APPLICATION OF NMR SPECTROSCOPY TO MONITORING MELAS TREATMENT: A CASE REPORT

HARALD E. MÖLLER, PhD,1,2,3 DIRK WIEDERMANN, PhD,1
GERHARD KURLEMANN, MD,4 THORSTEN HILBICH, PhD,1 and
GERHARD SCHUIERER, MD2

1 Instituto für Physikalische Chemie, Universität Münster, Münster, Germany
2 Instituto für Klinische Radiologie, Universität Münster, Münster, Germany
3 Max-Planck-Institut für Neuropsychologische Forschung, Leipzig, Germany
4 Klinik und Poliklinik für Kinderheilkunde, Universität Münster, Münster, Germany

Accepted 4 December 2001

A point mutation at base pair 3243 or 3271 of the mitochondrial transfer RNA (tRNA)Leu(UUR) gene is most frequently the underlying cause of the maternally inherited syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS).23 Common biochemical defects observed at muscle biopsy are impaired complex I or IV activities or multiple defects of respiratory chaining enzymes. The disease is characterized clinically by strokelike episodes associated with cerebral infarctions on magnetic resonance imaging (MRI) of the head; seizures; hemiparesis; visual loss; and progressive dementia, headaches, vomiting, and muscle weakness accompanied by accumulation of lactate. It is assumed that the infarcts are nonvascular and are due to transient oxidative-phosphorylation dysfunction within the brain parenchyma. The clinical course is variable, with patients becoming progressively disabled in a stepwise fashion. Therapeutic drug trials aim at the improvement of aerobic energy production and prevention of acidosis-related damage. However, their effectiveness is difficult to assess in view of the diversity of clinical manifestations and the fluctuating nature of the clinical course.

Localized magnetic resonance spectroscopy

ABBREVIATIONS: γ-ATP, γ-phosphate of ATP; Acq, acquisitions; ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; CK, creatine kinase; Cr, creatine; DANTE, delays alternating with nutations for tailored excitation; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAA, N-acetylaspartyl compounds; PCR, phosphocreatine; P, inorganic phosphate; PP, phosphorylation potential; ST, saturation transfer; STEAM, stimulated echo acquisition mode; tCr, total creatine; TA, acquisition time; TE, echo time; TR, repetition time; tRNA, transfer RNA; VOI, volume of interest

Key words: 1H magnetic resonance spectroscopy; 31P magnetic resonance spectroscopy; creatine kinase; creatine supplementation; MELAS

Correspondence to: H.E. Möller, Max Planck Institute of Cognitive Neuroscience, Stephanstraße 1a, D-04103 Leipzig, Germany; e-mail: moeller@cns.mpg.de

© 2002 Wiley Periodicals, Inc. Published online 11 March 2002 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.10084
(MRS) permits the noninvasive investigation of metabolic abnormalities in human tissues in vivo. In MELAS patients, previous proton MRS studies of the brain have demonstrated an elevation of the intracellular lactate level and a decreased concentration of N-acetylaspartyl compounds (NAA; i.e., mostly N-acetylaspartate with an additional contribution from N-acetylaspartyl glutamate). Phosphorus MRS investigations of brain and skeletal muscle have revealed a decreased concentration of phosphocreatine (PCr), whereas that of inorganic phosphate (P_i) was elevated. Unlike muscle biopsy, MRS permits noninvasive monitoring of treatment and may be a potentially useful tool for assessing therapeutic efficacy. In patients with primary mitochondrial disorders, serial 1H MRS examinations have demonstrated a decline of intracerebral lactate levels during treatment with sodium dichloroacetate. Using skeletal muscle 31P MRS, evidence for improved cellular energy state has been obtained in a MELAS patient following administration of riboflavin and nicotinamide.

Recently, Hagenfeld et al. reported reduced frequency and intensity of headaches and improvement of work performance in a MELAS patient during creatine (Cr) treatment. In normal subjects, it is well established that high-dose Cr intake for several days may elevate skeletal muscle Cr and PCr levels and improve performance during high-intensity, intermittent exercise. This beneficial effect was postulated to be due to higher pre-exercise PCr stores and an improved PCr resynthesis capacity. To study the physiological mechanisms underlying potentially beneficial effects of oral Cr supplementation, we used 31P MRS for monitoring the therapeutic response in a girl with MELAS. Resting muscle intracellular phosphates and fluxes through the creatine-kinase (CK) reaction were repeatedly investigated by static spectroscopy and saturation-transfer (ST) measurements. In parallel, additional 1H spectra of the brain were recorded before and 9 weeks after initiation of Cr treatment.

MATERIALS AND METHODS

Case Report. Our patient is a 14-year-old girl, the third child of nonrelated parents. Her siblings are healthy. Pregnancy and birth were uneventful, and the milestones of development were reached within the normal range. At the age of 9 years, epilepsy partialis continua with continuous twitches of the right face, arm, and leg occurred, accompanied by severe headache and “eye blinking” of the left eye. “Eye blinking” was the description given by the patient with reference to continuous visual flashes of lightning. The girl was functionally blind in her left eye. Electroencephalography showed continuous spikes in the left precentral and occipital region. Although resistant to all medical therapeutic trials with conventional antiepileptic drugs, the seizures stopped spontaneously after some days. They were followed by a Todd’s paresis that lasted for several days. Lactate level was elevated in the cerebrospinal fluid and plasma. A point mutation at base pair 3243 of the mitochondrial tRNA<sub>Leu(UUR)</sub> indicated MELAS syndrome. Therapeutic trials were started with thiamine, riboflavin, coenzyme Q<sub>10</sub>, and carnitine. Finally, Cr monohydrate at a dose of 5 g/day (body weight, 24 kg) was introduced for 10 weeks. All treatments were tolerated well, but none was successful in eliminating plasma lactate or seizure activity. The epileptic syndrome was resistant to all antiepileptic drugs. The patient is now receiving lamotrigine and phenobarbital, with blood levels in the therapeutic range. She is unable to walk alone, is blind, and has developed sensorineural hearing loss. She has to be tube-fed.

Magnetic Resonance Imaging. All MRI examinations were performed at 1.5 T (Magnetom 63SP; Siemens, Erlangen, Germany). The study was conducted according to the Declaration of Helsinki after informed written consent had been obtained. Conventional spin-echo sequences were employed for MRI. An axial double-echo proton-density and T2-weighted sequence (repetition time, TR 2700 ms; echo times, TE 15/90 ms; acquisitions, Acq 1; matrix 192 × 256; acquisition time, TA 8:42 min) and a coronal T1-weighted sequence (TR/TE 450/15 ms; Acq 2; matrix 256 × 256; TA 3:55 min) were performed. The field of view was 230 mm.

The first MRI study, which was performed before Cr treatment, showed multiple, mainly cortical, defects located in the cerebellar vermis, the right cerebellar hemisphere, the right occipital lobe, and the right parietal lobe. Furthermore, symmetric defects of the pallidum were found. The T2-weighted images showed edematous swelling of the cortex and mainly subcortical white matter of the right parieto-occipital operculum extending into the dorsal parietal cortex and of the left parietal operculum. These lesions showed only mild signal loss on T1-weighted images resulting in a pseudothickening of the cortex. The follow-study after 9 weeks of Cr treatment showed the same defects without any changes (Fig. 1). The opercular lesion on the left had resolved without any definite defect, the right opercular lesion resulted in small bright area on T2-weighted images, possibly resembling a gliotic defect smaller.
than the original lesion. However, a new left occipital lesion including the left calcarine fissure and extending into the parietal and paramedian cortex had evolved. The MRI changes of the new lesion resembled the acute opercular lesions of the first study. Pseudoterritorial lesions resulting only partially in defects on follow-up studies are common and quite typical in patients with MELAS. The symmetric defects of the basal ganglia resemble those seen after cerebral anoxia and may be considered evidence of a previous episode of a more global cerebral energy crisis in this patient.

**1H Spectroscopy.** Proton spectra were recorded with the standard imaging head coil immediately following the MRI examinations. Two 8-ml volumes of interest (VOI) with predominantly gray matter, one centered in the right opercular cortex and the other midline in the occipital cortex (Fig. 2) were repeatedly investigated 4 days before initiating Cr treatment and 9 weeks thereafter. All spectra were recorded applying the STEAM (stimulated echo acquisition mode) technique (TR/TE 3000/20 ms; 1-K complex data points, Acq 128).

**31P Spectroscopy.** An 80-mm diameter surface coil was employed for 31P MRS studies. A baseline examination was performed immediately before initiation of Cr treatment (4 days after the first 1H MRS study) and a second one after 10 weeks (9 days after the second 31P MRS study) on the last day of Cr treatment. Spectra were recorded mainly from the gastrocnemius muscle with a pulse-and-acquire technique (average flip angle 50°, TR 5 s, 1-K complex data points, Acq 32). An initial static spectrum served for measuring steady-state peak areas and positions. For kinetic measurements, selective saturation of the resonance of either PCr or the γ-phosphate of adenosine 5′-triphosphate (γ-ATP) was achieved by a DANTE (delays alternating with nuta-
ADP levels and V/Vmax were computed from the CK reaction was performed by LCModel using a data base MRS data to obtain absolute metabolite concentrations, the ratio of the mitochondrial status was assessed from the concentration of calibrated model spectra. Previously recorded 1H spectra (unpublished data) of normal cortical gray matter from six children (mean age, 14.3 years; range, 10–17 years) served as a basis for comparison.

Processing of the 31P data included zero-filling to 2 K, exponential multiplication (5-Hz line broadening), Fourier transformation, and Lorentzian line fitting to determine resonance positions and areas. The pH was calculated from the chemical-shift difference between Pi and PCr, and the intracellular free Mg2+ concentration, [Mg2+]i, was computed using the chemical shifts of the α- and β-phosphate of ATP (α-ATP, β-ATP). To obtain concentration estimates, the peak area of the β-ATP resonance was used as a reference and assumed to represent a cytosolic ATP level of 8.2 mmol/L. Functional mitochondrial status was assessed from the concentration of cytosolic free adenosine 5′-diphosphate (ADP), the phosphorylation potential (PP), defined as [ATP]/([ADP] × [Pi]), and the ratio of the mitochondrial ATP synthesis rate over its maximum value according to V/Vmax = 1/1 + (0.53 × [PCr]/[Pi]).

In skeletal muscle, ADP is micromolar and, hence, below the sensitivity of in vivo 31P MRS. Therefore, ADP levels and V/Vmax were computed from the CK reaction:

\[ \text{PCr}^{2-} + \text{MgADP}^- + \text{H}^+ \xrightarrow{k_{\text{for}}} \text{Cr} + \text{MgATP}^{2-}, \]

by exploiting the metabolite ratio Pi/PCr as advocated by Chance et al. For comparison, reference 31P spectra of the resting calf muscle were taken from a previous study (unpublished data) in nine healthy volunteers (mean age, 26.8 years; range, 21–33 years).

A detailed description of the ST technique and its underlying theory, including a thorough analysis of the experimental limitations, is presented in a separate publication. Briefly, pseudo–first-order rate constants for the exchange of phosphate between ATP and PCr through the CK reaction, can be defined as k+1 = k for × [ADP] × [H+] and k−1 = k rev × [Cr]. The corresponding fluxes are therefore given by v for = k+1 × [PCr] and v rev = k−1 × [ATP]. The time-dependent behavior of the PCr and the γ-ATP signals during the ST experiment are dependent on k+1 and k−1. Hence, the pseudo–first-order rate constants are obtained by plotting the peak areas of both resonances as a function of the mixing time for both ST experiments (i.e., the one in which the PCr signal is saturated and another in which γ-ATP is saturated) and simultaneous fitting of all four time courses using nonlinear least-squares regression. Reference data for the normal resting calf muscle were acquired in three healthy volunteers (mean age, 31.3 years; range, 31–32 years).

Differences between patient and control data were regarded as significant on the 1%, 0.01%, or 0.0001% level if they exceeded 2.5×, 4×, or 5× the standard deviation (SD) in the control group.

RESULTS

1H Spectroscopy. Figure 2 shows brain spectra from repeated investigations of the two VOIs. A doublet signal (splitting of 7 Hz) from intracerebral lactate at 1.33 ppm, which is not readily detected by 1H MRS at 1.5 T in normal brain, was clearly visible in all patient spectra. Metabolite concentrations are summarized in Table 1. Before Cr treatment, an acute strokelike lesion was found in the right parieto-occipital operculum (Fig. 2A). The spectrum from this area (Fig. 2B) indicates an extreme elevation of lactate and reduced NAA (31% of the mean control value). In addition, glutamate and myo-inositol were reduced, whereas the levels of other metabolites, such as glutamine, total creatine (tCr, i.e., Cr plus PCr), and choline compounds, were normal. After 9 weeks, the lesion had almost resolved (Fig. 2C). In the corresponding spectrum (Fig. 2D), the area of the lactate signal was markedly reduced (22% of the value of the first examination), though it was still abnormal. Additionally, NAA partly recovered (to 53% of normal) but still remained below control levels. Similar findings, that is, elevated lactate and decreased NAA (55% of the mean control value) plus reduced glutamate and myo-inositol, were observed in a 1H spectrum (Fig. 2F) acquired midline in the occipital cortex. Note that this location appeared normal at the first MRI examination (Fig. 2E), whereas a strokelike episode occurred during Cr treatment (Fig. 2G). The corresponding 1H spec-
trum from the second examination (Fig. 2H) demonstrated a large increase in lactate (210% of the initial value) and progressive reduction of NAA (to 49% of normal).

Static 31P Spectroscopy. Table 2 gives a summary of metabolic parameters extracted from 31P spectra without ST. The level of free intracellular Mg2+ and the cytosolic pH were within the normal range and did not change significantly upon Cr treatment. In the baseline examination, PCr was only 80% of the mean control value, whereas Pi was elevated at 153%. The estimated free ADP concentration was increased at 191% with a concomitant elevation in V/Vmax (165% of the mean control value). The phosphorylation potential was well below the minimum value found in the control group (135 mmol⁻¹) and was 38% of the mean control value. At the end of Cr treatment, PCr was increased by 11% but still did not reach the mean control level (89%). None of the other markers of an abnormal energy metabolism showed a trend towards normalization: P, ADP, and V/Vmax progressively increased as compared with the mean control values (260%, 279%, and 204%, respectively), whereas the phosphorylation potential further declined (21%).

Kinetic Investigations. Saturation-transfer 31P spectra from the patient’s calf muscle are presented in Figure 3. Kinetic parameters extracted from fitting the data are given in Table 3. The rate constants k⁺ and k⁻ and the fluxes v for and vrev were somewhat higher than in the control group (167%, 157%, 135%, and 157%, respectively) although only the differences for k⁻ and vrev were significant. None of the kinetic parameters was significantly affected by Cr treatment.

DISCUSSION

Our combined findings from 1H an 31P MRS underline the marked involvement of both brain and skeletal muscle in mitochondrial dysfunction. Results from proton spectroscopy coincide with previous observations in MELAS patients. The generalized increase in lactate may be ascribed to abnormal up-regulation of glycolytic activity throughout the brain, including regions that appear normal on MRI. This is consistent with impairment of oxidative phosphorylation due to defective mitochondrial complex I. N-acetyl-l-aspartate is synthesized in brain mitochondria from acetyl-CoA and aspartate and is believed to be present primarily in neurons. The decline in NAA seen in MELAS patients is therefore attributed to neuroaxonal damage. This is further supported by the accompanying decrease of glutamate by an amount similar to that of NAA, which was also ob-

Table 1. Cytosolic metabolite concentrations (in mmol/L) obtained from cortical gray matter with 1H MRS.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control data (n = 6)</th>
<th>MELAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>On treatment</td>
</tr>
<tr>
<td>Lactate</td>
<td>&lt;1.0</td>
<td>14.1*</td>
</tr>
<tr>
<td>NAA</td>
<td>11.8 ± 1.4</td>
<td>3.6*</td>
</tr>
<tr>
<td>Glutamate</td>
<td>11.0 ± 2.3</td>
<td>4.4‡</td>
</tr>
<tr>
<td>Glutamine</td>
<td>4.9 ± 1.2</td>
<td>6.0</td>
</tr>
<tr>
<td>tCr</td>
<td>6.9 ± 0.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Choline</td>
<td>1.1 ± 0.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>5.6 ± 0.3</td>
<td>2.6*</td>
</tr>
</tbody>
</table>

*Beyond ±2 SDs (i.e., P < .000001) of the mean value in controls.
†Beyond ±4 SDs (i.e., P < .000001) of the mean value in controls.
‡Beyond ±2.5 SDs (i.e., P < .001) of the mean value in controls.

Table 2. Cytosolic phosphate metabolite ratios and concentrations measured in the resting gastrocnemius muscle with 31P MRS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Control data (n = 9)</th>
<th>MELAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>On treatment</td>
</tr>
<tr>
<td>PCr/ATP</td>
<td></td>
<td>3.53 ± 0.19</td>
<td>2.81*</td>
</tr>
<tr>
<td>P/ATP</td>
<td></td>
<td>0.55 ± 0.08</td>
<td>0.84*</td>
</tr>
<tr>
<td>P/PCr</td>
<td></td>
<td>0.16 ± 0.02</td>
<td>0.30‡</td>
</tr>
<tr>
<td>[PCr] mmol/L⁻¹</td>
<td>28.9 ± 1.5</td>
<td>23.0*</td>
<td>25.6</td>
</tr>
<tr>
<td>[P] mmol/L⁻¹</td>
<td>4.5 ± 0.7</td>
<td>6.9*</td>
<td>11.7†</td>
</tr>
<tr>
<td>[ADP] μmol/L⁻¹</td>
<td>8.9 ± 1.3</td>
<td>17.0†</td>
<td>24.8‡</td>
</tr>
<tr>
<td>[Mg2+] mmol/L⁻¹</td>
<td>0.9 ± 0.3</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.04 ± 0.02</td>
<td>7.09</td>
</tr>
<tr>
<td>PP mmol⁻¹</td>
<td>204 ± 64</td>
<td>78</td>
<td>42*</td>
</tr>
<tr>
<td>V/Vmax</td>
<td></td>
<td>0.23 ± 0.03</td>
<td>0.38‡</td>
</tr>
</tbody>
</table>

*Beyond ±2.5 SDs (i.e., P < .01) of the mean value in controls.
†Beyond ±4 SDs (i.e., P < .000001) of the mean value in controls.
‡Beyond ±2.5 SDs (i.e., P < .01) of the mean value in controls.
served by Wilichowski and colleagues. Bates et al. demonstrated that partial inhibition of single complexes of the respiratory chain can result in decreased NAA production. Impaired mitochondrial function may thus contribute to the observed reduction in NAA without cell death.

Metabolic alterations are considerably pronounced in MRI-detectable lesions. Extensive accumulation of lactate is indicative of a severe energy failure and acute hypoxia during a stroke-like episode. This may, in turn, cause neuronal dysfunction, as evidenced by further reduction in NAA. Similar to previous observations, transient recovery of NAA and regression of the original lesion at MRI were found in serial investigations (Figs. 2A–2D). This may indicate changes in intracellular NAA as a result of a reversible impairment of mitochondrial function, consistent with the above hypothesis of a decreased NAA production due to defective respiratory chain complex. Comparing the time courses of metabolic changes in both VOIs, the possibility that the transient recovery of NAA and decline of lactate observed in the parieto-occipital operculum were related to Cr treatment may be discounted because the opposite trends were found in occipital cortex. This also indicates that evaluation of a single VOI is insufficient for reliably assessing response to therapy.

The cellular ATP concentration at rest is tightly regulated and was found to be unaffected upon Cr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Control data (n = 3)</th>
<th>MELAS Baseline</th>
<th>On treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_+$</td>
<td>s$^{-1}$</td>
<td>0.39 ± 0.13</td>
<td>0.65</td>
<td>0.53</td>
</tr>
<tr>
<td>$k_-$</td>
<td>s$^{-1}$</td>
<td>0.99 ± 0.09</td>
<td>1.55*</td>
<td>1.59*</td>
</tr>
<tr>
<td>$k_-/k_+$</td>
<td></td>
<td>2.6 ± 0.6</td>
<td>2.4</td>
<td>3.0</td>
</tr>
<tr>
<td>$v_{for}$</td>
<td>mmol/L·s$^{-1}$</td>
<td>11.1 ± 3.1</td>
<td>15.0</td>
<td>13.6</td>
</tr>
<tr>
<td>$v_{rev}$</td>
<td>mmol/L·s$^{-1}$</td>
<td>8.1 ± 0.7</td>
<td>12.7*</td>
<td>13.0*</td>
</tr>
</tbody>
</table>

*Beyond ±5 SDs (i.e., P < .000001) of the mean value in controls.
supplementation.\textsuperscript{18} The assumption of a normal ATP level has also been verified at muscle biopsy in single cases of mitochondrial myopathies.\textsuperscript{19,31} Therefore, using PCr/ATP and P\textsubscript{i}/ATP to calculate PCr and P\textsubscript{i} concentrations seems justified. Qualitatively and quantitatively, our \textsuperscript{31}P MRS investigations of the resting gastrocnemius muscle revealed typical features observed in patients with mitochondrial myopathies,\textsuperscript{2} including elevated ADP and P\textsubscript{i} coupled to reduced PCr. Again, these abnormalities reflect the inability of the mitochondria to efficiently utilize oxygen for ATP synthesis. Phosphocreatine and ADP are interrelated via the CK reaction (Eq. 1), which is activated by a rising level of ADP to restore ATP.\textsuperscript{8} Mitochondrial activity determines the phosphorylation potential, which is low, indicating a low energy reserve in the resting muscle. Finally, defective mitochondrial functionality is apparent in an abnormal transfer function for ADP control given by an increased $V/V_{\text{max}}$, which points to accelerated ATP production in functioning mitochondria to fulfill the energy demands of the cell.\textsuperscript{3,12} The rise in the resting muscle PCr by 11\% upon Cr supplementation is at the lower end of increases observed in previous studies of healthy volunteers.\textsuperscript{20,30,35} Besides the moderately increased PCr availability, Cr treatment did not improve any of the other parameters characterizing muscular energy status at rest (Table 2).

Our kinetic parameters from the control group describing flux through the CK reaction for the direction of ATP synthesis are within the range of previous literature results.\textsuperscript{14,28} The corresponding reverse reaction has not yet been investigated in human muscle. Consistent with findings in animals,\textsuperscript{6} the ratio $k_{-1}/k_{+1}$ was approximately 3. Fluxes $v_{\text{for}}$ and $v_{\text{rev}}$ were similar, suggesting near-equilibrium conditions of the CK reaction. It should be noted that kinetic parameters of the resting muscle do not adequately describe conditions of work or recovery from exercise.

An increased concentration of cytosolic ADP, as found in our patient (Table 2), would lead to a higher pseudo-first-order rate constant $k_{-1}$ consistent with the observed trend (Table 3). A similar effect on $k_{+1}$ might result from an increased availability of free Cr. However, as the CK reaction is near equilibrium in resting muscle (see above discussion), PCr resynthesis should not be limited by the CK reaction under out experimental conditions; hence, variations in Cr per se should not lead to pronounced changes in the CK kinetics. This is in keeping with our observation that Cr treatment did not produce significant effects on the unidirectional CK reaction rates and fluxes.

In summary, there was no clinical improvement from Cr treatment in our patient. Consistently, except for a slightly elevated intramuscular PCr concentration, no further beneficial effect on various parameters of cellular energy metabolism could be found with static or kinetic investigations using \textsuperscript{31}P MRS. Cerebral metabolic disturbances and their progress with time observed with \textsuperscript{1}H MRS were typical for MELAS without demonstrating indications of a beneficial influence from Cr treatment. In conclusion, follow-up MRS studies are capable of providing objective markers of treatment response in mitochondrial myopathies in vivo without the potential damage inflicted by biopsy. This is particularly important because of the necessarily limited number of patients with such rare disorders that can be studied with any given protocol.

A portion of this work was presented at the 8th scientific meeting of the International Society for Magnetic Resonance in Medicine in April 2000, Denver, Colorado.

\textbf{REFERENCES}


