Metabolic disturbances during short exercises in dermatomyositis revealed by real-time functional $^{31}$P magnetic resonance spectroscopy

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Objective. $^{31}$P magnetic resonance spectroscopy (MRS) is useful for evaluating metabolic disturbances in dermatomyositis (DM). However, short-term alterations of metabolic parameters such as Pi/PCr (inorganic phosphate/phosphocreatine) have not been assessed in detail, although they may reveal insights into the origin of the known long-term changes. We therefore performed real-time functional $^{31}$P MRS to find out if there are characteristic short-term alterations of metabolic dynamics during muscular exercise and if they are of diagnostic relevance.

Methods. MRS measurements were performed on lower calf muscles of 10 DM patients and 18 healthy subjects throughout five short (1 min) cycles of submaximal exercise (50% maximum voluntary contraction).

Results. Pi/PCr ratios during exercise increased in patients and controls. They rapidly returned to baseline values in the controls, but both Pi and PCr remained above baseline values in patients and resulted in irregular Pi/PCr ratios. This was true for each individual patient, but resulted in broad variation in individual Pi/PCr values. To compare groups with limited patient numbers, it was therefore more appropriate to use a recovery index, i.e. the quotient of the Pi/PCr ratio during and after exercise, which was independent of individual parameters, such as age and the work/energy cost ratio.

Conclusion. Evaluation of short-term changes by real-time functional $^{31}$P MRS provides insight into alterations of Pi/PCr ratios and could improve diagnostic parameters in DM.

Key words: Dermatomyositis, $^{31}$P magnetic resonance spectroscopy, Metabolism.

Dermatomyositis (DM) is a chronic autoimmune disease characterized clinically by signs of severe myopathy of skeletal muscles and by an erythematous rash [1]. It features generalized myopathy of proximal, distal and paravertebral skeletal muscles with characteristic histological [2–5] and electromyographic [6] changes in various muscle groups. The extent of muscular involvement differs between patients, but its detection and its timely and adequate therapy are of primary concern for the physician if irreversible damage is to be prevented. Assessment of muscular involvement in the course of disease is sometimes difficult because patients’ self-evaluation is not always reliable and serum levels of creatine kinase (CK) [7–10] and other muscle enzymes do not always reflect disease activity with sufficient sensitivity, specificity or accuracy. Because of these limitations in recognizing the onset, progression or improvement of myositis, it was desirable to find more adequate parameters to assess disease activity before and during therapy.

In recent years, knowledge and diagnostic assessment of muscle pathology has been enriched by $^{31}$P magnetic resonance spectroscopy (MRS). This non-invasive technique determines myositis-related deviations in metabolic parameters [inorganic phosphate (Pi), phosphocreatine (PCr), adenosine triphosphate (ATP) and pH] before and after exercise in diseased muscle in vivo. Not only has it been useful in the diagnosis of inflammatory muscle diseases, but it has also opened up the opportunity to gain new insight into their pathophysiology [11–14]. It has been found [2, 11, 13–15] that muscles of DM patients usually reveal a sustained increase in Pi at the expense of phosphate bound to organic creatine (PCr) after exercise, while this increase is less prominent in controls, depending on the exercise regimen and the time-points of measurement. The Pi/PCr ratio in muscles reflects an inverse measure of energy reserve [16–17]: the lower the ratio, the more energy is available for muscle contraction. Its increase in patients is a sensitive indicator of disturbed energy metabolism.

Besides presenting higher Pi/PCr ratios than controls [2, 11, 13–14, 17–18], DM patients also revealed prolonged recovery times for Pi and PCr before and after exercise [2]. The slow post-exercise recovery was found to be associated with slow H$^+$ efflux, indicating that DM is a skeletal muscle disease with defects in aerobic metabolism secondary to impaired blood supply and not to primary abnormalities of mitochondria [2].

While these studies provided further insight into metabolism of muscle before and shortly after exercise, none of them evaluated the short-term alterations in Pi and PCr during the entire cycle of dynamic muscle contractions. However, because DM patients show prolonged recovery times for Pi and PCr [2], we wondered how this prolonged time would influence muscle metabolism of Pi and PCr during exercise, especially when breaks are shorter than the time required for the reconstitution of PCr. We also wondered whether altered dynamics of Pi and PCr during exercise, and thus during a period when disturbances may be particularly noticeable,
would reveal a parameter enabling us to detect disturbances of diseased muscle earlier and with higher sensitivity than the Pi/PCr ratio at the end of exercise. Thus, we investigated this time interval more closely and performed short-term, real-time, dynamic functional (non-steady) state $^{31}$P MRS on the lower calf muscle during the complete exercise span at a high temporal resolution. Because appropriate patient evaluation in the course of disease and treatment is crucial, we assumed that such a protocol could also increase sensitivity in detecting early signs of DM as well as alterations or relapses under therapy.

**Materials and methods**

**Patients and controls**

The patient group comprised 10 subjects (age 57.2 ± 11.1 yr; nine females) and the control group 18 healthy subjects (age 38.9 ± 18.9 yr; 10 females). As differences in the physical condition of individuals are known to influence oxidative metabolism of muscles and results of MRS during exercise [19], and since our patients had been sedentary for some months prior to examination, we selected as healthy controls only volunteers in a normal, untrained condition who had not been exceptionally active for some months prior to their MRS scan.

The clinical features of the DM patients are summarized in Table 1. All DM patients had a characteristic erythematous rash. Four patients (patients 1, 5, 7 and 8) had received no treatment prior to the MR examinations. Two patients (patients 5 and 6) were evaluated several times (longitudinal measurements). They had been unresponsive to steroids and were therefore additionally treated with high doses of intravenous immunoglobulins (2 g/kg body weight every 4 weeks). Serum CK values were substantially elevated in only three patients (patients 1, 2 and 5); the CK values in the remaining patients were repeatedly normal (Table 1). Electromyographic analysis and muscle biopsies were performed in all patients; the findings were consistent with dermatomyositis. General condition and muscle weakness (ability to climb a certain number of steps and to comb or brush the hair, frequency and intensity of various leisure activities) were evaluated clinically and according to a modified MRC scale [2].

After detailed description of the study to the subjects, written informed consent was obtained. This study was approved by the local Ethics Committee for the Protection of Human Subjects of the University of Münster.

**Pre-exercise protocol**

The maximum voluntary contraction (MVC) for each patient’s calf muscles was determined outside the magnet [13]. In brief, the left leg was in full extension and the weight that the subject or patient could no longer raise was measured as the MVC. The mean MVC was 16.7 ± 5.2 kg for controls and 7.9 ± 4.7 kg for patients.

**Protocol for $^{31}$P MRS data acquisition**

Patients and healthy volunteers were positioned supine in the magnet (Magnetom SP 1.5 T; Siemens, Erlangen, Germany) with the left calf centred on a Siemens $^{31}$P surface coil (diameter 80 mm), and the foot positioned at the end of the table. A non-magnetic load equivalent to 50% of the MVC was then secured on an ergometer and the patient’s ankle was taped to the ergometer. The patient performed plantar flexions against the weight by flexing and extending the foot every 2 s (0.5 Hz) for 1 min, followed by a 1-min break. The contractions were isokinetic.

The protocol for data acquisition encompassed one spectrum at rest at the beginning and at the end of the examination with 100 acquisitions each; three spectra before the start of the first exercise (temporal resolution 17 s); two spectra (six acquisitions each) during 1 min of exercise (temporal resolution 17 s) and three spectra (six acquisitions each) during a 1-min break (with a total of five cycles); and 10 spectra (temporal resolution 17 s) and 10 subsequent spectra (temporal resolution 34 s) during the subsequent recovery interval.

The magnetic field was adjusted for maximum proton resolution. Spectra were acquired at 25.7 MHz with a rectangular pulse of 500 ms duration, a repetition time of 2.5 s and a resolution of 2 kHz. The surface coil comprised a relatively large volume of muscle, and the spectral data represented weighted means.

Our approach of performing short-term, real-time dynamic functional (non-steady) state $^{31}$P MRS during a complete exercise span was best accomplished on the lower calf muscle as it permits measurements during exercise with a high temporal resolution at an adequate signal-to-noise ratio. The calf muscle enables the detection by $^{31}$P MRS of characteristic alterations of DM in a similar way as the thigh muscle [2]. This is also consistent with the fact that similar histological [2–5] and electromyographic [6] changes were found in different muscle groups in DM patients. The fact that patients complain initially about myalgias and weakness of primarily their proximal muscles may be due to the higher mass and the intense use of these muscles, resulting in earlier awareness of alterations.

All spectra were phase-corrected and the size of the peak area was determined by integration using Microcal Origin 5.0 software (Microcal Software, Northampton, MA, USA).

Signal-to-noise ratios for all relevant peaks were always ≥ 3 and the quality of spectra was adequate. Control subjects who were tested several times had metabolite level variations <10% with our

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**Table 1. Clinical characteristics of patients at the time of their MRS examination**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Duration (yr)</th>
<th>CK (U/ml)</th>
<th>Prednisone</th>
<th>Other immuno-suppressive therapy</th>
<th>Muscle weakness</th>
<th>Quadriceps</th>
<th>Calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37/F</td>
<td>6</td>
<td>92</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>53/M</td>
<td>1</td>
<td>87</td>
<td>None</td>
<td>HIVIG* (2 mg/kg)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>49/F</td>
<td>12</td>
<td>43</td>
<td>None</td>
<td>HIVIG* (7.5 mg)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>58/F</td>
<td>7</td>
<td>14</td>
<td>5 mg</td>
<td>HIVIG* (2 mg/kg)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>56/F</td>
<td>5</td>
<td>194</td>
<td>None</td>
<td>HIVIG* (2 mg/kg)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>74/F</td>
<td>23</td>
<td>34</td>
<td>7.5 mg</td>
<td>HIVIG* (2 mg/kg)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>63/F</td>
<td>1</td>
<td>24</td>
<td>60 mg</td>
<td>None</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>74/F</td>
<td>1</td>
<td>33</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>54/F</td>
<td>6</td>
<td>23</td>
<td>5 mg</td>
<td>HIVIG* (2 mg/kg)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>55/F</td>
<td>4</td>
<td>20</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MRC values for patients 5 and 6 were obtained before high-dose intravenous immunoglobulin treatment (HIVIG); data for patient 1 represent values obtained 6 weeks after cessation of therapy.

*Normal range <80 U/ml.

*Muscle weakness is expressed according to general evaluation by the examiner and the modified MRC scale.
The work/energy cost ratio of muscles was calculated as the weight in pounds (50% MVC) divided by the mean Pi/PCr ratio during recovery after the last exercise cycle. For this purpose, Pi and PCr levels during recovery after the last exercise cycle were fitted as described elsewhere [11–13].

In addition, we determined the recovery time constants for $T_{Pi}$ and $T_{PCr}$ (time constants needed for PCr and Pi to reach about 63% recovery of their pre-exercise levels). For this purpose, Pi and PCr levels during recovery after the last exercise cycle were fitted using a mono-exponential function [20]:

$$PC(t) = \Delta PCr \cdot (1 - e^{-t/T_{PCr}})$$

$$Pi(t) = \Delta Pi \cdot e^{-t/T_{Pi}}$$

for Pi.

The work/energy cost ratio of muscles was calculated as the weight in pounds (50% MVC) divided by the mean Pi/PCr ratio during the five cycles of exercise, as described previously [11]. This yields a representation of the overall metabolic status.

Recovery index

Due to broad variation in Pi/PCr values among individuals, the mean Pi/PCr ratios during exercise increased less markedly in patients and controls than the intra-individual increases would indicate. For the same reason, differences in means between controls and patients were also less marked than suggested by the individual courses. Therefore, the average Pi/PCr ratio during exercise is not an optimal parameter per se for comparing disease-specific metabolic disturbances between individuals. This is probably due to the fact that the individual ratios were influenced by several patient-related parameters, such as age, muscle mass and cardiopulmonary status. The same is true for Pi/PCr ratios during recovery periods. The relative decrease in the Pi/PCr ratio, however (given by the quotient of the Pi/PCr ratio during and after exercise) is less affected by these additional parameters.

We therefore designed a new parameter representing the ratio between consumption and grade of replenishment, i.e. the mean Pi/PCr (15 spectra/5 cycles) during breaks between exercises, divided by the mean Pi/PCr of the second spectrum taken during exercise over five cycles: recovery index $= \frac{\text{mean}(\text{Pi}/\text{PCr})_{\text{recovery}}}{\text{mean}(\text{Pi}/\text{PCr})_{\text{exercise(2nd)}}}$

The second spectrum was taken because it always reflected the maximum Pi/PCr alteration during the course of each exercise cycle and gave a good general picture of the bioenergetic status of the muscles (Fig. 1A, 1B).

Results

Pi/PCr ratio in controls

In order to evaluate whether our data were dependent on age, controls were divided into two groups: group I, under 50 yr of age ($n = 11$); and group II, over 50 yr of age ($n = 7$). There were no significant differences between these two age groups for Pi/PCr at rest ($t$ test; $t = -0.8, P = 0.84$; group I, 0.11 ± 0.034, group II, 0.13 ± 0.024) or for the work/energy cost ratio ($t$ test; $t = 0.99$, $P = 0.34$; group I, 55.3 ± 20.9, group II, 45.6 ± 18.7). The only parameter to be significantly influenced by age was Pi/PCr at the end of the examination (ANCOVA, $F = 4.18, P = 0.035$), when older control subjects had a lower Pi/PCr than younger controls. Because our controls were, on average, slightly younger than the patients, the statistical difference in Pi/PCr between patients and controls at the end of the examination may have been even more pronounced than shown here (Table 2).

Metabolic results were not influenced significantly in the control group by gender. The only gender-related difference was a lower work/energy cost ratio for women (ANOVA; $F = 5.85; P = 0.028$; females $= 42.5 ± 15.4$, males $= 63.0 ± 20.5$). Consequently, as all but one of our patients were female, gender-matched controls were used for the comparison of work/energy cost ratios between patients and controls (Table 2).

During exercise with 50% MCV, all control subjects revealed a rapid and marked increase in Pi/PCr which returned almost to baseline levels within the 1-min rest period. A typical Pi/PCr curve for a healthy volunteer is shown in Fig. 1A, and the individual Pi and PCr changes during our protocol are presented in Fig. 1B. Monitoring their time course throughout the exercise programme, and thus during the quickly alternating periods of breaks and exercises, revealed an almost inversely related synchronization of Pi and PCr changes, a decrease in Pi being accompanied by an increase in PCr. PCr and Pi levels are represented as almost baseline levels within the 1-min rest period. A typical Pi/PCr curve for a control subject. Pi/PCr increased significantly during the exercise period (1 min) and returned to almost baseline levels within the 1-min rest period. (B) Corresponding individual changes in Pi and PCr during our protocol. These changes are well synchronized and the Pi decrease is accompanied by an increase in PCr. PCr and Pi levels are represented as PCr (mean PCr at rest) and Pi (mean Pi at rest) respectively. Pi levels are indicated at the left ordinate and PCr levels at the right ordinate. *Measurements during exercise with 50% MCV; circles, measurements during breaks between exercises; triangles, measurements before the start and after the end of the exercises.

Data collected

We evaluated peak areas for Pi, PCr and ATP, corrected them for saturation effects and determined the Pi/PCr ratios. Cytoplasmic pH (chemical shift difference between Pi and PCr) was calculated by several patient-related parameters, such as age, muscle mass and cardiopulmonary status. The same is true for Pi/PCr ratios during recovery periods. The relative decrease in the Pi/PCr ratio, however (given by the quotient of the Pi/PCr ratio during and after exercise) is less affected by these additional parameters.

Recovery index

Due to broad variation in Pi/PCr values among individuals, the mean Pi/PCr ratios during exercise increased less markedly in patients and controls than the intra-individual increases would indicate. For the same reason, differences in means between controls and patients were also less marked than suggested by the individual courses. Therefore, the average Pi/PCr ratio during exercise is not an optimal parameter per se for comparing disease-specific metabolic disturbances between individuals. This is probably due to the fact that the individual ratios were influenced by several patient-related parameters, such as age, muscle mass and cardiopulmonary status. The same is true for Pi/PCr ratios during recovery periods. The relative decrease in the Pi/PCr ratio, however (given by the quotient of the Pi/PCr ratio during and after exercise) is less affected by these additional parameters.
period was incomplete. Consequently, values for Pi/PCr did not reach baseline levels, and the subsequent rise also decreased steadily with each subsequent exercise cycle. A representative curve is presented in Fig. 2A. Separate evaluation for Pi and PCr (Fig. 2B) revealed that patients degraded only little PCr into Cr and Pi in their last exercise cycles and that the inverse synchronization between changes in PCr and Pi was no longer present. This was observed in each DM patient.

**Time constants**

As an estimate of the time required for replenishment of PCr and Pi, we attempted to determine the time constants $T_{Pi}$ and $T_{PCr}$ (Fig. 3A, B). $T_{Pi}$ and $T_{PCr}$ were available in 14 of 18 volunteers in whom reliable fits could be obtained.

In patients, only a few time constants could be determined. In all these cases $T_{PCr}$ and $T_{Pi}$ constants were markedly higher for patients: $T_{PCr}$ was $50.0 \pm 17.1$ s for patients ($n=2$) and $31.8 \pm 12.8$ s for controls ($n=14$), while $T_{Pi}$ was $74.1 \pm 13.1$ s ($n=4$) for patients and $38.4 \pm 10.4$ s for controls ($n=14$) ($P<0.001$; Mann–Whitney). In the other patients, a strong and sustained reduction in Pi did not allow $T_{Pi}$ to be calculated, but this low Pi peak itself clearly indicated metabolic disturbances.

**Work/cost ratio**

To correlate biochemical and functional abnormalities during exercise, we evaluated the work/energy cost ratio [11]. Patients had a lower work/cost ratio than controls ($P=0.017$ (Table 2). They also presented a significant correlation between the work/energy cost ratio and the Pi/PCr ratio, both at rest and at the end of the examination (Spearman’s $\rho^2 = 0.600$, $P=0.008$, and $\rho^2 = 0.687$, $P=0.003$, respectively). This linear correlation between high Pi/PCr and low work/energy cost ratio was valid.
over a wide range of ratios. In healthy subjects, the work/energy cost ratio was not significantly associated with the Pi/PCr ratio at the end of the examination (trend; \( r^2 = 0.166, P = 0.093 \)).

**Recovery index**

By dividing Pi/PCr ratios during exercise and recovery we obtained markedly and significantly higher values for patients than for controls (\( r \) test; \( t = -5.0, P < 0.001 \)) (Table 2). This was consistent with the observation that the Pi/PCr ratios would rise in each individual patient. This recovery index was independent of work/energy cost ratios in healthy subjects; \( (r^2 = 0.0078, P = 0.72) \) and patients \( (r^2 = 0.022, P = 0.69) \) (Fig. 5). It was also independent of age (patients, \( r^2 = 0.053, P = 0.95 \); healthy subjects, \( r^2 = 0.03, P = 0.59 \)) and of 50% MVC (patients, \( r^2 = 3 \times 10^{-3}, P = 0.96 \); healthy subjects, \( r^2 = 1 \times 10^{-5}, P = 0.99 \)).

Thus, the recovery index or relative decrease in Pi/PCr ratio is less affected by broad variations of interindividual Pi/PCr values which result from patient related parameters such as age, muscle mass and cardiopulmonary status.

**pH values and ATP**

There was no difference between patients and controls with regard to baseline pH levels (Table 3). During exercise, two different courses were observed among controls: while 13 control subjects (group I) revealed a decrease in pH levels during exercise (Fig. 6A), no exercise-related pH changes were observed in the other subjects (group II). Subjects in groups I and II differed significantly in two aspects: group II exhibited higher pH values before exercise and during breaks than group I (Table 3).

All DM patients exhibited unchanged pH values during exercise (Fig. 6B), a finding similar to that for control subjects of group II.

ATP was also measured at the beginning of our exercise regimen. For detailed measurements of ATP during the exercise protocol, a higher number of scans would have been needed to provide a reliable signal-to-noise ratio during exercise. However, due to the focus of our MRS analysis on rapid changes in PCr levels, our protocol was optimized to yield the highest possible temporal resolution at the expense of the number of scans. Using a lower number of scans resulted in large interindividual variations in ATP, so that statistically significant differences in ATP at rest, exercise and recovery time-points could not be detected.

**Example of longitudinal evaluation**

\( ^3 \)P MRS was repeated immediately before and after therapy in two patients (during three cycles of therapy with intravenous immunoglobulins). In patient 5, a 1.53 (± 0.16)-fold improvement in the work/energy cost ratio (from 30.3 to 46.5) was observed directly after therapy, as was a similar decrease in the quotient of the Pi/PCr ratio (from 0.66 to 0.43), indicating better replenishment of PCr and concomitant recovery. In contrast, the muscle strength of patient 6 showed no marked improvement under this therapy, with no marked change in the \( ^3 \)P MRS parameters. Her therapy was subsequently discontinued as she also showed no clinical sign of improvement, indicating irreversible muscle damage.

**Table 3. pH changes during the course of our exercise protocol in DM patients and in controls**

<table>
<thead>
<tr>
<th></th>
<th>pH at rest</th>
<th>pH during breaks$^a$</th>
<th>pH during exercise$^a$</th>
<th>pH at end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls I ( (n = 12) )</td>
<td>6.89 ± 0.07</td>
<td>6.71 ± 0.07</td>
<td>6.91 ± 0.07</td>
<td>6.97 ± 0.06</td>
</tr>
<tr>
<td>Controls II ( (n = 6) )</td>
<td>7.00 ± 0.07$^b$</td>
<td>7.03 ± 0.10$^c$</td>
<td>7.05 ± 0.17</td>
<td>7.03 ± 0.05</td>
</tr>
<tr>
<td>DM patients ( (n = 10) )</td>
<td>6.97 ± 0.07</td>
<td>6.92 ± 0.3</td>
<td>6.92 ± 0.25</td>
<td>6.92 ± 0.25</td>
</tr>
</tbody>
</table>

Thirty per cent (group II) of the controls exhibited no exercise-related changes in pH. This is in line with the results of Block et al. [12], who also found that healthy controls can be divided into two groups. Due to relatively large standard deviations, differences between patients and group I controls did not reach statistical significance.

$^a$Mean of five cycles.

$^b$Significant difference: control group I vs control group II (Mann–Whitney U test, \( P = 0.017 \)).

$^c$Significant difference: control group I vs control group II (Mann–Whitney U test, \( P = 0.002 \)).
be a result of damage to mitochondria consequent on an impaired
of cycles, degradation of PCr into Pi was progressively slower. The
this may indeed be the case. Moreover, with an increasing number
MRS during a complete exercise span, we provide evidence that
energy compounds such as PCr [2, 17, 19]. By our approach of
monitoring of the PCr and Pi course.
lar contractions over a course of several exercise cycles. Usually,
in DM, and that the degradation of PCr into Pi runs progressively
more slowly.

To our knowledge, no other studies have been published to date
on muscle metabolism during dynamic, short intermittent muscu-
lar contractions over a course of several exercise cycles. Usually,
measurements taken over longer time intervals yield an average
value valid for the whole exercise span, but do not attempt subtle
monitoring of the PCr and Pi course.
The more pronounced elevation of Pi/PCr in patients compared
with healthy controls during other exercise protocols has been
suggested to reflect inefficiency in producing or utilizing high-
energy compounds such as PCr [2, 17, 19]. By our approach of
performing short-term, real time dynamic functional state 31P-
MRS during a complete exercise span, we provide evidence that
this may indeed be the case. Moreover, with an increasing number
of cycles, degradation of PCr into Pi was progressively slower. The
inefficiency in producing or utilizing high-energy compounds may
be a result of damage to mitochondria consequent on an impaired
blood supply [2].

**Pi/PCr ratios**
Pi/PCr ratios during breaks, recovery or rest did not always reveal
significant differences between patients and controls in the various
studies. This may be due to differences in the severity of disease,
the presence or absence of treatment, or the muscular condition of
the person concerned (various degrees of inactivity). Although
there are differences in study design with regard to time-points,
frequency of 31P MRS measurements during exercise cycles, type
and duration of exercise, and muscle groups that were examined,
the mean Pi/PCr ratio of healthy subjects at rest in our study
(0.10 ± 0.03) was consistent with findings from other studies which
reported Pi/PCr ratios of 0.15 [11], 0.11 [13], 0.12 [12] or 0.10 [2]. Pi/PCr
ratios at rest were not statistically different between DM
patients and healthy controls, though patients exhibited higher
values (P = 0.08). This was similar to the results obtained with
juvenile DM [11], while other studies revealed significantly higher
values in DM patients [2, 13].

Although it is commonly accepted that Pi rises and PCr falls
during muscular activity [16], and although increased Pi/PCr ratios
under exercise are reported in most studies for DM patients [6, 11,
13] as well as for healthy subjects [11–13, 16], the range of increase
and especially the question of whether there is a significant
difference between patients and controls produced different out-
comes. While one study [11] found significant differences between
both groups only during recovery, others observed significant
alterations under exercise [2, 13].

Our observations suggest that these differing results are likely to
be due to differences in intervals between measurements. When
measurements are taken at short intervals during exercise, such as
in the present study, they are able to detect short-term alterations
(and differences) in Pi/PCr in both patients and controls. Measurements
taken over longer intervals reveal an average value valid for the whole exercise span and may thus miss the
differences occurring between exercise periods. As already pointed
out, our healthy controls revealed a rapid and marked increase
in Pi/PCr ratio during the exercise period, but the ratio began to
return towards baseline levels immediately at the start of the 1-min
break period. This is in line with the findings of those authors
who also performed measurements in healthy subjects at shorter
intervals during exercises [2, 16].

**Recovery times**
The times required for replenishment of PCr and Pi (T_Pi and T_PCr)
are prolonged in patients relative to controls. Our results are
consistent with data of Cea _et al._ [2], who reported T_PCr times in
DM patients that were almost twice as long as in controls. While
T_PCr could be calculated for 14 of 18 control persons, recovery
curves in our patients were often irregular at the end of the
examination, so that reliable fits for T_PCr could be obtained for
only two patients. As all patients could easily complete our exercise
protocol, and as they had similar MVCs to those reported in other
studies [11, 13–14], this reaction pattern is not dependent on
differences in the patients’ efforts but may be characteristic of DM

**Discussion**

**Time-course of exercise cycles**
This study analysed metabolic changes in muscles of DM patients
by real-time functional 31P MRS during a submaximal dynamic
exercise regimen at a higher temporal resolution of measurements
than most previous studies [2, 11, 13]. Our exercise regimen was
able to detect relevant changes in muscular metabolism during
DM, similar to the way in which functional MRS imaging of
the brain revealed disturbed cerebral metabolism. It allowed the
detection of an inversely related synchronization of changes in Pi
and PCr in healthy subjects (Fig. 1B), which indicated that in
healthy muscles PCr is predominantly degraded to or replenished
and PCr in healthy subjects (Fig. 1B), which indicated that in
healthy muscles PCr is predominantly degraded to or replenished
from Cr and Pi, and that muscles can adapt rapidly to changing
exercise conditions. In DM patients this inverse synchronization
between changes in PCr and Pi was no longer present after two
cycles of exercise with an intermittent 1-min break (Fig. 2B);
patients subsequently degraded less PCr into Cr and Pi than
healthy controls. This suggests that the two systems are decoupled
in DM, and that the degradation of PCr into Pi runs progressively
more slowly.

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T_PCr could be calculated for 14 of 18 control persons, recovery
curves in our patients were often irregular at the end of the
examination, so that reliable fits for T_PCr could be obtained for
only two patients. As all patients could easily complete our exercise
protocol, and as they had similar MVCs to those reported in other
studies [11, 13–14], this reaction pattern is not dependent on
differences in the patients’ efforts but may be characteristic of DM

**Functional 31P-MRS in dermatomyositis**

**FIG. 6. pH curves during our exercise protocol for (A) a control and (B) a patient (patient 5, Table 1). During exercise, pH decreased
to 6.8 (6.67 ± 0.18) in controls and then increased by the end of exercise to 7.12 ± 0.07. Remarkably, pH values neither decreased nor
increased in DM patients during exercise.**
patients when using our specific exercise regimen. Likewise, the fact that \( T_1 \) could not be obtained in all patients because Pi at the end of the exercise cycles was too low for integration also clearly indicates metabolic disturbances in DM.

Recovery index

The recovery index, a new parameter which reflects differences in muscle metabolism between patients and controls more clearly than individual Pi/PCr ratios, seems to be a very sensitive marker for disturbances in muscle metabolism caused by DM (Table 2). This quotient of the mean Pi/PCr during intermittent breaks divided by the mean Pi/PCr during exercise gives a good overall picture of the muscle's ability to provide enough energy in synchronisation with exercise cycles and to replenish energy efficiently and rapidly during short breaks. We showed that this measure reduces the impact of individual parameters, such as age, which influence Pi and PCr (and the ratio Pi/PCr) and thus weaken the sensitivity of the mean of solely the Pi/PCr ratios. The quotient was also independent of the work/energy cost ratio and 50\% MVC in both healthy subjects and patients. In contrast, Pi/PCr ratios at rest and at the end of the examination were dependent on the work/energy cost ratio, for example. The recovery index therefore gives a more stable picture, focusing on the ability of the muscle to recover after a short period of intensive exercise. The higher the recovery index, the more PCr and Pi are decoupled.

In summary, this new index is very useful for comparing small groups of patients with healthy individuals and should offer a better diagnostic tool for the examination of DM than the ratio of Pi/PCr alone [2, 11, 13–15]. Longitudinal studies of two patients demonstrate this potential, because an improvement in one patient’s condition was related to a clear reduction in the recovery index while no change in the other patient’s condition was reflected by no change in this parameter. This should be followed up in further studies.

pH measurements

Utilization of PCr is often accompanied by a decrease in intracellular pH due to lactate production [20], but only after 60\% PCr has been degraded. In our control subjects the values for pH in calf muscle during exercise either decreased or showed no change. These results are consistent with those of previous studies on muscular pH and are reported to reflect individual variations in the utilization of slow twitch type I fibres or fast twitch type II fibres in the calf muscle [12]. Type I fibres have a high oxidative capacity, so that less high-energy compounds are hydrolytically cleaved in response to exhaustive exercise, resulting in only minor accumulation of protons [21–22]. Type II fibres show a higher glycolytic activity and subsequently produce more protons. The individual differences in utilization of type I or type II fibres are independent of training or maximum strength [12]. It was remarkable that such different behaviours in pH appeared to be absent in all DM patients. However, such a homogeneous lack of decrease in pH occurring concomitantly with the prolonged degradation and resynthesis of PCr (Table 3) is more likely to indicate a pathological condition (e.g. insufficient initiation of glycolysis [23]) than predominance of type I fibres in patients.

Influence of other potential cofactors

Markedly reduced muscle mass may result in a reduced signal-to-noise ratio in the MRS spectra, but in our examinations the quality of MRS spectra on calf muscles was sufficient (signal-to-noise ratios for relevant peaks were > 3). None of our patients or controls had serious cardiovascular risk factors that might have influenced oxidative metabolism [24]. No dependency of our MRS data on age (except Pi/PCr at end of the exam) or gender (except work/energy cost ratio) was observed. There have been reports of an age-related increase in Pi/PCr during rest, but these differences were not consistent and may only apply when differences between ages are markedly larger [24–26] than encountered in our groups of patients or controls. This should be re-evaluated when performing these types of examinations in childhood DM. Various degrees of inflammation or adipose tissue around the muscles could also be excluded as confounding factors, as our patients were not obese and they did not have clinically apparent signs of marked perimuscular or subcutaneous inflammation. In addition it has been reported that neither inflammation nor fatty infiltration of the muscle affects the discovery of MRS-detectable abnormalities [2].

Although our observation was based on a restricted number of patients and controls, it yielded in all DM patients a consistent finding that was absent in all controls. There were limitations in our methodological approach with regard to evaluating the entire energy metabolism of muscle. While it was perfectly appropriate to observe short-term changes in PCr, it was not ideally suitable to monitor changes in ATP. We were able to measure ATP at the beginning of our measurements but would have needed a higher number of scans to obtain reliable signal-to-noise-ratios during exercise. Increasing the number of scans would have been possible only at the expense of our high time resolution and thus of our selected parameters. Consequently, our study does not allow conclusions to be drawn on alterations in ATP.

Also, as in other studies [11, 13–14], it is difficult to prove that all patients and controls maintained their initial contraction force. However, it is very likely that they did because (i) we monitored them carefully while they performed the exercises, (ii) the therapy regularly improved their PCr values, and (iii) very similar results were observed when measurements on patients were repeated (data not shown). It would have been difficult for patients to apply a consistent level of reduced force over repeated examinations. In addition, we had patients who were initially suspected to have DM but subsequently proved to have another form of myopathy. These patients showed no effect either clinically or in MRS in response to DM-directed therapy with immunoglobulins. Thus, there is good evidence that our exercise regimen can be performed repeatedly with comparable efforts.

In summary, our methodological approach enabled relevant changes in muscular metabolism to be detected during DM. Reproducible alterations in Pi/PCr ratios and corresponding Pi and PCr curves during exercise in all DM patients compared with controls were recorded. A new measure (the recovery index) which is more sensitive to metabolic disturbances and therefore better reflects disturbances in muscle metabolism was introduced. The improvement in the recovery index and in the work/energy cost ratio in one patient under successful therapy and the lack of change in a non-responding patient indicate that \(^1\)P MRS may be useful for the longitudinal management of DM patients and may serve as a reliable guide for tapering or increasing immunosuppressive therapy [18]. This is important in view of the high risk or the high costs involved in some therapies.

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References


