
OBJECTIVE. The purpose of our study was to show how, despite pathognomonic signs of cerebral involvement in chronic progressive external ophthalmoplegia (CPEO), mitochondrial respiratory chain insufficiency is associated with increased lactate and reduced $N$-acetyl-laspartate. CPEO and mitochondrial myopathy are caused by mitochondrial DNA mutations leading to impaired oxidative phosphorylation. Cortical and subcortical metabolites, cerebral diffusivity, and structural MRI were assessed to characterize possible subclinical cerebral pathology in CPEO.

SUBJECTS AND METHODS. Ten patients with CPEO ($n = 8$), mitochondrial myopathy ($n = 1$), and Kearns-Sayre syndrome ($n = 1$) and 13 control group volunteers were studied by MRI, both long TE (144) proton MR spectroscopic imaging ($^1$H MRSI), and diffusion-weighted imaging. Relative concentrations of $N$-acetyl-laspartate, choline, creatine, and lactate were estimated by Linear Combination of Model Spectra (LCModel) in healthy-appearing white matter, gray matter, and white matter hyperintensities.

RESULTS. Of five patients with cortical atrophy, it was moderate in three and severe in two. One patient had severe and four had moderate cerebellar atrophy. Six of 10 patients showed unspecific white matter lesions, whereas the remainder had hyperintensities in the pyramidal tract ($n = 2$) and middle cerebellar peduncle ($n = 1$) despite clinical signs. No basal ganglia lesions were found. Physiologic metabolite ratios were normal and lactate was absent in supratentorial healthy-appearing cortex and subcortical white matter. Global diffusion histogram metrics revealed no abnormalities.

CONCLUSION. Normal spectroscopic imaging in radiologic unaffected brain and healthy global brain parenchymal diffusion findings do not support the hypothesis of a generalized cerebral energy loss in CPEO. Bilateral structural alteration of central motor pathways in two patients without clinical pyramidal signs may, however, reflect subclinical axonal injury in predilection sites in some patients.

Mitochondrial myopathy, chronic progressive external ophthalmoplegia (CPEO), CPEO-plus, and the Kearns-Sayre syndrome [1–6] are caused by mutations of mitochondrial DNA and probably reflect a clinical continuum. Mitochondrial myopathy and CPEO are pure myopathies, the latter with ptosis and external ophthalmoplegia as hallmarks of the disease. CPEO-plus has one or more additional nonmyopathic symptoms like ataxia, dementia, or sensorineural hearing loss. Kearns-Sayre syndrome, the most severe form, is a multisystem disorder characterized by an early onset of external ophthalmoplegia and pigmentary retinopathy. It may also involve ataxia, heart block, and elevated cerebral spinal fluid protein.

Skeletal muscle biopsies in patients with mitochondrial myopathy, CPEO, and Kearns-Sayre syndrome reveal abnormally proliferating mitochondria that cause ragged red fibers, a hallmark of the severe biochemical defects of oxidative phosphorylation in many mitochondrial encephalomyopathies, including MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) syndrome [7–12] and MERRF (myoclonus epilepsy with ragged red fibers) syndrome [13, 14].

Patients with CPEO and Kearns-Sayre syndrome are reported to show cortical and cerebellar atrophy, peripheral white matter signal abnormalities, and involvement of the deep gray matter nuclei in MRI [15, 16]. However, these findings are nonspecific and in the absence of clear-cut symptoms and laboratory results, the imaging features cannot be considered pathognomonic. Carlow et al. [17] reported a significant reduction of extracocular muscle size in CPEO patients that...
may facilitate the differential diagnosis of extraocular muscular paresis. At present, MR spectroscopy data on mitochondrial myopathy, CPEO, and Kearns-Sayre syndrome are rare and controversial. Some studies found increased lactate, mostly in Kearns-Sayre syndrome patients, whereas others did not observe lactate increases in CPEO. Decreased N-acetylaspartate/creatine was also noted in only some patients [4, 18–20]. One study reported increased amino acids in three of four CPEO patients [21].

However, the presence of lactate has been found more consistently in two other mitochondrial disorders, MELAS and MERRF, regardless of MRI data. This suggests that MR spectroscopy might be more sensitive for detecting abnormalities associated with mitochondrial disorders. MR diffusion has recently been shown to be sensitive in detecting subclinical ischemic events [22] and provides additional information in mitochondrial disorders [23].

Our study assesses possible subclinical cerebral pathology in mitochondrial myopathy, CPEO, and Kearns-Sayre syndrome patients by using hydrogen-1 MR spectroscopic imaging (1H MRSI) and MR diffusion measurements thought to be particularly sensitive to acute or chronic energy failure. We hypothesized that mitochondrial respiratory chain insufficiency is associated with increased lactate and reduced N-acetylaspartate and total creatine. Also, mean diffusivity changes can be expected either as a decrease in acute lesions reflecting cytotoxic edema or as an increase in patients with chronic lesions.

Subjects and Methods

Patients

Ten patients (5 women and 5 men; mean age, 37.9 years; range, 20–73 years) with established diagnoses of mitochondrial myopathy [1], CPEO [4], CPEO-plus [4], or Kearns-Sayre syndrome [1] and 13 age- and sex-matched healthy volunteers were included in this study. The diagnosis was based on clinical and laboratory findings and muscle biopsy and genetic characterization. Ptosis and ophthalmoplegia were present in all CPEO, CPEO-plus, and Kearns-Sayre syndrome patients. The patient with mitochondrial myopathy, however, showed only a myopathy. All CPEO-plus patients, two CPEO patients, and the Kearns-Sayre syndrome patient showed additional symptoms of mitochondrialopathy. Two CPEO-plus patients and the Kearns-Sayre syndrome patient presented a pigmentary retinopathy. One CPEO-plus patient also suffered from cognitive impairment and hypothyroidism. The muscle biopsy disclosed ragged red fibers in all patients. Genetic analysis disclosed deletion in three CPEO and one CPEO-plus patient; no mutations were found in two CPEO-plus patients and the mitochondrial myopathy patient. In three patients, genetic analysis was unavailable. All subjects and volunteers gave their informed written consent according to institutional guidelines.

MRI, Diffusion-Weighted Imaging, and 1H MRSI

MRI and spectroscopy were performed with a 1.5-T whole-body scanner (Signa Echospeed, GE Healthcare) using axial T2-weighted fast spin-echo images (TR/TE, 4,500/72; number of excitations, 3; echo-train length, 8; slice thickness, 3 mm; spacing, 1 mm), axial

Fig. 1—Axial FLAIR images of patient 4, 26-year-old man with chronic progressive external ophthalmoplegia plus. A, Arrows show signal hyperintensities in pyramidal tract. B, Arrows show signal hyperintensities in subcortical white matter that can be traced through pyramidal tract.
fast FLAIR scans (TR/TE, 10/133; inversion time [TI], 2,200 milliseconds; thickness, 5 mm; no spacing), or sagittal TI-weighted 3D fast spoiled gradient-recalled echo acquisition (TR/TE, 10.4/3,400; TI, 500 milliseconds; thickness, 1.1 mm; no spacing). Image interpretation was done by two experienced neuroradiologists, one with more than 15 years of experience and one with more than 5 years, by consensus on a 4-point scale in terms of atrophy. No atrophy was judged as 0; mild, moderate, and severe atrophy were judged as 1, 2, and 3, respectively.

Diffusion imaging was done using a modified spin-echo echo-planar sequence (single shot [TR/TE, 2,200/120; flip angle, 90°; bandwidth, 83 kHz; field of view, 24 × 24 cm²]) and applying a tetrahedral technique [24] suitable for calculating the trace of the diffusion tensor and thus the mean diffusivity maps (D). To reduce partial volume effects from free fluid, a dummy acquisition was obtained before image acquisition in seven patients and volunteers. Three patients and volunteers received non–fluid-attenuated diffusion images for technical reasons and thus had to be excluded from the quantitative analysis.

Image processing was performed offline using IDL programs (ITT Visual Information Solutions). To extract brain structures from extracerebral signal intensity, we used a region-growing algorithm on the mean diffusivity maps and manually traced and suppressed the remaining extracerebral structures. We then calculated the average mean diffusivity value for whole-brain parenchyma on fluid-attenuated images.

Hydrogen-1 MRSI data were acquired by chemical shift imaging (TR/TE, 2,500/144; bandwidth, 2,500 Hz; number of points, 2,500) with a point-resolved spectroscopy technique for volume-selective excitation. Water suppression was achieved using three chemical shift selective pulses. The chemical shift imaging dimension was 24 × 24 × 1 (acquired number of voxels in a grid), field of view = 24 × 24 × 1.5 cm, yielding a voxel size of 1.5 mL. The point-resolved spectroscopy box was positioned in the semioval center, yielding spectra from both possibly involved cortex and white matter with a resolution of 1.5 mL, as described previously [25]. Postprocessing was performed with SAGE (Spectroscopy Analysis, GE Healthcare), including high-pass convolution filtering (bandwidth, 20 Hz), spectral apodization (gaussian; line broadening, 2 Hz), spatial apodization (diameter, 90%; transition, 50%), automatic zero-order autophasing, and 2D Fourier transformation. All spectra were visually analyzed by two spectroscopists for the presence of an inverted peak at 1.3 ppm reflecting lactate.

For quantitative analysis, three voxels were selected from left and right hemispheric white matter and from the cortex in each subject. Only voxels predominantly containing healthy-appearing white matter on T2-weighted and FLAIR images were chosen. Cortical voxels were selected from posterior midline predominantly containing gray matter. Spectra from voxels contaminated by fat were zeroed out. Metabolite concentrations were estimated by use of the LCModel (Linear Combination of Model Spectra) program [26], a fitting algorithm applying an automatic linear combination of model spectra. The program estimates absolute metabolite concentrations in institutional units and the fitting error is expressed as the SD. In addition, information on the spectra quality is given by providing line width and signal-to-noise ratio (SNR). This approach was found to be superior to peak area calculation based on the automated peak picking routine supplied by SAGE. Only metabolite information with an error below 20% SD was included in the final analysis.

The concentrations of total phosphocreatine, choline-containing compounds, and N-acetylaspartate moieties were estimated and expressed in international units allowing direct comparison between spectra ac-
MRI of Ophthalmoplegia

![Box plots showing N-acetylaspartate (NAA) normalized to creatine (CR) in three different brain regions (cortex and right and left white matter (WM)) for patients with chronic progressive external ophthalmoplegia (gray boxes) and age-matched controls (white boxes). O = outliers.](image)

**Fig. 3**—Box plots showing N-acetylaspartate (NAA) normalized to creatine (CR) in three different brain regions (cortex and right and left white matter [WM]) for patients with chronic progressive external ophthalmoplegia (gray boxes) and age-matched controls (white boxes). O = outliers.

required on the same system. In addition, data were expressed as ratios to choline or creatine metabolite concentrations for comparison with published data.

**Statistics**

Relative and absolute resonance intensities in arbitrary units were considered abnormal if they were more than 2 SDs outside the normal mean for the age-adjusted brain volume in different brain regions. Statistical analysis was performed using SPSS version 10.2 (Statistical Package for the Social Sciences) for Windows (Microsoft). One-way analysis of variance was used to test for group differences in age, sex, and region of interest of the spectroscopic voxels. Comparisons between groups and hemispheres for patients only were performed by applying a multivariate analysis of variance with N-acetylaspartate/creatine, choline/creatine, and N-acetylaspartate/choline as dependent variables and age, sex, and white matter and cortex as covariates, followed by a univariate F test (significant at $p < 0.05$).

For the reduction of type I error, all tests were followed by Bonferroni post hoc correction for multiple tests.

**Results**

**Imaging Results**

Cortical atrophy was severe in two patients, moderate in three, and mild in one. Cerebellar atrophy was mild in one, moderate in four, and severe in one. One patient had only cerebellar signs of atrophy and a cerebellar arachnoidal cyst. The same patient showed signal hyperintensities in the dentate nuclei as an MR sign for increased deposition of paramagnetic substances. Another patient also had a temporo-parapolar arachnoidal cyst. Two patients had hyperintensities in the corticospinal and corticofugal tracts, which could be traced through the internal capsule up to the subcortical white matter (Fig. 1). Prominent signal hyperintensities in the pyramidal tract were also seen in two other patients but could not be traced up to the subcortical white matter. Additional hyperintensities were found in the lamina quadrigemina ($n = 1$) and the cerebellar peduncles of the brainstem ($n = 2$), thalamus ($n = 1$), and globus pallidus ($n = 1$) (Fig. 2).

**Diffusion Findings**

Quantitative analysis of diffusion-weighted images was obtained in seven patients and volunteers. The average mean diffusivity of whole-brain parenchyma deploying fluid attenuation revealed no difference between the two groups. The average mean diffusivity of CPEO patients was $0.713 \times 10^{-3} \text{ mm}^2/\text{s}$ in contrast to a mean of $0.708 \times 10^{-3} \text{ mm}^2/\text{s}$ in the volunteer group. Qualitative evaluation of diffusion images revealed focal abnormalities only in the one patient with T2 signal hyperintensities in the cerebellar peduncles. Mean diffusivity maps showed focal enhancement in the same localization, probably reflecting demyelination.

$^1$H MR Spectroscopic Imaging

Visual analysis of all point-resolved spectroscopy–chemical shift imaging spectra disclosed no lactate in either white or gray matter. We used 108 spectra from healthy-appearing white matter for statistical analysis of both the right and left semiovale center in patients ($n = 54$) and controls ($n = 54$). Metabolite ratios for N-acetylaspartate/creatine, N-acetylaspartate/choline, and choline/creatine did not differ significantly between the two groups from all three regions (multivariate Wilks test; Wilks lambda, 0.956; and degrees of freedom (df) = 3, 51; $F = 0.776; p = 0.513$), as shown in Figure 3. Neither did we observe differences in absolute metabolite concentration estimates.

There were no differences between the right and left hemispheres in any patients.

Patients and control subjects showed no differences in the cortex for relative or absolute quantification ($p > 0.05$ for all metabolites).

Of note, one patient with signal hyperintensities in the cerebellar peduncles (Fig. 2A) agreed to undergo an additional scan to explore the metabolic profile of the lesion using single-voxel spectroscopy revealing a distinct lactate peak despite lack of lactate in his healthy-appearing centrum semiovale.

**Discussion**

As previously reported, we found signs of cortical and cerebellar atrophy in CPEO, CPEO-plus, and mitochondrial myopathy. The T2 hyperintensities seen in our patients may reflect the pathologic findings of spongiform encephalopathy with involvement of supranuclear, nuclear, and internuclear structures. Clearly visible T2 hyperintensities passing from the cerebellar peduncles via the lamina quadrigemina up to the corona radiata and extending into the pericentral subcortical white matter, middle cerebellar peduncle, and adjacent white matter in one patient might reflect secondary demyelination that was found to be associated with both elevated lactate and mean diffusivity in the cerebellar peduncle despite other detectable MR signs of mitochondrial impairment in the healthy-appearing supratentorial white matter.

The signal hyperintensities we found in the dentate nuclei of one patient likely represent calcification because of the primary disorder or because of the often-associated hypoparathyroidism, as discussed by Pellock et al. [27] and Berkovic et al. [13].

In contrast to earlier reports on Kearns-Sayre syndrome, we could not detect a lactate peak in either the white or gray matter of the centrum semiovale applying a chemical shift imaging technique. However, other authors located spectra in the visual cortex [14, 19] or affected white matter [14, 20] and used single-voxel spectroscopy.
copy. Although these technical differences may account for some of the discrepant findings, the main explanation is likely to come from heterogeneity of the disease and regional heterogeneity in keeping with imaging findings. Against this background, our main study aim was to search for indicators of generalized cerebral mitochondrial impairment, and hence chemical shift imaging including gray and white matter was felt to be the most appropriate approach. Salvan et al. [21], who investigated patients with CPEO, were likewise unable to detect a lactate peak in unaffected white matter but found anomalies of brain metabolism with no uniform pattern. The group hypothesized that, according to a characteristic threshold effect in mitochondrial disorders, the level of the gene defect in the brain might be sufficient to express a metabolic disturbance but not yet high enough for clinical expression. Kapeller et al. [20] suggested that MRI abnormalities would increase parallel to the neurologic progression of Kearns-Sayre syndrome supporting the mitochondrial respiratory chain insufficiency causing parenchymal alterations. Matthews et al. [14] found healthy metabolite ratios in patients with pure mitochondrial myopathies. In this study, we investigated only one patient with Kearns-Sayre syndrome, but we had four patients each with CPEO and with CPEO-plus and one patient with mitochondrial myopathy. It is thus noteworthy that only one patient with CPEO-plus was found to have lactate in affected white matter, which is generally well in line with the fact that imaging and spectroscopic abnormalities are more common in the more severe clinical phenotypes. This underlines the notion of a continuous pathophysiological process with more widespread (multigorgan) involvement and more severe local biochemical defects in Kearns-Sayre syndrome and CPEO-plus than in CPEO and mitochondrial myopathy. However, this was the only patient in whom a large enough white matter hypersensitivity allowed for proton spectroscopy of the affected region in addition to the main study.

Moreover, the lack of metabolic findings in our Kearns-Sayre syndrome patient underscores the highly variable presentation of even one clinical phenotype. Time-varying expression of subclinical energy failure may also contribute to these inconsistencies, because unknown external factors are likely to trigger suprathreshold mitochondrial respiratory chain failure with conceivably transient imaging and spectroscopic correlatives.

In conclusion, cerebral atrophy is the most common cerebral manifestation of mitochondrial myopathy, CPEO, CPEO-plus, and Kearns-Sayre syndrome. MRSI in the centrum semiovale and global mean diffusivity histogram metrics did not reveal any subclinical cerebral manifestation in these mainly myopathic subjects. Future studies applying proton or phosphorus spectroscopy at higher field strength in conjunction with neuronal or metabolic challenge would be required to further enhance the sensitivity that is likely to detect subclinical cerebral involvement in patients presenting with mitochondrial myopathy and CPEO.

Acknowledgments

We would like to express our gratitude to Astrid Hofbauer and Rosa Hemauer for analysis of spectra and to all subjects who participated in the study.

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