Urinary levels of creatine and other metabolites in the assessment of polymyositis and dermatomyositis

Y.-L. Chung1,2, W. S. Wassif2, J. D. Bell3, M. Hurley4 and D. L. Scott1

Background. A simple and reliable method is needed to assess disease activity and monitor the efficacy of therapy in polymyositis (PM) and dermatomyositis (DM). This study used in vitro proton (1H) magnetic resonance spectroscopy (MRS) to explore whether excretion of urinary metabolites can be used as a reliable marker of disease in PM and DM patients.

Methods. Urine samples were obtained from PM/DM patients (n = 34), healthy controls (50) and subjects with known muscle-wasting conditions including adult-onset muscular dystrophy (8), stroke patients (10), rheumatoid arthritis (RA) patients on steroids (13) and not on steroids (16) and patients with alcoholic myopathy (12). Levels of urinary metabolites were then correlated with creatine kinase (CK) activities and quadriceps muscle strength.

Results. Creatine was detected in the urine in 26 of 35 patients with PM/DM, four of 60 cases with other medical disorders (including one with adult-onset dystrophy, one with a stroke and two with RA who were not on steroids) and 10 of 50 healthy controls. The urinary creatine/creatinine ratio exceeded 0.4 in 20 patients with PM/DM but no patients with other medical disorders and no healthy controls. These differences were highly significant (P < 0.001) by Kruskal–Wallis test (comparing all groups) and by Mann–Whitney U-tests (comparing individual groups with PM/DM cases). Citrate, glycine, choline-containing compounds and taurine levels were significantly increased in PM/DM when compared with controls. There were positive correlations between CK activities and choline-containing compounds (r = 0.78, P = 0.0006) and also between CK activities and betaine (r = 0.57, P = 0.026).

Conclusions. This study shows significant differences in the urinary levels of creatine, choline-containing metabolites, betaine and citrate in PM/DM subjects compared with controls, although further work is required to elucidate the underlying metabolic processes.

Key words: Polymyositis, Dermatomyositis, Proton magnetic resonance spectroscopy, Urinary creatine.

Disease assessment in dermatomyositis (DM) and polymyositis (PM) is conventionally based on clinical evaluation [1–3], muscle biopsy [4], serum enzyme levels such as creatine kinase (CK) [2] and electromyography (EMG) [5, 6]. These all have limitations. CK activities are influenced by many factors over and above disease...
activity including racial group, exercise immediately before taking blood samples and general physical activity and fitness levels [7]. EMG is subjective and 20% or more of cases have no definite abnormalities [5, 6]. Muscle biopsies can appear normal despite active disease as involved muscles can contain many normal areas [8].

Muscle abnormalities have been detected in PM and DM patients by in vivo magnetic resonance imaging (MRI) [9–13] and spectroscopy (MRS) [14–19]. However, these methods are unsuitable for routine use because they are relatively expensive, are not widely available and appear relatively insensitive for detecting disease progression. This is an especial problem in chronic myositis when fatty infiltration is a dominant feature on magnetic resonance imaging and proton (1H) spectroscopy [15]. Imaging specific areas of muscle in myositis can also be misleading as the abnormalities in DM and PM are unevenly distributed. Simpler methods may be of more clinical value, especially if they reflect overall changes in the muscles.

Measuring metabolic products in the urine may meet this need. The aim in this study was to use in vitro 1H MRS [20] profiles of urine to explore whether excretion of metabolites can be used as a reliable marker of disease in PM and DM patients.

Patients and method

PM/DM patients

These met the criteria recommended by Bohan and Peter [21]. They had clinical features of muscle weakness, muscle biopsy-proven disease and elevated serum CK activities at presentation. The PM/DM patient group comprised 34 patients. There were four males and 30 females of mean age 43 ± 6 yr (range 23–72 yr). The mean interval since presentation of the disease was 6 yr, ranging from 4 weeks to 27 years. Serum was collected (without haemolysis) from the first 18 PM/DM patients enrolled in the study for total CK activity measurement (mm isoform) assayed on the DAX-48 analyser (Bayer Diagnostics, Newbury, UK) by standard methods. The study was approved by King’s Healthcare Research Ethics Committee and all patients and controls gave oral informed consent.

Control groups

Urines were collected from 50 healthy controls (11 males and 39 females of mean age 43 ± 6 yr, range 37–77 yr). Urine was also collected from five patient-control groups. These patient-control groups had disorders in which there could be muscle wasting from a variety of different causes. The patient-control groups comprised: (i) 10 wheelchair-bound stroke patients (six males and four females of mean age 72 ± 8 yr, range 61–87 yr) to assess the effects of muscle wasting due to disuse atrophy; (ii) 13 rheumatoid arthritis (RA) patients receiving long-term systemic steroids (> 7.5 mg daily) (four males and nine females of mean age 58 ± 12 yr, range 26–70 yr) to determine the effects of steroids; (iii) 16 RA patients not on steroids (four males and 12 females of mean age 60 ± 12 yr, range 25–84 yr) to evaluate the effect of persisting systemic inflammation; (iv) 12 subjects with alcohol-related muscle myopathy (12 males of mean age 48 ± 7 yr, range 32–56 yr) to define the effect of toxic muscle damage; and (v) eight patients with adult-onset myotonic diseases and muscular dystrophies, including dystrophy myotonica, limb girdle and distal dystrophies (three females and eight males of mean age 31 ± 9 yr, range 23–50 yr) to assess the effects of other types of muscle-wasting disease.

Sample preparation

Urine samples were collected from all subjects and stored at −30°C prior to analysis. A 0.5 ml aliquot of each sample was placed in 5 mm NMR tubes with 0.1 ml of D2O. Sodium 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate (TSP) was added as a reference compound for accurate chemical shift calibration.

1H MRS of urine

Proton MRS was performed on a JEOL 500 MHz spectrometer. Spectra were acquired at pulse angle of 45°, repetition time of 3.5 s and 64 averages. The water resonance was suppressed by the use of homogated decoupling centred at the water frequency. The levels of major metabolites were calculated relative to the creatinine peak at 4.07 ppm. The creatinine signal at 4.07 ppm was used for quantification, as the signal at 3.05 ppm was poorly resolved from the creatine signal in a number of spectra. Likewise, the relative level of creatine was determined from the resonance at 3.93 ppm, instead of 3.03 ppm.

Quadriceps muscle strength

This was assessed in the first 18 PM/DM patients enrolled in the study. Patients were seated on a specially constructed chair with their hips and knees flexed to 90° and a restraining strap across their pelvis to minimize unwanted hip, pelvis girdle and lower trunk movement. A non-extendible strap was placed around their lower leg just above the malleoli, the other end was attached to a strain gauge that was clamped on to the frame of the chair. The patients were instructed to straighten their knee, pushing as hard as they could against the ankle strap. Because the non-extendible strap prevented joint movement, an isometric maximum voluntary contraction (MVC) was produced. The signal from the strain gauge was amplified, processed by an A-D converter, displayed on a personal computer using ‘Chart’ data handling software and collected for off-line analysis using ‘Signal Averager’ software (all from C.E.D., Cambridge, UK). Previous measurements of isometric muscle strength have been shown to be highly reliable and provide quantitative information in the assessment of patients with myositis [22] and have been used to monitor changes in the clinical variables of patients with PM/DM [23, 24].

Statistical analysis

All data are expressed as medians with interquartile ranges. Analysis of variance (ANOVA) and the Mann–Whitney U-test was used to compare changes between each subject group. The Kruskal–Wallis test was used to compare all subject groups. The Spearman rank correlation was used to correlate parameters within the same group. Differences were considered statically significant at P < 0.05.

Results

The expanded aliphatic region (2.95–4.15 ppm) of a 1H MR spectrum of urine collected from a healthy volunteer and a polymyositis patient are shown in Fig. 1. Resonances have been assigned to creatinine (Cr), creatine (Cr), trimethylamine oxide (TMAO), glycine...
were not on steroids) and 10 of 50 healthy controls. The urinary creatine/creatinine ratio exceeded 0.4 in 20 patients with PM/DM but no patients with other medical disorders and no healthy controls. These differences were highly significant by the Kruskal–Wallis test (comparing all groups) and by Mann–Whitney U-tests (comparing individual groups with PM/DM cases). Figure 2 shows these differences between groups.

There were large differences between groups in urinary betaine, citrate, glycine, choline-containing compounds and taurine and these were significantly increased in patients with DM/PM compared with healthy controls (Table 1). There were no changes in urinary lactate (results not shown) and only marginal changes in urinary TMAO. Choline-containing compounds were significantly higher in the DM/PM cases, although they were above normal levels in RA patients not on steroids. Betaine levels were also elevated in the dystrophy cases and RA patients not on steroids, taurine levels were elevated in patients with alcoholic myopathy and strokes.

In the first 18 consecutive PM/DM patients studied, urinary metabolites were correlated with serum CK and quadriceps strength. There were positive correlations between CK activities and choline-containing compounds ($r = 0.78$, $P = 0.0006$) and also between CK activities and betaine ($r = 0.57$, $P = 0.026$). Citrate was inversely correlated with quadriceps strength ($r = -0.81$, $P = 0.0012$). No significant correlations were found between creatine level with either CK activities or quadriceps strength. Five of these 18 patients with DM/PM did not have elevated CK activities (>200 units/ml) and median urinary creatine levels were similar in patients with normal and those with elevated CK activities (0.80 and 0.70, respectively). By contrast, median urinary choline-containing compounds and betaine levels were lower in patients with normal CK levels (for choline-containing compounds 0.06 and 0.15, respectively, and for betaine 0.06 and 0.23, respectively).

### Table 1. Creatine and other urinary metabolites in PM/DM and control groups

<table>
<thead>
<tr>
<th></th>
<th>Normal controls $(n=50)$</th>
<th>PM/DM $(n=34)$</th>
<th>Adult-onset myopathy $(n=12)$</th>
<th>Alcoholic myopathy $(n=8)$</th>
<th>Chronic stroke $(n=10)$</th>
<th>RA on steroids $(n=13)$</th>
<th>RA not on steroids $(n=16)$</th>
<th>Overall difference Kruskal–Wallis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine Median</td>
<td>0</td>
<td>0.54*</td>
<td>0**</td>
<td>ND**</td>
<td>0**</td>
<td>ND**</td>
<td>0**</td>
<td>0.00001</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.00–0.00</td>
<td>0.04–0.81</td>
<td>0.00–0.00</td>
<td>–</td>
<td>0.00–0.00</td>
<td>–</td>
<td>0.00–0.00</td>
<td></td>
</tr>
<tr>
<td>Choline Median</td>
<td>0.02</td>
<td>0.07*</td>
<td>0**</td>
<td>0.01**</td>
<td>0.02**</td>
<td>0.01**</td>
<td>0.04**</td>
<td>0.00001</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.00–0.04</td>
<td>0.06–0.10</td>
<td>0.00–0.02</td>
<td>0.00–0.06</td>
<td>0.00–0.06</td>
<td>0.00–0.04</td>
<td>0.03–0.05</td>
<td></td>
</tr>
<tr>
<td>Taurine Median</td>
<td>0.00</td>
<td>0.08*</td>
<td>0**</td>
<td>0.12</td>
<td>0.09</td>
<td>0.06**</td>
<td>0**</td>
<td>0.001</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.00–0.05</td>
<td>0.00–0.15</td>
<td>0.00–0.05</td>
<td>0.08–0.25</td>
<td>0.09–0.13</td>
<td>0.03–0.10</td>
<td>0–0.07</td>
<td></td>
</tr>
<tr>
<td>Betaine Median</td>
<td>0.00</td>
<td>0.12*</td>
<td>0.07</td>
<td>ND**</td>
<td>ND**</td>
<td>ND**</td>
<td>0.05</td>
<td>0.00001</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.00–0.05</td>
<td>0.04–0.28</td>
<td>0.02–0.14</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.02–0.09</td>
<td></td>
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<tr>
<td>Citrate Median</td>
<td>0.36</td>
<td>0.67*</td>
<td>0.39**</td>
<td>0.28**</td>
<td>0.38**</td>
<td>0.21**</td>
<td>0.34**</td>
<td>0.0008</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.27–0.51</td>
<td>0.36–0.80</td>
<td>0.32–0.47</td>
<td>0.21–0.36</td>
<td>0.30–0.50</td>
<td>0.09–0.32</td>
<td>0.19–0.55</td>
<td></td>
</tr>
<tr>
<td>Glycine Median</td>
<td>0.13</td>
<td>0.19*</td>
<td>0.09**</td>
<td>0.09**</td>
<td>0.15</td>
<td>0.09**</td>
<td>0.16</td>
<td>0.0076</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.10–0.19</td>
<td>0.12–0.25</td>
<td>0.05–0.11</td>
<td>0.03–0.12</td>
<td>0.12–0.22</td>
<td>0.09–0.14</td>
<td>0.09–0.20</td>
<td></td>
</tr>
<tr>
<td>TMAO Median</td>
<td>0.09</td>
<td>0.07</td>
<td>0.05</td>
<td>0.15</td>
<td>0.32</td>
<td>0.07</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.04–0.17</td>
<td>0.04–0.18</td>
<td>0.04–0.08</td>
<td>0.10–0.27</td>
<td>0.18–0.37</td>
<td>0.05–0.09</td>
<td>0.04–0.08</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different ($P < 0.01$) from healthy control group by Mann–Whitney U-test.

**Significantly different ($P < 0.03$) from myositis group by Mann–Whitney U-test.

ND, not detected in any patient in group.
Urinary MR assessment of PM/DM

In PM/DM subjects, there appears to be no evidence of increased creatine biosynthesis, suggesting an alternative mechanism for the creatinuria observed in this study. Moreover, in vivo magnetic resonance spectroscopy studies have shown that although skeletal muscle of PM/DM subjects has a reduced inorganic phosphate to phosphocreatine ratio compared with healthy subjects, they have normal levels of total creatine [15–18]. This suggests that in PM/DM there is a derangement in the retention of intracellular creatine, rather than a reduction in its uptake by skeletal muscle cells, but the possibility of abnormal renal reabsorption in PM/DM cannot be fully dismissed and may be a contributory factor. Thus, the creatinuria observed in PM/DM most likely results from cell damage, with creatine leakage increasing from skeletal muscle cells. This increase in circulating creatine appears to exceed the renal threshold leading to creatinuria. The possibility of using urinary creatine in the PM/DM diagnosis requires further discussion. The fact that increased urinary creatine can be observed in other conditions and that its level does not correlate with clinical measurements of the disease, including serum CK and muscle strength, suggest that its use as a diagnostic marker may be limited [36]. However, the fact that urinary creatine levels do not correlate with serum CK may arise from the fact that the mechanism of CK release is different to that of creatine in damaged muscle cells.

A number of other metabolites, including choline-containing compounds, taurine and citrate, were also shown to be significantly increased in the urine from PM/DM subjects. However, these changes showed lesser specificity than creatine, since abnormalities in the excretion of these metabolites have been previously reported in a number of conditions [35–38]. The pattern of excretion of these metabolites suggests a common source and mechanism, probably involving increased cellular release coupled to decreased renal reabsorption. Choline, phosphorylcholine and glycerophosphocholine are present in high concentration in most cells and would be released once cell membrane integrity is compromised. This fits well with the positive correlation between these metabolites and serum CK activities, as CK is released simultaneously into the blood when cell membranes are disrupted [2]. It is also likely that alteration in membrane metabolism leads to changes in the total choline pool, which in turn may affect the levels of many choline-containing compounds. Similarly, urinary citrate excretion has been used as a marker for renal mitochondrial metabolism [39] and its excretion is sensitive to changes in systemic acid–base balance [40, 41]. In PM/DM, urinary citrate was increased when compared with controls and inversely correlated with muscle strength. As with choline-containing metabolites, plasma citrate may be significantly raised by increased muscle leakage, leading to elevated cytosolic citrate in renal cells and therefore increased urinary excretion. Thus, the correlation observed between serum CK activities and some of these metabolites might be part of a non-specific change in metabolism, principally poor tissue retention.

**Discussion**

$^1$H MRS of urine from patients with PM/DM shows consistent, highly significant changes in their metabolic profiles when compared with controls. Urinary creatine was only present in substantial levels in PM/DM patients, while betaine, citrate, glycine, choline-containing compounds and taurine levels were significantly increased. Taurine levels were also raised in patients with strokes and alcoholic myopathy. Although elevated urinary creatine levels in PM/DM patients have been previously reported [25], changes in their concentration in relation to disease status, serum CK activity and objective measures of quadriceps strength have not been described. Elevations of urinary taurine, citrate, glycine, betaine and choline-containing compounds in PM/DM have not previously been reported.

The origin of high levels of urinary creatine in PM/DM subjects is not clear. Creatine is synthesized from amino acid precursors in the liver, kidneys and pancreas, and transported in the blood to the target tissues [26, 27]. Most of the body creatine pool is located in the muscle and neural tissue and its uptake into muscle cells occurs by an active mechanism against a concentration gradient, and shows a sodium dependence [28]. The human Na$^+$-dependent creatine transporter has now been cloned allowing better understanding of how cells regulate their intracellular creatine stores [29, 30]. This appears to occur at the level of creatine biosynthesis as well as the direct regulation of the creatine transporter by extracellular creatine levels. In healthy subjects, circulating creatine is efficiently maintained by the kidneys with a threshold for creatine excretion of about 0.05 mmol/l [31], which is slightly above the plasma concentration. Conditions that lead to significant increases in circulating creatine, either by increased biosynthesis, dietary supplementation, reduced muscle absorption or cell leakage, can readily lead to creatinuria [26, 32, 33]. Similarly, creatinuria can also arise from decreased renal reabsorption [34, 35].

**Fig. 2.** Urinary creatine/creatinine ratios by diagnostic groups.
Taurine (2-amino-ethyl sulphonic acid), a ubiquitous free amino acid prominent in type I muscle fibres [42], is an end product of methionine and cysteine metabolism. Its urinary levels have been proposed as a potential biochemical marker of total body protein status [43]. Waterfield et al. [43] suggested that decreased protein synthesis leads to an increase in the pool of amino acids available for taurine synthesis, which in turn would lead to elevated urinary taurine. Improved protein synthesis appears to reverse this metabolic change. The increased levels of urinary taurine in PM/DM, stroke and alcoholic myopathy patients may therefore indicate reduced levels of protein accretion in skeletal muscle. The reduction in taurine excretion in PM/DM subjects on steroid treatment may reflect improved muscle protein synthesis. However, this potential marker needs further validation before it can be used as a non-invasive method for assessing skeletal muscle atrophy.

In conclusion, we have shown significant differences in the urinary levels of creatine, choline-containing metabolites, betaine and citrate in PM/DM subjects compared with controls, although further work is required to elucidate the underlying metabolic processes. Now that specific metabolites associated with myositis have been identified using MR spectroscopy, the next step is to measure one or more of these using routine analytical methodologies, such as HPLC, that are readily available in a clinical biochemical laboratory.

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References