Positron Emission Tomography and Magnetic Resonance Spectroscopy of Cerebral Glycolysis in Children with Congenital Lactic Acidosis

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Congenital lactic acidosis with neurological symptoms may be due to a variety of disorders of energy metabolism. We investigated whether positron emission tomography (PET) and proton magnetic resonance spectroscopy (1H MRS) are capable of demonstrating specific changes to facilitate diagnosis. A corresponding increase of cerebral lactate (with MRS) and rate of glycolysis (with PET) was observed in 2 children with biochemical evidence of defective mitochondrial respiration. No such increase was noted in a child with lactic acidosis due to stress and exercise but normal respiratory chain activity, and in a control case with an epilepsy syndrome without evidence of primary changes of energy metabolism. The results suggest that defects of oxidative phosphorylation may cause a massive increase of glycolysis to cover energy requirements, with corresponding accumulation of lactate in brain tissue. This mechanism can now be demonstrated in vivo and, with further experience, may potentially be used as a diagnostic marker of respiratory chain disorders in brain tissue.


Congenital lactic acidosis (CLA) is a symptom that can occur as a result of an inborn error in the pathway responsible for the metabolism of pyruvate. Common etiologies include defects of the pyruvate dehydrogenase complex, pyruvate carboxylase, mitochondrial respiratory chain enzymes, or the Krebs cycle [1, 2]. The accompanying clinical signs and symptoms may vary considerably depending on the type and the severity of defect. Diagnosis is usually based on biochemical, radiological, and pathological studies. However, evaluation is often invasive, time consuming, and is not always conclusive.

Positron emission tomography (PET) has been used in the evaluation of mitochondrial diseases because it provides a quantitative tracer method for regional measurements of glucose and oxygen metabolism in brain tissue as well as blood flow. A study using 2-[18F]-fluoro-2-deoxyglucose (FDG), [15O] oxygen, and [15O] carbon dioxide in a series of adult patients with mitochondrial encephalomyopathy showed an altered stoichiometry in glucose and oxygen utilization resulting from aerobic glycolysis to lactate [3]. A similar study examining patients with a clinical phenotype of CLA known by the acronym MERRF for myoclonus epilepsy and ragged-red fibers [4], however, revealed no signs of increased aerobic glycolysis. These authors found a chronically low rate of oxidative metabolism [5]. An adult patient with a mitochondrial encephalomyopathy (probable MERRF) also showed a severe decrease in the cerebral metabolic rate of glucose (CMRg) [6].

Another imaging tool that may be helpful in the noninvasive examination of normal and abnormal brain development is image-guided localized proton magnetic resonance spectroscopy (1H MRS). The information obtained from localized proton MR spectroscopy can be supplemented by information on the spatial distribution of metabolites using 1H MR spectroscopic imaging (SI). In several studies [7-10] increased peaks for lactate have been described in children with Leigh disease [11], a form of CLA that can be caused by a defect in oxidative phosphorylation or pyruvate metabolism.

FDG PET and 1H MRS are complementary with...
regard to investigating the rate of glycolysis. The former
basically measures the rate of FDG phosphorylation
by hexokinase, which is nearly irreversible and
therefore reflects total, oxidative and nonoxidative, gly-
colytic activity; the latter assesses lactate, which is the
principal product of nonoxidative glycolysis. We there-
fore performed both, FDG PET and \(^{1}H\) MRS, for di-
rect comparison of glycolytic rates and lactate levels in
4 children, 2 of whom had biochemical evidence of
impaired mitochondrial respiration.

Materials and Methods
This study was performed in accordance with the policies of
the Ethics Committee of the Medical Faculty of Cologne
University. With the informed consent of parents, all patients
were initially examined with PET and MRS under the tena-
tive diagnosis of a congenital metabolic disturbance. CMR
values were compared with literature data for normal chil-
dren of birth age to 2 year and 1 to 2 year [12], as listed in
Table 1. MRS data of patients were compared with MRS
data obtained in children without neurological disorders [13].
Amino acid screening in urine was performed using thin-
layer chromatography, or quantitatively in urine and serum
using a reverse-phase high-performance liquid chromato-
graphic (HPLC) method. Urinary organic acids were deter-
mined quantitatively using gas chromatography and identified
by gas chromatography–mass spectroscopy. Lactate, pyru-
vate dehydrogenase complex, and citrate synthetase were
measured in skeletal muscle specimens (taken from musculus
quadriceps femoris) at the Department of Pediatrics, Univer-
sity Hospital Nijmegen, The Netherlands, using the proce-
dures described by Fischer and colleagues [14] and Korenke
and associates [15].

 Positron Emission Tomography
CMR was studied using FDG PET [16] on a 7-slice posi-
tron camera (Scanditronix PC384) with an in-plane resolu-
tion of 7.8 mm at a slice thickness of 11 mm or, in one
case (Patient 3), on a 47-slice camera (Siemens-CTI ECAT
EXACT 921) with 6-mm transaxial and 5-mm axial resolu-
tion. After a rapid intravenous bolus injection of FDG
(3.7–7.4 MBq/kg of body weight), patients were scanned for
a total period of 50 to 60 minutes in a dimly lit room with
low ambient noise. Images were recorded with the 7-slice
scanner in two table positions from 30 to 40 and 40 to 50
minutes after injection, respectively, yielding a total of 14
slices comprising nearly the entire brain; whereas with the
47-ring scanner, the whole brain was assessed without chang-
ing the table position from 20 to 60 min. Plasma activity
was measured in multiple arterial blood samples. CMR was
calculated pixel by pixel with rate constants \(K_i\) and \(k_3\)
adjusted to measured tissue activity [17], and a fixed lumped
constant value of 0.42.

All images were initially analyzed visually. Each image was
then coregistered three-dimensionally to a reference PET im-
age in standard position (transaxial plane parallel to the inter-
commissural line) using a multipurpose match program [18]
with striatum and thalamus as major landmarks. Regions of
interest (ROIs) were placed on multiple transaxial slices using
a PC adaptation of an interactive mapping program [19],
guided by the atlas of Matsui and Hirano [20], and CMR was
calculated for major brain structures including cerebral
lobes, caudate nucleus, lentiform nucleus, thalamus, cerebel-
lar cortex, and brainstem.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Case 1 (Cerebral Palsy)</th>
<th>Case 2 (Possible Complex IV Defect)</th>
<th>Reference Values(^a)</th>
<th>Case 3 (Complex I Defect)</th>
<th>Case 4 (Congenital Epilepsy Syndrome)</th>
<th>Reference Values(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>20 mo</td>
<td>14 mo</td>
<td>1–2 yr</td>
<td>2 wk</td>
<td>1 mo</td>
<td>Birth to 1 yr</td>
</tr>
<tr>
<td>Cerebral hemisphere</td>
<td>15.53</td>
<td>53.13</td>
<td>27.36 ± 6.46</td>
<td>96.43</td>
<td>19.30</td>
<td>20.35 ± 4.79</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>15.76</td>
<td>48.13</td>
<td>29.65 ± 7.30</td>
<td>84.57</td>
<td>18.20</td>
<td>19.68 ± 4.84</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>16.88</td>
<td>54.86</td>
<td>28.76 ± 7.08</td>
<td>87.79</td>
<td>19.22</td>
<td>20.23 ± 5.03</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>15.85</td>
<td>55.63</td>
<td>28.67 ± 8.54</td>
<td>82.29</td>
<td>20.29</td>
<td>20.00 ± 4.23</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>15.86</td>
<td>59.65</td>
<td>30.39 ± 0.00</td>
<td>78.58</td>
<td>17.55</td>
<td>20.54 ± 5.08</td>
</tr>
<tr>
<td>Sensorimotor cortex</td>
<td>17.13</td>
<td>50.26</td>
<td>29.73 ± 7.44</td>
<td>103.02</td>
<td>21.51</td>
<td>22.17 ± 5.11</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>15.73</td>
<td>54.86</td>
<td>29.61 ± 6.40</td>
<td>108.62</td>
<td>21.60</td>
<td>20.53 ± 4.89</td>
</tr>
<tr>
<td>Caudate nuclei</td>
<td>8.67</td>
<td>69.81</td>
<td>28.77 ± 7.34</td>
<td>189.70</td>
<td>20.08</td>
<td>22.95 ± 7.06</td>
</tr>
<tr>
<td>Lenticular nuclei</td>
<td>14.04</td>
<td>81.51</td>
<td>32.36 ± 8.04</td>
<td>199.24</td>
<td>26.16</td>
<td>26.42 ± 7.14</td>
</tr>
<tr>
<td>Thalamus</td>
<td>14.00</td>
<td>39.40</td>
<td>29.48 ± 5.90</td>
<td>216.96</td>
<td>19.03</td>
<td>27.15 ± 7.86</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>15.61</td>
<td>26.66</td>
<td>19.50 ± 5.00</td>
<td>77.24</td>
<td>17.60</td>
<td>17.59 ± 2.70</td>
</tr>
<tr>
<td>Brainstem</td>
<td>12.53</td>
<td>37.11</td>
<td>21.17 ± 3.60</td>
<td>145.10</td>
<td>23.09</td>
<td>18.63 ± 3.81</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SD, from Chugani and colleagues (1987).
The patient was a normal boy until the age of 3 months when he developed progressive hypotonicity and ataxia. MRI at 4 months showed cortical atrophy, psychomotor retardation, and widening of the cerebrospinal fluid (CSF) spaces. Analysis for chromosomal defects was negative. The diagnosis was cerebral palsy due to an unidentified metabolic disorder. The lactic acidosis was probably caused by severe physical stress and exercise. Brain autopsy revealed a symmetrical glial polymicrogyria of cortex.

In Case 2, additional spectroscopic images were produced from a representative slice of 2.8-cm thickness using a combination of localized excitation and phase-encoded acquisition, applying $32 \times 32$ phase-encoding steps over a field of view of $225 \times 225$ mm. The data can be presented as maps representing the distribution of specific biochemical compounds with a nominal in-plane resolution of 7.0 mm, or as sets of spatially resolved spectra, to allow a more precise evaluation of specific foci of interest. Technical details have been described elsewhere [22]. The metabolite resonances were corrected for relaxation and saturation effects.

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For PET and MRS studies, patients were sedated using pentobarbital with an initial dose of 3 mg/kg of body weight followed by 1 to 2 mg/kg of body weight per hour intravenously.

Patients and Results

Case 1

The patient was a normal boy until the age of 3 months when he developed progressive hypotonicity and ataxia. Parents were consanguineous (Grade I). At the age of 1.5 years, he had severe psychomotor delay and a spastic paraplegia. Laboratory evaluation demonstrated an elevated lactate level (Table 2); however, a pyruvate loading test demonstrated a normal pyruvate clearance from blood, which indicates normal pyruvate metabolism [24]. MRI at 20 months showed increased signal on the T$_2$-weighted image and decreased signal on the T$_1$-weighted image in the lateral putamen. MRS was normal. PET at 20 months revealed a global decrease in glucose metabolism with markedly decreased values in the caudate nuclei, lentiform nuclei, thalamus, and frontal cortex (see Table 1, Fig 1). Electrophysiological studies were without abnormality. A muscle biopsy was unremarkable and no mitochondrial abnormalities were found (Table 3). An analysis for chromosomal defects was negative. The patient's condition progressively worsened and he died at the age of 2 years due to sepsis. The final clinical diagnosis was cerebral palsy due to an unidentified metabolic disorder. The lactic acidosis was probably caused by severe physical stress and exercise. Brain autopsy revealed a symmetrical glial polymicrogyria of cortex.
Mitochondrial encephalopathy:

Fig 1. Transaxial metabolic maps at the basal ganglia level of all 4 patients obtained with 2-[\textsuperscript{18}F]fluoro-2-deoxyglucose positron emission tomography. The individual range of values (in \textmu mol/100 g/min) displayed with a rainbow scale is shown below each image. (Top row) Cases 2 and 3 with mitochondrial enzyme disorder and increased $\text{CMR}_\text{gl}$, most pronounced in the basal ganglia. (Bottom row) Cases 1 and 4 without evidence of mitochondrial impairment and low metabolism.

The basal ganglia demonstrated increased lactate in the entire volume (Fig 2). Evaluation with PET (see Fig 1) at 14 months showed remarkably increased metabolic rates of glucose in all regions, with the largest increases in the caudate nuclei, lentiform nuclei, and parietooccipital cortex (see Table 1). Evoked potential (EP) studies were abnormal. Electroencephalogram (EEG) was slow without focal abnormalities. Electromyography (EMG) and nerve conduction velocity (NCV) examinations were normal. A muscle biopsy revealed a mild nonspecific myopathy. Analysis of mitochondrial respiratory chain enzymes revealed a defect in complex IV (cytochrome $c$ oxidase). The pa-
Table 3. Activities of Respiratory Chain Enzymes and Pyruvate Dehydrogenase Complex

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Tissue</th>
<th>Cytochrome c Oxidase</th>
<th>NADH:Q&lt;sub&gt;1&lt;/sub&gt; Oxidoreductase</th>
<th>Succinate: Cytochrome c Oxidoreductase</th>
<th>Pyruvate Dehydrogenase Complex</th>
<th>Citrate Synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cerebral palsy (lactic acidosis due to physical stress)</td>
<td>Muscle, frozen</td>
<td>81</td>
<td>27</td>
<td>7.5</td>
<td>3.0</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>Possible complex IV defect</td>
<td>Muscle, frozen</td>
<td>67</td>
<td>16</td>
<td>9.7</td>
<td>4.6</td>
<td>113</td>
</tr>
<tr>
<td>3</td>
<td>Complex I defect</td>
<td>Muscle, fresh</td>
<td>78</td>
<td>1.1</td>
<td>22</td>
<td>1.6</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59</td>
<td>1.7</td>
<td>n.d.</td>
<td>n.d.</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117</td>
<td>0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>223</td>
</tr>
<tr>
<td>4</td>
<td>Congenital epilepsy syndrome</td>
<td>No biopsy taken</td>
<td>Normal range</td>
<td>52–186</td>
<td>8.9–27</td>
<td>8.2–44</td>
<td>2.7–8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muscle, frozen</td>
<td>68–437</td>
<td>4.4–26</td>
<td>22–88</td>
<td>2.7–8.2</td>
<td>48–162</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>13–108</td>
<td>4.7–9.2</td>
<td>—</td>
<td>—</td>
<td>13–96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain</td>
<td>26–181</td>
<td>5.1–19</td>
<td>—</td>
<td>—</td>
<td>53–207</td>
</tr>
</tbody>
</table>

Enzyme activities given in milliunits per mg protein (600 g of supernatant was used in case of fresh muscle; total homogenate was used in case of frozen muscle). Citrate synthetase was used as mitochondrial reference enzyme.

<sup>a</sup>Denotes post-mortem tissue.


tient's condition deteriorated rapidly at the age of 18 months, and she died shortly thereafter due to sepsis and cardiovascular collapse. An autopsy was not performed.

Case 3
The patient was a term male newborn delivered spontaneously to consanguineous parents (Grade I) after an uncomplicated pregnancy. On the fifth day postpartum he developed hypotonia as well as increasing hyperglycemia and metabolic acidosis. He eventually required mechanical ventilation secondary to increasing respiratory insufficiency. At 7 days, he developed a severe lactic acidosis (see Table 2) and suffered generalized seizures. Magnetic resonance imaging (MRI) at 2 weeks revealed increased signal intensity predominantly on T<sub>2</sub>-weighted images in the frontal lobes. MRS of the basal ganglia (Fig 3) revealed markedly decreased NAA/Cho and Cr/Cho ratios with a large lactate signal (8 ± 1 mmol/L). PET at 2 weeks demonstrated globally elevated CMR<sub>glc</sub> in all regions with the largest increases in the thalamus, caudate and lenticular nuclei, and brainstem (see Table 1). EP studies suggested a generalized central nervous system (CNS) disturbance. EEG showed highly irregular background activity with superimposed multifocal epileptic foci. Muscle biopsy was normal. Analysis of mitochondrial respiratory chain enzymes in skeletal muscle, and post-mortem studies of liver and brain tissue, revealed a defect in complex I (NADH−coenzyme Q<sub>1</sub> oxidoreductase) (see Table 3). A chromosome analysis was unremarkable. The child experienced a rapid decline during the third week and died on his 24th day due to cardiovascular collapse. Neuropathological examination revealed symmetrical spongy necrotizing lesions in both hemispheres, the basal ganglia, the thalamus, and the lower brainstem.

Case 4
The patient was the daughter of nonconsanguineous parents, born at term after an uneventful pregnancy and delivery. On the second day postpartum, she began experiencing cyanotic episodes that progressively increased in frequency and later became associated with tonic−clonic movements of the arms and legs. Seizure−like episodes decreased after the patient was treated with corticotropin (ACTH). She had hypotonia, tachypnea, and increased respiratory tract sections. Laboratory tests were essentially normal. MRI was unremarkable. MRS showed no abnormal metabolism products, but compared with other signals, NAA signal intensity was below normal level. PET at 1 month revealed a normal metabolic rate of glucose in all regions except for mildly decreased rates in the thalamus (see Table 1). Upon visual evaluation there was an area of increased metabolism in the right mesiotemporal region that could have represented an epileptic focus. EEG showed multifocal spikes with alternating areas of focal
Fig 2. $^1$H Magnetic resonance spectroscopic study of Patient 2. (A) Axial proton density-weighted image with volume of interest (VOI) indicated. (B) Lactate map within VOI. The numbers indicate the origin of spatially resolved spectra displayed in C. The bright areas near the frontal border of the VOI originate from contaminations by signal of retroorbital and skull fat. (C) Selected spectra demonstrating lactate as a doublet peak with a relative maximum in locations 8 and 9, whereas the broader signal at the same position in locations 1 and 16 indicates contaminations from fat.

epileptic activity in the right and left rolandic area. All other neurophysiologic studies were normal. A muscle biopsy and chromosome analysis were both normal. At 4 months she experienced a rather rapid decline that was accompanied by tachycardia and tachypnea. There was a gradual increase in her arterial Pco$_2$ over several days, which eventually required intubation. Shortly after intubation she developed asystole, which was refractory to all resuscitative measures. Brain autopsy revealed a right-sided frontoparietal polymicrogyria as well as a moderate enlargement of the ventricular system. Necrosis was found in the area of the thalamus, lower brainstem, and bilateral occipital lobes.

Discussion
Results obtained from PET measurements on cerebral glucose metabolism and MRS provide evidence that patients with congenital lactic acidosis due to mito-
The extent of increase in the metabolic rates may depend on the type and severity of defect [26]. The substantially higher rates of glucose metabolism and, correspondingly, the higher cerebral lactate concentration in Case 3 compared with Case 2 agree with the much more decreased activity of the respiratory chain capacity in Case 3. The cytochrome c oxidase activity in Patient 2's muscle was reduced only when expressed per citrate synthetase activity. In fibroblasts this enzyme showed a normal activity.

Glucose uptake and lactate accumulation are two aspects of glycolysis. The large resonance peaks for lactate in Cases 2 and 3 show that high amounts of lactate were present in brain tissue but not in Cases 1 and 4. There was some contamination from CSF lactate signal, because the volumes of interest (VOIs) also contained parts of the ventricles (9% and 4% of total VOI in Cases 2 and 3, respectively). CSF concentrations measured by lumbar puncture (see Table 2) were in the same range (3—8 mmol/L) as values in the whole VOI, indicating that there were no large differences between tissue and CSF concentrations, and that the error of tissue concentration measurements due to CSF contamination was small. Presence of lactate in the MR spectra seems to correlate with increased FDG uptake. This indicates that in these cases accumulated lactate is a consequence of increased glycolysis and does not result from impaired lactate clearance. In addition, the reductions in NAA in both Cases 2 and 3 suggest a process of ongoing neuronal damage. The low NAA signal in Case 4 may indicate neuronal damage or delayed myelination as a consequence of experienced cyanotic episodes.

Previous PET studies in adults with mitochondrial disorders showed globally decreased rates in metabolism [3, 5, 6]. Two of these studies also demonstrated relatively larger decreases in the thalamus [5, 6] and basal ganglia [5]. Most likely that type of hypometabolism is the result of chronic brain damage. A similar pattern was seen in our Patient 1. Chronic energy failure has been reported in a recent 13P MRS study [27]. In one study [5] oxidative metabolism was compared with glucose metabolism and oxidation turned out to be relatively more impaired than glycolysis, probably still indicating increased nonoxidative glycolysis in that chronic stage. Possibly, our Patients 2 and 3 had a more severe form of the disease with a massive increase of glycolysis that could not be maintained for a long time. Theoretically, massive lactate accumulation could exert toxic effects [28] and thus contribute to the unfortunate outcome; but increased cerebral lactate signals have also been described in chronic cases of mitochondrial encephalomyopathies [29].

PET and MRS now are able to demonstrate the met-
abolic consequences of impaired mitochondrial respiration noninvasively in brain tissue. Although mitochondrial diseases usually can be diagnosed by biochemical analysis of muscle biopsies, the severity of impairment may be different in muscle and brain, as indicated by our Case 2. Furthermore, the severity of the clinical symptoms is often not well correlated with the residual activity of the affected enzyme. Therefore, with further experience, the imaging techniques may possibly provide an estimate of the severity of brain involvement and may permit diagnosis also in cases with little or no muscle involvement. In addition, effectiveness of therapy could be investigated by these techniques.

References