Part II: Innovative MR techniques
Chapter 8

Phosphorus-31 MR spectroscopy of skeletal muscle in maternally inherited diabetes and deafness A3243G mitochondrial mutation carriers

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ABSTRACT

Purpose
To investigate high-energy phosphate metabolism in striated skeletal muscle of patients with Maternally Inherited Diabetes and Deafness (MIDD) syndrome.

Materials and Methods
In 11 patients with the MIDD mutation (six with diabetes mellitus (DM) and five non-DM) and eight healthy subjects, phosphocreatine (PCr) and inorganic phosphate (Pi) in the vastus medialis muscle was measured immediately after exercise using 31P-magnetic resonance spectroscopy (MRS). The half-time of recovery (t1/2) of monoexponentially fitted (PCr+Pi)/PCr was calculated from spectra obtained every 4 seconds after cessation of exercise. A multiple linear regression model was used for statistical analysis.

Results
Patients with the MIDD mutation showed a significantly prolonged t1/2 (PCr+Pi)/PCr after exercise as compared to controls (13.6 ± 3.0 vs. 8.7 ± 1.3 sec, p = 0.01). No association between the presence of DM and t1/2 (PCr + Pi)/PCr was found (p = 0.382).

Conclusion
MIDD patients showed impaired mitochondrial oxidative phosphorylation in skeletal muscle shortly after exercise, irrespective of the presence of DM.
INTRODUCTION

The maternally inherited diabetes and deafness (MIDD) syndrome is known as a phenotype of the adenosine to guanine mutation at position 3243 (A3243G) in the tRNA gene (1,2). The key identifying features of MIDD patients are characterized by a triad of diabetes mellitus (DM), developing in 80% of patients with the MIDD mutation carriers, sensorineural deafness, and a history of these conditions in maternal relatives (3-6). Diagnosis of the MIDD syndrome is based on the pattern of inheritance, the presence of clinical features, and DNA analysis. Although MIDD is often unrecognized, it is estimated that MIDD affects between 0.6-1.5% of DM patients (7).

Besides the characteristic triad, MIDD patients may present with other symptoms such as renal disease, cardiomyopathy, gastrointestinal complaints, and muscle cramps or muscle weakness (3). A transition of adenosine to guanine at nucleotide position 3243 affects the encoding of mitochondrial proteins. This mutation causes the formation of dysfunctional mitochondria and subsequently reduced mitochondrial oxidative adenosinetriphosphate (ATP) energy production (4). The striated skeletal muscle depends largely on mitochondrial oxidative phosphorylation for generation of high-energy phosphates. Associations have been suggested between (sub)clinical myopathies and mitochondrial dysfunction in the MIDD syndrome (5,6).

Phosphorus-31 magnetic resonance spectroscopy (31P-MRS) is a sensitive and specific noninvasive method for the assessment of skeletal muscle mitochondrial ATP production. Observations at rest are not specific for mitochondrial disorders. During exercise patients with mitochondrial myopathies will display rapid phosphocreatine (PCr) depletion. During recovery, 31P-MRS measurements are the most sensitive and the most specific indices used to assess skeletal muscle mitochondrial ATP production (8-11). To our knowledge, only a limited number of MIDD patients have been studied by skeletal muscle 31P-MRS and the potential confounding effect of the presence of DM has not been systematically evaluated before (12-14). As the A3243G mutation potentially results in decreased oxidative phosphorylation and some MIDD patients show signs of muscle involvement, we hypothesized that alterations in phosphate energy metabolism of the striated muscles can be detected in these patients after exercise.

Accordingly, the objective of the current study was to investigate with 31P-MRS whether the presence of the A3243G mitochondrial mutation in the MIDD phenotype affects high-energy phosphate metabolism in the striated skeletal muscle of patients with the A3243G MIDD mutation. As alterations in phosphate metabolism in MIDD patients may be attributed to DM (15-19), we included both DM and non-DM MIDD patients.
MATERIALS AND METHODS

Study participants
The local medical ethics committee approved the study and informed consent was obtained from all participants prior to enrolment in the study.

A $^{31}$P-MRS examination was performed in 11 MIDD patients (five male; age 36 ± 10 years; six with DM and five non-DM) and eight healthy control subjects (five male; age 35 ± 8 years), matched for age, height, and body weight. Patients were recruited from the local MIDD database of the DM outpatient clinic of our institution. A standard oral glucose tolerance test (OGTT) was performed in the MIDD patient group - except for the DM patients - to differentiate between DM and patients with normal glucose tolerance (non-DM). The patient group showed no signs of increased muscle weakness or fatigue during normal daily activities. Control subjects and patients were not actively engaged in sports or training activities. Other exclusion criteria comprised general contraindications to MRI.

MR spectroscopy protocol
In order to apply an individually adjusted exercise load, the maximal isokinetic potential of the leg muscles was measured prior to MRS in a standardized setting with a dynamometer (EnKnee, Enraf Nonius, Delft, The Netherlands). Subjects were positioned in the test chair with the lower leg secured above the ankle, and hip and thigh strapped down to avoid involuntary movements. After extending the knee 15 times in rapid succession the isokinetic muscle strength was expressed as the maximum peak torque produced in these knee extensions.

The subjects were subsequently instructed to exercise by leg extension in the scanner with a weight attached to the ankle of the dominant leg. The weight consisted of lead-containing rubber strips and corresponded to 25% of the individually assessed maximal isokinetic potential. The knee of the subject was supported to enable extension. Exercise was performed by repeated extension once per second during 3 minutes to reach a steady state, which was validated in a pilot study before onset of this study, depicted in Figure 1. During exercise the subjects were in a supine position in a 1.5 T MRI-system (Gyroscan ACS/NT15; Philips, Best, The Netherlands). A 6 cm diameter circular surface coil was positioned at the anatomical localization of the vastus medialis muscle. After exercise the subject kept the leg immobilized for 3 minutes, in which the spectra were obtained. The whole procedure was repeated after 15 minutes of rest.

Immediately after cessation of the exercise, $^{31}$P-MRS from the vastus medialis muscle were obtained every 4 seconds (sweep width 2000 Hz, 1024 samples, flip angle 20°, 6 cm diameter circular surface coil, four signal averages obtained at TR = 1 sec). Shimming of the magnetic field was performed with the proton imaging body coil, yielding a water resonance of 0.2-0.3 parts/million full-width at half-maximum. Relative concentrations of high-energy phosphates (PCr and inorganic phosphate (Pi)) were measured.
Data analysis
Ratios of \((\text{PCr+Pi})/\text{PCr}\) were calculated from spectra obtained during the recovery period using time-domain spectral fitting (MRUI-software, AMARES) (20). Recovery rate, reflecting efficiency and rate of oxidative phosphorylation (21), was characterized by the half-time of recovery \(t_{1/2}\) of monoexponential fitted \((\text{PCr+Pi})/\text{PCr}\) (22).

As each subject performed exercise twice in the scanner, we obtained two phosphorus spectra from each individual. From each obtained phosphorus spectrum the half-time of PCr recovery was calculated. Subsequently the average of the two measurements in each subject was used for statistical analysis.

Laboratory assessment
Venous blood samples were obtained prior to and after exercise to determine blood glucose, insulin, connecting-peptide (C-peptide), glycated hemoglobin (HbA1c), creatine-phosphokinase (CPK), and lactate levels.

Statistical analysis
Multiple linear regression analysis and one-way analysis of variance (ANOVA) were used for statistical evaluation. Results are expressed as mean ± standard deviation. A multiple linear regression analysis was performed for analysis of the \(^{31}\text{P}-\text{MRS}\) results. To identify independent predictors of skeletal phosphorus-metabolism, \(t_{1/2}\) \((\text{PCr+Pi})/\text{PCr}\) was entered as a dependent variable and MIDD mutation (yes/no) and DM (yes/no) were subsequently entered as independent variables into the model. For comparison of isokinetic potentials and laboratory measurements between groups one-way ANOVA analysis was used, with post-hoc Bonfer-
roni correction when comparing healthy subjects with patients both with DM and without DM. Statistical significance was indicated by a p-value less than 0.05.

RESULTS

The results of patients and healthy subjects are summarized in Table 1.

Table 1. Clinical characteristics and 31P-MRS results of the study population

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Healthy subjects (n=8)</th>
<th>DM (n=6)</th>
<th>Non-DM (n=5)</th>
<th>Total MIDD (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>5 / 3</td>
<td>3 / 3</td>
<td>3 / 2</td>
<td>6 / 5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35 ± 6</td>
<td>39 ± 6</td>
<td>36 ± 5</td>
<td>38 ± 5</td>
</tr>
<tr>
<td>Body mass Index (kg/m²)</td>
<td>23.7 ± 2.4</td>
<td>22.6 ± 3.6</td>
<td>24.4 ± 3.1</td>
<td>23.4 ± 3.3</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.5 ± 0.4</td>
<td>7.2 ± 2.5*</td>
<td>5.0 ± 0.2*</td>
<td>6.2 ± 2.1*</td>
</tr>
<tr>
<td>Max isokinetic potential (Newton)</td>
<td>103 ± 27</td>
<td>88 ± 47</td>
<td>137 ± 70</td>
<td>110 ± 61</td>
</tr>
<tr>
<td>Ankle load (kg)</td>
<td>6.6 ± 1.3</td>
<td>5.6 ± 2.7</td>
<td>7.3 ± 2.0</td>
<td>6.4 ± 2.5</td>
</tr>
<tr>
<td>31P-MRS result</td>
<td>8.7 ± 1.3</td>
<td>14.2 ± 3.8*</td>
<td>12.9 ± 1.9*</td>
<td>13.6 ± 3.0*</td>
</tr>
</tbody>
</table>

Laboratory measurements

| Glucose prior exercise (mmol/l) | 5.0 ± 0.8 | 6.7 ± 2.1 | 4.7 ± 0.4 | 5.8 ± 1.9 |
| Glucose after exercise (mmol/l) | 5.2 ± 1.0 | 6.4 ± 1.8 | 5.8 ± 1.3 | 6.1 ± 1.5 |
| Insulin prior exercise (mU/l)   | 17 ± 19    | 12 ± 8    | 15 ± 6    | 13 ± 7    |
| Insulin after exercise (mU/l)    | 13 ± 18    | 37 ± 60   | 19 ± 18   | 29 ± 45   |
| C-Peptide prior exercise (nmol/l) | 1.05 ± 0.71 | 0.61 ± 0.43 | 1.06 ± 0.24 | 0.81 ± 0.41 |
| C-Peptide after exercise (nmol/l) | 0.79 ± 0.67 | 0.67 ± 0.53 | 1.36 ± 1.07 | 0.97 ± 0.84 |
| Lactate prior exercise (mmol/l)  | 1.1 ± 0.3  | 1.5 ± 0.4 | 1.9 ± 1.4 | 1.7 ± 1.0 |
| Lactate after exercise (mmol/l)  | 1.6 ± 0.7  | 2.1 ± 0.9 | 2.5 ± 2.2 | 2.3 ± 1.5 |
| CPK prior exercise (U/l)         | 99 ± 29    | 239 ± 191 | 210 ± 134 | 226 ± 159 |
| CPK after exercise (U/l)          | 95 ± 31    | 221 ± 183 | 191 ± 131 | 208 ± 153 |

Data are expressed as mean ± standard deviation. Results were significantly different from healthy subjects (p < 0.05). A3243G: adenosine to guanine mutation at position 3243; MIDD: maternally inherited diabetes and deafness; DM: diabetes mellitus; HbA1c: glycated hemoglobin; Max: maximal; 31P-MRS: 31-phosphorus magnetic resonance spectroscopy; t½: recovery half time; Pi: inorganic phosphate; PCr: phosphocreatine; C-peptide: connecting-peptide; CPK: creatine-phosphokinase

Patient characteristics

Comparison of the study groups shows similar age and body mass index (BMI) due to matching (Table 1). Four of the DM patients used insulin, one of them in combination with an oral glucose-lowering sulfonylurea derivate; the other two DM patients showed abnormal glucose levels on OGTT. HbA1C levels were significantly higher in the MIDD group, reflecting the
inclusion of MIDD patients diagnosed with DM in this group. Differences in values of maximal isokinetic potential in exercise and the subsequently used ankle loads were nonsignificant. All subjects tolerated the exercise well.

**Skeletal muscle mitochondrial function**

$^31$P-MRS was performed successfully and a steady state was reached in all participants. Figure 2 shows typical examples of skeletal muscle $^31$P-MRS recovery curves of an MIDD patient and a healthy subject from which PCr recovery $t_{1/2}$ was calculated. The MIDD mitochondrial mutation showed a statistically significant association with half-time of PCr recovery after exercise (beta: 0.611, $p = 0.01$). In the multiple linear regression model no association of the presence of DM was found with $t_{1/2}$ $(PCr + Pi)/PCr$ ($p = 0.382$).

**Figure 2.** Phosphorus metabolite ratio recovery curves. Example of an MIDD patient ($t_{1/2} = 18.0$ sec) and a healthy control subject ($t_{1/2} = 6.9$ sec). Solid lines indicate fitted curves.

**Laboratory measurements**

Glucose, insulin, and C-peptide measurements before and after exercise did not significantly differ between all groups. Lactate levels were normal in all subjects, before and after exercise. High baseline CPK levels were seen in A3243G MIDD mutation carriers (borderline significance with controls, $p = 0.058$), but in both groups CPK levels did not increase after the exercise.

**DISCUSSION**

This study revealed that mitochondrial function is impaired in the skeletal muscle in carriers of the MIDD mutation compared to healthy subjects. Furthermore, in the mutation carriers
no additional effect of DM on mitochondrial function was demonstrated. The latter observation, however, could be due to insufficient statistical power in the small study sample size of this rare mutation.

In the present study PCr recovery half-time was significantly prolonged in the MIDD mutation carriers as compared to healthy controls. During recovery from exercise, PCr is resynthesized as a consequence of oxidative ATP synthesis (23). Therefore, \( t_{1/2} (\text{PCr+Pi})/\text{PCr} \) provides information about mitochondrial function. Our results support the studies of Chinnery et al, who observed mitochondrial dysfunction in the calf muscle in a case report (13) and in A3243G mutation families (14). However, in their study not all A3243G mutation carriers were of the MIDD phenotype, in contrast to our carefully selected MIDD patient population. Furthermore, those authors speculated that physical training or the use of coenzyme Q (12) or dichloracetate contributed to the \( ^{31}\text{P}-\text{MRS} \) detected impaired oxidative phosphorylation rather than the mutation itself. In our study none of the participants used this medication and none were actively engaged in sports or training activities, excluding the potential confounding effect of these factors. In the current study all participants performed individually adjusted exercise to equal the exercise level. Similar maximal isokinetic potentials at a succession of knee extensions were seen, which confirms comparable baseline physical exercise capacity between the groups. So we suggest that MIDD patients show subclinical muscular impaired mitochondrial phosphorylation. This is supported by the high CPK baseline levels in the patient group, without clinical signs of muscle weakness.

We did not find an association of DM and PCr recovery values, as no effect of DM in our MIDD patient population on skeletal oxidative phosphorylation was observed. Recently, Schrauwen-Hinderling et al (18) applied \( ^{31}\text{P}-\text{MR} \) spectroscopy in the vastus lateralis muscle to study mitochondrial function in overweight type 2 DM patients and BMI-matched control subjects. They reported a longer PCr recovery half-time in the type 2 diabetes group when compared to the control group. On the basis of their results we expected an additional effect of DM in the MIDD patient group with DM as compared to the non-DM MIDD subgroup, which was not confirmed by our results. The recently published results of de Feyter et al (15) did not show an effect of insulin resistance or type 2 DM on mitochondrial function when compared to healthy normoglycemic controls, which is in accordance with our study results. In the current study we did not perform proton MRS to further evaluate mitochondrial dysfunction and the association with insulin resistance, as shown in previous studies (15,18). Thus, the exact relationship between DM per se and skeletal muscle phosphate metabolism remains to be clarified, but our results clearly indicate an effect of the MIDD mutation itself on phosphate metabolism.

Our study has limitations. We studied a limited number of patients and controls, although the MIDD phenotype of the A3243G mutation is a very rare entity and previous reports in the literature described mainly case reports. As the mutation is not a common entity, international collaboration is required to collect larger numbers of MIDD subjects, including DM and non-DM patients.
In conclusion, the present study demonstrates subclinical mitochondrial dysfunction of the skeletal muscle in MIDD patients. The presence of DM does not seem to affect the mitochondrial function of the skeletal muscle in the MIDD population independently.
REFERENCES


