Lactate Detection by MRS in Mitochondrial Encephalopathy: Optimization of Technical Parameters

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ABSTRACT
Mitochondriopathies are a heterogeneous group of diseases with variable phenotypic presentation, which can range from subclinical to lethal forms. They are related either to DNA mutations or nuclear-encoded mitochondrial genes that affect the integrity and function of these organelles, compromising adenosine triphosphate (ATP) synthesis. Magnetic resonance imaging (MRI) is the most important imaging technique to detect structural and metabolic brain abnormalities in mitochondriopathies, although in some cases these studies may present normal results, or the identified brain abnormalities may be nonspecific. Magnetic resonance spectroscopy (MRS) enables the detection of high cerebral lactate levels, even when the brain has normal appearance by conventional MR scans. MRS is a useful tool for the diagnosis of mitochondriopathies, but must be correlated with clinical, neurophysiological, biochemical, histological, and molecular data to corroborate the diagnosis. Our aim is to clarify the most relevant issues related to the use of MRS in order to optimize its technical parameters, improving its use in the diagnosis of mitochondriopathies, which is often a challenge.

Introduction
The mitochondrion plays an important role in cellular metabolism. Enzyme disorders related to mutations in mitochondrial DNA or nuclear-encoded mitochondrial genes may affect the integrity and function of these organelles, compromising ATP synthesis. Mitochondrial disorders frequently manifest during childhood and early adulthood. Several distinct syndromes have been recognized on the basis of their phenotype, histological, biochemical, or genetic manifestations, sharing unique characteristics of mitochondrial inheritance and the normal deterioration of mitochondrial function with aging. Some well-defined disorders include, among others, MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes), MERRF (myoclonus epilepsy, and ragged red fibers), Kearns-Sayre syndrome (chronic external ophthalmoplegia plus, with retinal pigment abnormalities), Leber’s hereditary optic neuropathy, Alper’s disease (progressive infantile poliodystrophy), and Leigh’s disease (subacute necrotizing encephalomyelitis). Each one of these entities is associated with a different point mutation of the mtDNA causing a defect in the mitochondrial protein synthesis.

MELAS syndrome is characterized by stroke-like episodes often preceded by treatment-resistant partial seizures. Short stature, diabetes mellitus, and slowly progressive mental impairment leading to dementia are common features. The Kearns-Sayre syndrome is a nonfamilial disease characterized by ataxia, ophthalmoplegia, and retinitis pigmentosa. The syndrome may include elevated protein levels in the cerebrospinal fluid (CSF), heart block, dementia, short stature, sensorineural hearing loss, and endocrine dysfunction. In Alper’s or pseudo-Alper’s disease, patients present with early-onset seizures and a progressive course, with myoclonic jerks, developmental delay, and failure to thrive. Leigh syndrome is characterized by hypotonia, psychomotor impairment, and brain stem dysfunction. Further considerations concerning complete classification and all clinical aspects of mitochondriopathies are beyond the scope of this article. In some cases, the neurological features remain unaltered for a long period of time, until acute deterioration occurs, which can be precipitated by stressful situations such as infections or trauma. MR is the most important technique for noninvasive brain evaluation in patients suspected of having mitochondrial diseases, both for the presumptive diagnosis and for monitoring disease evolution and response to therapies.

Leigh syndrome tends to manifest typical MR findings. The diagnosis is characterized by vascular proliferation and demyelination, which lead to necrosis and cavitation in typical locations, including the basal ganglia, midbrain, pons, and posterior column of the spinal cord. MR lesions consist in bilateral and symmetrical putaminal and periaqueductal gray

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matter involvement. Lesions are also seen in the caudate nuclei, globus pallidi, and thalami, but never in the absence of putaminal lesions. Extensive changes in white matter, most related to microcystic cavitation after necrosis, are also characteristic of Leigh syndrome.

In Kearns-Sayre syndrome, gray and white matter are affected, most often the brain stem tegmentum, basal ganglia, and the white matter of the brain and cerebellum. Calcifications are commonly seen in the basal ganglia. Involvement of the subcortical "U" fibers with sparing of the periventricular white matter is typical.12-14

Stroke-like lesions, often transient and not confined to the vascular territories are the imaging hallmark of MELAS.12 The stroke-like cortical lesions affect mainly the basal ganglia and parieto-occipital regions.

MR spectroscopy is useful in this setting because it frequently detects abnormal positive lactate peaks that correlate highly with other clinical markers in all mitochondriopathies.5

Muscle biopsy is often diagnostic, although some patients with mitochondrial myopathy may show normal results.1 In such cases, the diagnosis is presumptive and must also be correlated with clinical, laboratory, and MR data.2 In mitochondriopathies, the disruption of the respiratory chain causes the predominant catabolic metabolism to shift from the Krebs cycle to anaerobic glycolysis, with an accumulation of lactate. An increase in tissue lactate may be related to local oxidative stress due to a reduction in oxygen supply, an increase in oxygen demand, or an impairment in oxidative metabolism.2 MRS is a feasible technique, which enables a number of biochemical metabolites to be detected in vivo. MRS can identify metabolic abnormalities even when brain parenchyma appears normal by conventional MR scans, and has been helpful for both diagnosis and follow-up of mitochondrial disorders. The laboratory evaluation included lactate dosage and its quantification in the CSF through lumbar puncture, carried out by our service, using the automated enzyme method (Dimension R AR Dade Behring, Germany). Lumbar puncture is an invasive procedure, and as almost all patients are candidates for MR, MRS is a feasible noninvasive option in this setting. The frequency of lactate detection in brain parenchyma and CSF is unknown. Lin et al. observed a good correlation between high lactate levels detected by MRS and other markers of mitochondrial disease. In MRS, the lactate doublet peak is located at a chemical shift of 1.33 ppm, and needs to be clearly visible above the noise background for its detection.

The detection of lactate is neither specific nor found in all patients in the setting of mitochondrial disorders. Elevated brain lactate is only one of the helpful information in the evaluation of these patients, and its sensitivity shows temporal and regional variation, depending on the type of mitochondrial disorder.2 Other MRS abnormalities can be seen, as elevated levels of alanine and glucose.15 Elevated cerebral glucose is ascribed to a high degree of monoxidative glycolysis. In patients with pyruvate dehydrogenase complex deficiency, elevated cerebral pyruvate16 may be seen in addition to the lactate elevation.

The comprehension of the correlation between elevated cerebral lactate detected by MRS and parenchymal abnormalities depicted by MR scans is very useful for the neurological diagnosis of mitochondriopathies. Some authors have demonstrated a higher sensitivity of MRS compared to conventional MR scans in the detection of abnormalities in symptomatic patients and individuals at risk, who still have not developed neurological deficit.12,15,17-19 Normal individuals present lower lactate levels in the CSF than in the serum, and this metabolite is not detected by routine MRS neither in brain parenchyma nor in CSF. The serum lactate detection presents low sensitivity in the diagnosis of mitochondriopathies, because it is transported to the liver where it is normally reoxidized into pyruvate. Therefore, the quantification of this metabolite in the CSF provides important information about the presence of anaerobic metabolism in encephalic tissues. Nissenkorn et al.20 have demonstrated similar sensitivity between the CSF lactate detection by MRS and other biochemical, genetic, and histopathological markers of these diseases.

Our aim is to clarify the most relevant aspects related to the use of MRS in mitochondriopathies. For this purpose, we analyze the MRS in a series of 7 patients with mitochondrial disorders (Table 1), discussing technical parameters in order to optimize this method, improving the diagnosis of mitochondriopathies, which is often a challenge.

Discussion
Development of Parenchymal Lesions
Patients with mitochondrial disorders may present intermittent bouts and some authors have demonstrated that high levels of lactate in the CNS generally correlate to periods of exacerbation of neurological symptoms.21-22 Thus, MRS is more sensitive during episodes of clinical exacerbation. Besides being useful for the diagnosis, MRS is also highly applicable for monitoring the development of brain lesions. However, the elevation of cerebral lactate is usually variable in different clinical forms of mitochondrial disorders, and it is not unequivocally present in all patients and in all brain parenchyma.

MRS always demonstrates lactate elevation in acute lesions of mitochondriopathies that occur with stroke-like episodes (MELAS), as would be expected in ischemic insults. In subacute lesions, this finding is occasional, while in chronic lesions, lactate is not usually demonstrated.15,22,23 (Fig 1). The diagnosis of mitochondriopathy does not depend only on the demonstration of encephalic lesions by conventional MRI scans. On the other hand, the detection of increased CSF lactate levels by MRS is a consistent finding that can replace the search for this metabolite by lumbar puncture.

Concentration of Metabolite
Cross et al.21 described that lactate must have a concentration above 4.0 mmol/L to be detected by MRS. However, using proper pulse sequences and timing, presumably lactate can be detected at minor concentrations, particularly using higher fields and specialized methods available. Despite this, the detection of lactate is not specific for the mitochondrial disorders. A normal MRS neither excludes the diagnosis of this condition nor replaces the quantification of lactate levels in the CSF in all cases.
<table>
<thead>
<tr>
<th>Cases</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical</th>
<th>MRI</th>
<th>MRS</th>
<th>Lactate (mmol/L)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>05 y</td>
<td>F</td>
<td>Ataxia, convergent strabismus, cardiac arrhythmia and increased protein levels in the CSF</td>
<td>Bilateral putaminal, periaqueductal, subthalamic and tegmental lesions</td>
<td>Lactate peak on right putaminal lesion</td>
<td>4,44 (CSF)</td>
<td>Kearns-Sayre</td>
</tr>
<tr>
<td>2</td>
<td>08 y</td>
<td>F</td>
<td>Choreo-athetosis, walking problems, loss of NPM acquisitions</td>
<td>Bilateral striatal, periaqueductal and ION lesions</td>
<td>Lactate peak on right putaminal lesion</td>
<td>3,02 (Blood)</td>
<td>Mutation SURF1*</td>
</tr>
<tr>
<td>3</td>
<td>16 m</td>
<td>F</td>
<td>Myopathy and lactic acidosis</td>
<td>Mild atrophy</td>
<td>Lactate peak on normal left basal ganglia</td>
<td>5,88 (CSF)</td>
<td>Pyruvate dehydrogenase deficiency*</td>
</tr>
<tr>
<td>4</td>
<td>06 y</td>
<td>F</td>
<td>Myopathy and lactic acidosis</td>
<td>Mild cerebellar atrophy, right basal ganglia lesion</td>
<td>Lactate peak on normal left basal ganglia</td>
<td>2,13 (Blood)</td>
<td>Lactic acidosis myopathy</td>
</tr>
<tr>
<td>5</td>
<td>16 y</td>
<td>M</td>
<td>Weight/stature deficiency and epilepsy</td>
<td>Brain atrophy and parenchymal posterior lesions (stroke-like pattern)</td>
<td>Lactate peak in both parenchymal posterior lesion and intraventricular</td>
<td>–</td>
<td>MELAS</td>
</tr>
<tr>
<td>6</td>
<td>06 y</td>
<td>M</td>
<td>Weight/stature deficiency</td>
<td>Basal ganglia, thalamus and brainstem lesions</td>
<td>Lactate peak only in intraventricular voxel</td>
<td>1,17 (Blood)</td>
<td>Non-specific mitochondrialopathy</td>
</tr>
<tr>
<td>7</td>
<td>05 y</td>
<td>M</td>
<td>Low stature, NPM retardation, epilepsy</td>
<td>Brain atrophy, basal ganglia and multifocal parenchymal lesions (stroke-like pattern)</td>
<td>Lactate peak in both right putaminal lesion and intraventricular</td>
<td>7,54 (CSF)</td>
<td>MELAS</td>
</tr>
</tbody>
</table>

NPM = neuropsychomotor; ION = inferior olivar nucleus. Reference values to blood analysis = 0.63 - 2.44 and CSF = 1.1 – 2.2 mmol/L. *molecular diagnosis.
Fig 1. Patient 5: MELAS. (A) Single-voxel MRS (PRESS/TE = 144 ms) in the abnormal hyperintense left parieto-occipital region shows a lactate peak at a chemical shift of 1.33 ppm in the spectral curve (arrow). (B) Single-voxel MRS (PRESS/TE = 144 ms) in the left striatum. The axial FLAIR image shows a faint hyperintense left putaminal focus, but the spectral curve was normal. (C) Single-voxel MRS (PRESS/TE = 144 ms) at the enlarged atrium of the left lateral ventricle shows a higher lactate peak in the spectral curve compared to the peaks obtained from brain parenchyma.
Table 2. Short Guidelines for MRS Use in Mitochondriopathies

<table>
<thead>
<tr>
<th>When</th>
<th>During clinical exacerbations</th>
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<tbody>
<tr>
<td>Where</td>
<td>CSF and parenchyma</td>
</tr>
<tr>
<td></td>
<td>(hyperintense parenchymal lesions on DWI)</td>
</tr>
<tr>
<td>TE</td>
<td>144 ms (confirms lactate)</td>
</tr>
<tr>
<td>&quot;SVS&quot;</td>
<td>If enlarged lateral ventricles</td>
</tr>
<tr>
<td></td>
<td>If normal lateral ventricles, include both frontal horns</td>
</tr>
<tr>
<td>&quot;MVS&quot;</td>
<td>Including CSF and brain parenchyma</td>
</tr>
<tr>
<td></td>
<td>(First choice Allows small voxels into the normal ventricles using TE 144 ms)</td>
</tr>
</tbody>
</table>

*SVS = single voxel spectroscopy; **MVS = multivoxel spectroscopy.

A good correlation between spectroscopy and lumbar puncture in the detection of CSF lactate levels is obtained only when the CSF lactate level is abnormally high by MRS. If MRS shows normal results, we need to perform lumbar puncture and CSF analysis because a small amount of lactate might not be detected by spectroscopy alone. Thus, the MRS analysis can replace lumbar puncture only when high levels of lactate are depicted by spectroscopy. The lactate detection depends on the clinical course. Some authors described a MERRF-MELAS patient that showed high lactate levels depicted by MRS only in the follow-up examination.

Nevertheless, the metabolic evaluation of different regions may increase the MRS sensitivity. Elevated brain lactate is only one piece of helpful information for both diagnosis and follow-up of mitochondrial disorders.

**Selecting the Region of Interest and Technical Parameters**

The regions of interest and technical parameters are also relevant variables in this setting (Table 2). We performed single voxel (TE of 35 and 144 ms) and multivoxel (TE = 144 ms) spectroscopy using PRESS technique in all patients. The voxel location followed the criteria below:

- **Single voxel**: We included parenchymal lesions, mainly those hyperintense on diffusion-weighted imaging (DWI); lateral ventricles when they were enlarged and both frontal horns of the lateral ventricles when there was no ventricular enlargement. We also included CSF in the lateral ventricles for multivoxel analysis. All these situations are shown in the figures.

Several technical parameters of MRS can be modified and it depends on the MR equipment specifications. The MR

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Fig 2. Patient 1: Kearns-Sayre. Axial FLAIR (A) and diffusion-weighted (B) images show bilateral and symmetrical lentiform hyperintensities. (C) Single-voxel MRS (PRESS/TE = 35 ms) in the right lentiform nucleus shows a lactate peak at a chemical shift of 1.33 ppm in the spectral curve. (D) Single-voxel MRS (PRESS/TE = 144 ms) obtained from the same location demonstrates the lactate peak below the baseline.
Patient 6: Nonspecific mitochondriopathy. (A) Axial DWI demonstrate hyperintense lesions in the dorsal pons, midbrain, thalami, basal ganglia, and frontoparietal cortex. (B) Single-voxel MRS (PRESS/TE = 35 ms and 14 ms) in the abnormal hyperintense left striatum shows no significant metabolic abnormality in the spectral curves. (C) Single-voxel MRS (PRESS/TE = 35 ms and 144 ms) in both frontal horns of the normal-sized lateral ventricles shows a lactate peak at a chemical shift of 1.33 ppm in the spectral curves (arrows).

Equipment provides automatic or manual postprocessing data. The most important variation regards to the optimal pulse sequence, and it is also related to single or multivoxel acquisition. Because CSF is not stationary but flowing, single voxel and spectroscopic imaging techniques actually null out much of the CSF signal, presumably underestimating the lactate concentration.

Voxel size and echo time acquisition (TE) can also be modified, from 20-30 ms, 135-144 ms, and 288 ms. Short TE (20-30 ms) are preferred when the detection of glutamine, glutamate, and myo-inositol is important, however, there are substantial amounts of lipid signal present between 1 and 2 ppm and macromolecules contributing to the baseline. High TE has lower signal-to-noise ratio (S/N) and is useful to confirm lactate presence by nulling out lipid peaks. Using TE = 144 ms, the resonance shows an inverted doublet peak of lactate, whereas TE = 288 ms gives a positive doublet peak. Using TE = 288 ms would make the baseline flatter, but with lower S/N and the scan time or voxel size must be increased to get spectra with the same S/N as TE = 144 ms.

Inao et al. have demonstrated that lactate presents a biphasic pattern of elevation. Firstly, the parenchymal lactate elevation probably occurs secondary to a response by the organism to a certain brain injury. Secondly, the CSF lactate accumulation occurs consequently to the clearance of this metabolite from the damaged brain tissue. Thus, the window for the detection of high CSF lactate levels is larger than the window for the detection of this metabolite in the brain parenchyma. So, the CSF is the preferred evaluation site, increasing the MRS accuracy.
There is a regional variability in the increased levels of cerebral lactate. This metabolite is usually more pronounced in regions where MR shows recent structural lesions, such as those hyperintense on DWI (Fig 2). However, we do not necessarily detect parenchymal lactate in patients with mitochondrial disorders, even in areas that appear abnormal by MR scans. Thus, the CSF evaluation by MRS is recommended. In individuals with enlarged ventricles, the positioning of the intraventricular voxel is facilitated. However, in patients without ventricular enlargement, the use of a single voxel technique may decrease the sensitivity of the method, because it is very difficult to include only the CSF in the analyzed sample, using a voxel size of 8 cm$^3$. The reduction of the voxel dimensions should be a plausible alternative in this situation; however, it is limited by the dropout of the S/N and by the increase in the MRS acquisition time. Our useful solution was the positioning of a single voxel including both frontal horns of the lateral ventricles (Fig 3). Because of its dimensions, single voxel technique usually includes both parenchyma and ventricular or subarachnoid CSF. In patients with high lactate levels, we cannot determine the precise origin of this metabolite in this setting.

Using a single voxel technique, you will need more than one acquisition to study different areas, for example, ventricular CSF and brain parenchyma. Multivoxel technique has some advantages, because it enables a wider evaluation of different parenchymal regions and intraventricular CSF in a single acquisition, with less acquisition time and greater S/N (Fig 4).

**Conclusion**

MRS is an important complementary tool for the detection of metabolic abnormalities in mitochondrial disorders, mostly the lactate elevation. The analysis of our series and the revision of the literature allow us to conclude that MRS should be optimized during episodes of clinical exacerbation, in acute lesions and in the ventricular CSF. When the patient exhibits normalized lateral ventricles, we prefer to use a single voxel with high

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**Fig 4.** Patient 7: MELAS. (A) Axial FLAIR image demonstrates cortical and right basal ganglia hypeintensities. (B) Single-voxel MRS (PRESS/TE = 35 ms) in the right basal ganglia shows a lactate peak at a chemical shift of 1.33 ppm in the spectral curve (arrow). (C) Single-Voxel MRS (PRESS/TE = 144 ms) at the same location does not separate the lactate peak from the background noise in the spectral curve (circle). (D and E) Multivoxel MRS allows a wider metabolic evaluation of both brain parenchyma and CSF. There is a huge lactate peak below the baseline in the spectral curve that corresponds to the small intraventricular voxel in D.
Multivoxel MRS showed some advantages characterized by the inclusion of both brain parenchyma and ventricular CSF in only one acquisition. Our study has some limitations, including the small number of patients and the absence of simultaneous correlation between the detection of CSF lactate levels by MRS and by laboratorial analysis in some of them. The optimization of MRS parameters is crucial for the use of MRS as both confirmatory and follow-up noninvasive test in this setting.

References