleted mtDNA and complementation between wild-type and deleted mtDNA.

The lactate decrease observed in our patients was not associated with the percentage of deleted mtDNA determined in muscle biopsy before the training. On the contrary, the extent of such a decrease appeared to be related to the number of COX- and RRF fibers as well as the extent of COX activity reduction observed in these patients in basal conditions. Although Shoubridge [11] and Goto [8] demonstrated that, in CPEO, RRF and COX- myofiber segments principally contained deleted mtDNA while mutated mtDNA was extremely rare in normal myofiber segments, the spatial distribution of normal vs enzyme defective areas along the myofiber appears the most important hallmark for functional expression of competent / incompetent mitochondrial population in the cell. Also the authors previously underlined [14] that the residual COX activity of isolated muscle is a good predictor for the abnormal precocious activation of anaerobic metabolism during incremental exercise. Surprisingly the present results indicate that one’s own the patients showing the lowest levels of COX activity were those which better can benefit from the aerobic training.

In conclusion, aerobic training seems to enable CPEO patients to improve lactate kinetics from exercising muscle. The mechanisms underlying this effect of the training are still unknown, even if events linked to some gene shifting have been postulated [18] in addition to the reversal of some of the consequences of chronic deconditioning [17].

The predictivity of skeletal muscle alterations for the positive effects of the training can not be considered conclusive at the present, but an investigative approach including repeated biopsies will further clarify the basic mechanisms of muscle adaptation to aerobic training in CPEO subjects.

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