The aerobic forearm exercise test, a non-invasive tool to screen for mitochondrial disorders

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Abstract
The diagnosis of a mitochondrial disorder is often difficult. Therefore, new approaches and diagnostic criteria are being developed. One of these tests is the aerobic forearm exercise test, a screening tool that can contribute to assess whether or not the patient suffers from a mitochondrial myopathy. With this simple, non-invasive test, the oxidative metabolism of muscle can be evaluated in rest and during exercise. We performed the aerobic forearm exercise test in patients with a mitochondrial disorder and an identified pathogenic gene mutation, in patients with a suspected mitochondrial disorder based on their clinical presentation and biochemical results, but without a molecular diagnosis, and in patients with atypical fatigue and no characteristics of a mitochondrial myopathy. In the first two groups, abnormal oxygen extraction from the blood during exercise was observed in four out of twelve patients. In the third group no abnormalities were found.

The number of patients that we could test so far was limited, but all the patients experienced the aerobic forearm exercise as an easy test. We would like to stimulate clinicians to perform this test whenever a mitochondrial myopathy is suspected, as it can be a valuable diagnostic screening tool.

Key words: Aerobic forearm exercise ; diagnosis ; mitochondrial disease ; muscle ; oxygen extraction.

Introduction
Mitochondrial disorders present a broad clinical spectrum, ranging from an isolated myopathy or cardiomyopathy to a multisystemic disease. This heterogeneity complicates making a correct diagnosis on clinical grounds only. Because a muscle biopsy is invasive for the patient, much effort is being put in the development of non-invasive techniques to evaluate the mitochondrial function, for instance by monitoring the oxidative phosphorylation during exercise using 31P-magnetic resonance spectroscopy (Barbiroli et al. 1995), near infrared spectroscopy (Chance et al. 1998), or cycle ergometry. However, these tests require sophisticated setups and expensive equipment that is not widely available. A good alternative is the aerobic forearm exercise test, introduced a few years ago as a sensitive tool to detect and quantify impaired muscle oxidative metabolism in patients with mitochondrial myopathies (Taivassalo et al. 2002 ; Jensen et al. 2002). The measurement of the partial pressure of oxygen (pO2) and the oxygen saturation level in venous blood at rest and during exercise, may reveal impaired oxygen use and provide evidence for an underlying mitochondrial defect. In normal subjects, the level of oxygen in venous blood decreases during exercise, because the mitochondria need oxygen for their aerobic energy metabolism and the muscles extract it from the blood. When the oxidative phosphorylation is blocked, the ability of muscle to increase the rate of extraction of oxygen from blood is impaired. As a result pO2 levels and oxygen saturation remain high during exercise because the increase in oxygen delivery by the circulation is higher than the oxygen use. Thus, by measuring blood gases and lactate before, during and after the exercise, an evaluation of the mitochondrial function can be made.

We recently introduced the aerobic forearm exercise test in our hospital. In this report we evaluate the results of the first 20 patients that performed the test.

Materials and methods

Patients
A total of 20 patients performed the aerobic forearm test, 9 males and 11 females, all between 14 and 51 years of age. The patients were subdivided in three groups : patients with a mitochondrial disorder and a well characterized genetic defect (group 1, n = 5), patients with a suspected mitochondrial disorder based on their phenotype and biochemical results, but without proven molecular defect (group 2, n = 7) and patients with an atypical fatigue syndrome or congenital muscular dystrophy, whose phenotype or biochemical results...
were not suggestive of a mitochondrial myopathy (group 3, n = 8) (Table 1).

**Biochemical and Molecular Analyses**

Spectrophotometric analyses to determine the enzymatic activity of the respiratory chain complexes were performed on skeletal muscle tissue as described previously (Van Coster et al., 2001).

Total DNA was isolated from lymphocytes and skeletal muscle tissue using standard procedures. The presence of common point mutations in mitochondrial tRNALeu and tRNALys genes was assessed by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), while the presence of the NARP m.8993T > G/C mutation and large DNA rearrangements was studied using Southern Blot analysis. In case all the 22 mitochondrial encoded tRNAs needed to be evaluated for the presence of a nucleotide alteration, the polymerase chain reaction and single stranded conformation polymorphism (PCR-SSCP) techniques were used. For analysis of the complete mitochondrial genome, denaturing High Performance Liquid Chromatography (dHPLC) in combination with the Mito screen assay kit (both from Transgenomic, Omaha, USA) was used (van den Bosch et al., 2000). All fragments showing aberrant migration patterns were subsequently sequenced using the BigDye Terminator v1.1 Cycle sequencing Kit sys-

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**Table 1**

Clinical, molecular and physiological characteristics of the patients with a proven mitochondrial disorder (1-5), suspected with a mitochondrial disorder (6-12) and atypical fatigue (13-20)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age(y)/gender</th>
<th>Symptoms</th>
<th>MVC (kg)</th>
<th>Biochemical analysis</th>
<th>Molecular analyses</th>
<th>Genetic % Mutant mtDNA</th>
<th>MU–LN</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>51f</td>
<td>moderate proximal weakness, fatigability</td>
<td>26</td>
<td>normal</td>
<td>common mutations</td>
<td>m.3251A &gt; G</td>
<td>90-50</td>
</tr>
<tr>
<td>2</td>
<td>28f</td>
<td>myoclonic epilepsy</td>
<td>42</td>
<td>nd</td>
<td>common mutations</td>
<td>m.3251A &gt; G</td>
<td>nd-50</td>
</tr>
<tr>
<td>3</td>
<td>51f</td>
<td>exercise intolerance and fatigue</td>
<td>27</td>
<td>normal</td>
<td>complete mtDNA</td>
<td>2.5kb deletion</td>
<td>15-0</td>
</tr>
<tr>
<td>4</td>
<td>47m</td>
<td>MELAS</td>
<td>33</td>
<td>nd</td>
<td>common mutations</td>
<td>m.3243A &gt; G</td>
<td>nd-40</td>
</tr>
<tr>
<td>5</td>
<td>38f</td>
<td>exercise intolerance, ptosis, RRF</td>
<td>35</td>
<td>nd</td>
<td>common mutations</td>
<td>m.16002T &gt; C</td>
<td>70-0</td>
</tr>
<tr>
<td>6</td>
<td>14m</td>
<td>proximal myopathy</td>
<td>7</td>
<td>normal</td>
<td>complete mtDNA</td>
<td>un</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>19m</td>
<td>MR, exercise intolerance</td>
<td>23</td>
<td>↓ cI and cIV</td>
<td>complete mtDNA</td>
<td>un</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>27f</td>
<td>CPEO</td>
<td>9</td>
<td>↓ cI and cIV</td>
<td>complete mtDNA</td>
<td>un</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>14m</td>
<td>exercise intolerance</td>
<td>12</td>
<td>↓ cI complete</td>
<td>mtDNA</td>
<td>un</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>15f</td>
<td>exercise intolerance, RRF</td>
<td>14</td>
<td>nd</td>
<td>common mutations</td>
<td>un</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>48f</td>
<td>dystonia</td>
<td>18</td>
<td>normal</td>
<td>complete mtDNA</td>
<td>un</td>
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<tr>
<td>12</td>
<td>29f</td>
<td>fatigability, ptosis</td>
<td>29</td>
<td>general ↓ of all enzymes</td>
<td>common mutations</td>
<td>un</td>
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<tr>
<td>13</td>
<td>20f</td>
<td>atypical fatigue</td>
<td>32</td>
<td>nd</td>
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<tr>
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<td>15m</td>
<td>congenital muscular dystrophy</td>
<td>5</td>
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<td>common mutations</td>
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<td>atypical fatigue</td>
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<td>un</td>
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<td>18</td>
<td>18f</td>
<td>atypical fatigue</td>
<td>26</td>
<td>nd</td>
<td>nd</td>
<td>un</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>19f</td>
<td>atypical fatigue</td>
<td>34</td>
<td>normal</td>
<td>complete mtDNA</td>
<td>un</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>18m</td>
<td>atypical fatigue</td>
<td>42</td>
<td>nd</td>
<td>nd</td>
<td>un</td>
<td></td>
</tr>
</tbody>
</table>

* MELAS : Mitochondrial Encephalomyopathy and Lactic Acidosis and Stroke like episodes ; MR : mental retardation ; CPEO : Chronic Progressive External Ophthalmplegia ; RRF : ragged red fibers.

* Maximal voluntary contraction.

* Respiratory chain enzymes were measured in muscle tissue ; nd = not determined.

* Molecular analyses on DNA isolated from muscle tissue and/or lymphocytes ; complete mtDNA = analysis of the entire mitochondrial genome ; common mutations = analyses of the most common deletions, the tRNA<sup>Leu</sup> and tRNA<sup>Lys</sup> genes and the NARP mutations ; nd = not determined.

* un = unknown.

* MU = DNA from muscle tissue ; LN = DNA from lymphocytes.
AERobic forearm exercise protocol

The exercise was always performed with the dominant arm. Thirty minutes before the start of the actual forearm exercise, the maximal voluntary contraction (MVC) was determined by taking the mean of three brief maximal handgrip efforts. For the exercise itself, patients performed a continuous submaximal, aerobic handgrip exercise consisting of one second of grip force alternating with one second of rest during three minutes. The target grip force was 40% of MVC, in order not to induce muscle stress. A specific software system (E-LinkH400) was linked to the precision handgrip dynamometer (Biometrics Ltd dynamometer and software, UK). This interactive evaluation system provided the exercised subjects motivation, and visual feedback on a computer screen allowed them to produce a steady effort and increased their compliance during the test.

BLOOD ANALYSES

All subjects had an intravenous catheter inserted in a median cubital vein of the exercised arm from which venous blood was drawn for analysis at rest, during the second minute of exercise, during the third minute of exercise, and 10 minutes post exercise. Samples for lactate were collected in a 2.7 ml sodium fluoride tube, and samples for pO2, partial pressure of CO2 (pCO2), oxygen saturation of hemoglobin and pH in a 1.5 ml heparinized syringe. The samples were taken with care to avoid air bubbles, mixed thoroughly and placed immediately on ice. Blood gases were determined on an ABL 735 blood gas analyzer (Radiometer, Denmark) within 20 minutes after collection. Plasma lactate was measured on a Vitros 950 AT analyzer (Ortho-Clinical Diagnostics Inc., USA).

STATISTICS

Differences with time (per group) were evaluated with a Student’s t-test, while differences between group means were evaluated using one-way analysis of variance. For both analyses, a p-value less than 0.05 was considered statistically significant.

RESULTS

The MVC was relatively low in all the patients, which could be expected since all the patients suffered from exercise intolerance and fatigue (Table 1). It has to be said that the mean age of the patients in group one is higher compared to group two and three (43 y versus 24 y and 17 y respectively). To our knowledge, the aerobic forearm exercise test has never been used in a large group of patients younger than 25 years old. Some of the younger patients had less strength than the older ones. However, it was already shown in the article of Hanisch (Hanisch et al. 2006) that less strength has no influence on the results of the test.

None of the patients complained about premature fatigue or unusual discomfort during the test. Because the exercise was monitored using a computer system, it was clear that all the patients performed the exercise at the correct intensity.

Looking at the mean values for pH, pO2, and oxygen saturation, a statistically significant decrease between the levels in rest and during exercise was seen in all groups (p < 0.05 except for pO2 in group 1). Moreover, in the same time difference, mean pCO2 and lactate levels showed a statistically significant increase in all groups (p < 0.05 except for lactate in group 1) (Table 2). Differences between group means were not statistically significant.

The first group consisted of five patients with a mitochondrial disorder caused by a well-characterized and pathogenic mutation in the mtDNA. Patients 1 and 2 (mother and daughter) showed a normal oxygen extraction pattern during exercise. The index patient of this family is a 23-year-old boy, suffering from progressive proximal myopathy with severe muscle pain. Because of the severity of his symptoms, he was not able to perform the aerobic forearm test. This boy had a very high mutant load in muscle tissue (98%) and showed severe complex I deficiency. Patient 3 had mild symptoms and a low mutation load, which correlates with the low normal oxygen extraction defect in this subject. Biochemical analyses were performed on muscle tissue of this patient, but no significant decrease, isolated or combined, of the activities of the oxidative phosphorylation complexes could be found. Patient 4 had a classical MELAS presentation, but without a very high mutation load. However, his pO2 and oxygen saturation level remained almost unchanged during the aerobic forearm exercise, clearly indicating a muscle extraction problem. No muscle tissue of this patient was available for biochemical analyses. Patient 5 was described previously (Seneca et al. 2000). Despite his severe symptoms and relatively high mutant load in muscle, no abnormal results were detected.

In the second group of patients (6 → 12) with a clinical and biochemically suspected mitochondrial disorder, patients 7 and 8 (brother and sister) showed an increase of pO2 and oxygen saturation during the exercise, suggesting a mitochondrial dysfunction. Patient 8 also showed a clearly increased lactate value at rest (4.7 mmol/L versus normal venous lactate at rest = ≤ 2.2 mmol/L). None of the other patients in this group nor in the
third group \((13 \rightarrow 20)\) with atypical fatigue showed an abnormal course of pO2 and oxygen saturation (Fig. 1).

**Discussion**

Over the years, a number of mitochondrial disorders were linked to specific clinical symptoms, like for example MELAS, MERRF or LHON presentation. However, for most patients, a battery of laboratory investigations is necessary to sustain a clinical diagnosis of mitochondrial dysfunction. To make this process less complicated, novel and sensitive screening techniques are being developed. The aerobic forearm exercise test is an example of such a new technique. For many years now, a diagnostic forearm test has been used to evaluate inborn errors of muscle glycolysis. The difference with the current forearm test is that in the original one, mainly used to diagnose McArdle disease, the exercise is performed anaerobically or ischemic, instead of aerobically. Although the test is not invasive and both sensitive and specific for the detection of muscle glycolysis disorders by measurement of the plasma ammonia and lactate levels, it was frequently experienced as unpleasant by the patients because of muscle cramps and pain in the exercised arm during the test (Kazemi-Esfarjani et al. 2002).

To evaluate patients with a (possible) mitochondrial myopathy, the group of Taivassalo developed the aerobic forearm test (Taivassalo et al. 2002). Using this test, the ability of muscle to extract more oxygen from blood during exercise than in rest, can be evaluated.

Several research groups demonstrated that patients with a mitochondrial dysfunction, often show impaired oxygen extraction during exercise (Taivassalo et al. 2002; Jensen et al. 2002; Jeppesen et al. 2007). However, the outcome of the test is said to be dependent on the level of muscle oxidative impairment rather than on the type of mutation, (Taivassalo et al. 2002) and thus the sensitivity of the test is less than 100%.

Looking at the results of the five patients with a mtDNA mutation, we found only two abnormal patterns of oxygen extraction. These two patients (patient 3 and 4) had respectively 15% of mutant mtDNA in muscle and 40% of mutant mtDNA in blood. This corresponded with the results described by Taivassalo et al. 2002 where patients with a low

<table>
<thead>
<tr>
<th>Group</th>
<th>pH Rest</th>
<th>pH Ex</th>
<th>pH Rec</th>
<th>pCO2 (mmHg) Rest</th>
<th>pCO2 (mmHg) Ex</th>
<th>pCO2 (mmHg) Rec</th>
<th>pO2 (mmHg) Rest</th>
<th>pO2 (mmHg) Ex</th>
<th>pO2 (mmHg) Rec</th>
<th>O2 saturation (%) Rest</th>
<th>O2 saturation (%) Ex</th>
<th>O2 saturation (%) Rec</th>
<th>Lactate (mmol/L) Rest</th>
<th>Lactate (mmol/L) Ex</th>
<th>Lactate (mmol/L) Rec</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.35</td>
<td>7.32</td>
<td>7.35</td>
<td>45.2</td>
<td>53.4</td>
<td>45.4</td>
<td>38.6</td>
<td>29.4</td>
<td>37.8</td>
<td>69.8</td>
<td>54.1</td>
<td>67.8</td>
<td>1.1</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>7.34</td>
<td>7.30</td>
<td>7.36</td>
<td>44.4</td>
<td>53.1</td>
<td>41.1</td>
<td>38.6</td>
<td>27.4</td>
<td>14.0</td>
<td>69.9</td>
<td>45.3</td>
<td>69.3</td>
<td>2.3</td>
<td>3.1</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>7.36</td>
<td>7.28</td>
<td>7.35</td>
<td>41.3</td>
<td>54.6</td>
<td>41.5</td>
<td>46.3</td>
<td>28.8</td>
<td>16.1</td>
<td>71.0</td>
<td>47.3</td>
<td>78.6</td>
<td>1.1</td>
<td>2.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Ex : after 1 minute of exercise ; Rec : after recovery ; SD : standard deviation.

\* different from rest, \( p < 0.01 \).

\* different from rest, \( 0.01 < p < 0.05 \).

![Fig. 1. — Difference in venous pO2 (A) and oxygen saturation level (B) between exercise and rest in patients with a mitochondrial disorder (group 1), patients with a suspected mitochondrial disorder (group 2), and patients with atypical fatigue. 3, 4, 7 and 8 refer to the patients in table 1.](image-url)
Several explanations are possible: the applied number of patients with normal lactate levels. However, patients 1 and 5 had a significant higher mutant load in muscle mtDNA, 90% and 70% respectively, but did not show an abnormal oxygen extraction pattern. Although the number of analysed patients was too low to draw final conclusions, these results may indicate that the aerobic forearm test is mutation specific.

In the group of patients with a suspected mitochondrial disorder based on their phenotype and biochemical results, two siblings turned out to have an increased PO2 and oxygen saturation level during exercise. Patient 8, who was the most severely affected from the two, also showed abnormal levels of pCO2 and lactate. She was diagnosed of having a mitochondrial myopathy with a clinical picture of chronic progressive external ophthalmoplegia. Her brother (patient 7), suffered from epilepsy and mental retardation since infancy, and started only recently at the age of 15 years to complain of exercise intolerance. All biological parameters such as lactate were normal, only the aerobic forearm exercise test was abnormal in this boy. A muscle biopsy revealed a deficiency in complex I and IV. Although these results clearly suggest a mitochondrial problem, so far no molecular defect has been identified, even after an extensive search, analyzing the whole mitochondrial genome by DHPLC and sequencing a number of important nuclear encoded mitochondrial genes. With the identification of new genes involved in the mitochondrial machinery, the molecular defect in these patients may eventually be found, but until then it can only be assumed that they suffer from a mitochondrial dysfunction. It cannot be excluded that other patients in group 2 have a mitochondrial disorder. Here as well, further molecular analysis is needed to reveal the true nature of their disease.

In the group of patients with atypical fatigue and/or exercise intolerance (group 3), no abnormal results were observed. However, there have been several reports of patients with mitochondrial dysfunction and exercise intolerance as a single symptom (Andreu et al. 1999; DiMauro et al. 2001), so it is important to test these patients too.

Although the number of patients in each group was limited, significant differences between rest and exercise could be detected for all the parameters, except for pO2 and lactate in group I. This means that indeed the pO2 decreased less during exercise in the group of patients with a pathogenic mutation, indicating an oxygen extraction problem, caused by a defect in the mitochondrial metabolism. Lactate did increase in group 1 during exercise, but not significantly. An elevated level of lactate is not specific for a mitochondrial disorder. As was already shown in other studies using different types of lactate stress testing, there are always a number of patients with normal lactate levels. Several explanations are possible: the applied workload is too low to reach the anaerobic threshold, especially after one minute of exercise; the specific mitochondrial defect of the patient does not go along with a lactate increase during slight exercise; the clearance rate of lactate from the serum is compensatory increased in some patients; or the muscle is only affected focally (Finsterer and Milvay 2004).

Compared to other investigations like MR spectroscopy, SATET, the lactate stress test and muscle analyses, the aerobic forearm exercise test is less invasive, less expansive and easier to perform. It was beyond the scope of this paper to evaluate the sensitivity of all these techniques. However, Hanisch (Hanisch et al. 2006) showed that measuring a lactate increase in cycle ergometry was as specific as the aerobic forearm test, but the sensitivity was moderate. Jeppesen (Jeppesen et al. 2007) showed that 31P MRS of skeletal muscle is not a sensitive test for mitochondrial disorders. The modified SATET, thoroughly evaluated by Hammaren in 2004 (Hammaren et al. 2004) showed a sensitivity of 78% and a specificity of 100% compared to normal controls, but can not discriminate between patients with a mitochondrial defect or another muscle disease.

In conclusion, the aerobic forearm exercise test can give important information on the oxidative metabolism in muscle. To determine the sensitivity and specificity of this test, the number of patients with a mitochondrial disorder confirmed by a molecular diagnosis should be increased. However, it can be very difficult to motivate patients to perform extra tests once the diagnosis is made. We would like to recommend all clinicians working with patients suffering from a mitochondrial disorder, with or without molecular diagnosis, to perform the aerobic forearm exercise test in their patients. The test is easy to perform and patients experience it as a pleasant test compared to other, more invasive procedures. However, for young children, below the age of ten, the handgrip dynamometer is too difficult to handle.

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