# Assessment and Therapy Monitoring of Leigh Disease by MRI and Proton Spectroscopy

Ingeborg Krägeloh-Mann, MD\*, Wolfgang Grodd, MD<sup>†</sup>, Gerhard Niemann, MD\*, Gerhard Haas, MD\*, and Wim Ruitenbeek, PhD<sup>‡</sup>

In a 2-year-old boy with Leigh disease, spasticity, dysarthria, and optic atrophy gradually developed. Computed tomography and magnetic resonance imaging disclosed progressive, symmetric basal ganglia lesions. In muscle tissue, a defect of pyruvate dehydrogenase complex was found. Magnetic resonance volume selective proton spectroscopy (MRVS) of the basal ganglia demonstrated an abnormal lactate peak. A ketonemic diet coincided with clinical stabilization and arrest of progressive brain lesions. Lactate could no longer be demonstrated by MRVS. It reappeared with a new brain lesion coinciding with discontinuation of the diet. MRVS, therefore, appears to be a sensitive tool to evaluate pathologic lactate production in metabolic brain disease with disturbed energy metabolism and allows noninvasive therapy monitoring.

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# Introduction

Leigh disease is an autosomal recessive disease with onset in infancy or early childhood. The onset usually is insidious and the course intermittently progressive for several years. Clinical manifestations vary considerably and are nonspecific (i.e., feeding problems, psychomotor regression, ataxia, dystonia, spasticity, visual deterioration) [1]. Metabolic acidosis is a frequent, but not constant, laboratory finding [2]. Definite diagnosis of Leigh disease has been based on pathologic findings which typically demonstrate necrotizing lesions that are most commonly found symmetrically in the tegmentum of the brainstem. as well as the basal ganglia, thalamus, cerebellum, medulla, optic nerves, and posterior columns of the spinal cord [3]. Computed tomography (CT) [4-6] and, with higher sensitivity, magnetic resonance imaging (MRI) [2,7,8] detect these lesions and most authors [4-8] stress the importance of symmetric basal ganglia defects, especially of the putamina. Deficiencies of specific mitochondrial enzymes - especially pyruvate dehydrogenase complex (PDHc), pyruvate carboxylase, NADH dehydrogenase, cytochrome c oxidase - have been demonstrated in fibroblasts, liver, muscle, and brain of patients with Leigh disease. The deficiencies in brain tissue were found only in combination with evidence of defects in other organs [3,8-12]. An abnormality of PDHc is believed to be the most consistent biochemical finding in Leigh disease [13]. Morphologic studies of Leigh disease have revealed mitochondrial abnormalities in brain and other organs of autopsied cases [14]; the brain abnormalities are regarded as evidence that Leigh disease is a cerebral mitochondriopathy.

Previously, it was supposed that the metabolic defect in Leigh disease may be restricted to brain [8,13] as normal mitochondrial enzyme activity may be found in muscle tissue and cultured skin fibroblasts despite elevated cerebrospinal (CSF) lactate and pyruvate levels. We report a boy whose clinical findings, CT and MRI studies, and biochemical analyses of muscle tissue were suggestive of mitochondrial encephalopathy with deficiency of the PDHc. Further diagnostic support of a defect in the oxidative metabolism involving mainly the brain was given by MRVS volume selective proton spectroscopy which demonstrated an abnormal peak of lactate in the region of the most progressive lesions (heads of the caudate nucleus).

# **Case Report**

This boy was the first child of nonconsanguineous parents. After uneventful pre-, peri- and post-natal periods and normal early development until the age of 2 years, deterioration of motor functions slowly developed. He exhibited staggering and walked stiffly on his toes. Hand functions became clumsy and speech slurred. Mental development was normal. During the first  $2\frac{1}{2}$  years of the disease, neurologic examinations demonstrated a slightly progressive spasticity which was greater in the lower than in the upper extremities (i.e., increased tone, hyperactive tendon reflexes, extensor plantar responses) with dystonic posturing and progressive dysarthria. This spastic tetraplegia was first predominant on the left side and then gradually became symmetric during the fifth year of life. One year later, his gait and speech stabi-

From the Departments of \*Developmental Neurology and Neuroradiology; University of Tübingen; Tübingen, Germany;

<sup>&</sup>lt;sup>1</sup>Institute of Pediatrics: University Hospital of Nijmegen; Nijmegen, The Netherlands.

Communications should be addressed to:

Dr. Krägeloh-Mann; Universitäts-Kinderklinik; Frondsbergstrasse 23; D 74 Tübingen. Germany.

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Figure 1. Disease course with respect to neuroradiologic investigations and diet.

lized, he could walk again without falling, and dysarthria was less pronounced. Height and weight were at the 3rd percentile and head circumference the 25th percentile at age 21/2 years. Growth rate decreased to below the 3rd percentile during the fourth and fifth years of life, then reached the 3rd percentile during the sixth year, leaving the child's height below the 3rd percentile.



A

Figure 2. (A)  $T_1$ -weighted cranial images at 39 months of age (1, corresponding to MRII in Fig 1) disclosing bilateral defects of the putamina rigure 2. (A) 11-weighted cranial images at 59 months of age (1, corresponding to MRI1 in Fig 1) alsolosing bilateral defects of the putamina and the caudate head on the right side, defects being hypointensive; at age 50 months (2, corresponding to MRI2) disclosing an increase in volume of the left head of the caudate nucleus with decreased signal intensities; at age 56 months (3, corresponding to MRI3) disclosing a decrease in volume of the left head of the caudate nucleus and a hyperintense area; at age 64 months (4, corresponding to MRI4) revealing no additional tissue defects; at age 74 months (5, corresponding to MRI5) unchanged; at age 78 months (6, corresponding to MRI6) disclosing a defect of the right caudate head. (B) T2-weighted images of the same examinations disclosing reversed signal intensities. The hyperintense areas of the caudate head visualized on the T1-weighted images are masked here which excludes remnants of blood and favors a paramagnetic effect in the necessitic area effect in the necrotic area.

### Table 1. Serum lactate and pyruvate levels

	Age (years)						
	21/2	31/4	41/2	51/4	51/2	<b>6</b> <sup>1</sup> / <sub>2</sub>	Normal
Lactate (mmol/L)	2.2	1.4	1.7	1.7	1.8	1.2	≤ 1.8
Pyruvate (µmol/L)	147	84	116	120	114	91	≤ 120
Lactate/pyruvate ratio	15	16	14.7	14.2	15.8	12.2	≤ 15

At the age of 2<sup>1</sup>/<sub>2</sub> years, normal results were demonstrated by neurophysiologic studies (i.e., electroencephalography, electromyography, motor nerve conduction velocity studies), electrocardiography, echocardiography, and abdominal ultrasonography. Fundoscopy was normal, but demonstrated bilateral partial optic atrophy without deterioration of vision 2 years later and proved unchanged at the age of 6 years.

At 4 years of age, oral treatment with thiamine (500 mg daily) was begun and continued for 6 months. A diet with increased saturated short-chain fatty acids then was introduced in order to obtain ketonemia (i.e., diet consisted of 40% fat; Fig 1).

Differential Diagnosis. Normal values were found for ceruloplasmin, copper excretion, biotinidase, phytanic acid, and very long-chain fatty acids, for lysosomal enzymes in leukocytes (arylsulfatase A,  $\beta$ -galactosidase,  $\beta$ -hexosaminidase A and B), and serum ( $\beta$ -glucuronidase, fucosidase,  $\alpha$ - and  $\beta$ -mannosidase,  $\beta$ -hexosaminidase A and B). CSF contained normal cell number, protein content, and protein immunoelectrophoretic pattern. Organic acids and amino acids in urine were normal; there was no excess of glutaric acid and 3-hydroxyglutaric acid.

Energy Metabolism. The serum lactate and pyruvate levels were slightly elevated at the time of the first examination (age  $2\frac{1}{2}$  years; Table 1) and returned to normal at the time of subsequent examinations. CSF lactate was elevated (3.0 mmol/L; normal:  $\leq 2.2$  mmol/L).

Diet Control. Serum acetoacetate was low before diet administration (53  $\mu$ mol/L) and elevated on the diet (110  $\mu$ mol/L); hydroxybutyrate demonstrated the same changes (111 and 283  $\mu$ mol/L, respectively). Urine ketones remained normal or were at 5-10 mg/dl. At 61/2 years of age, acetoacetate decreased to 67  $\mu$ mol/L and hydroxybutyrate to 118  $\mu$ mol/L, which resulted from discontinuation of the diet by the child's mother. During the diet, lactate response to glucose loading was normal (lactate 1.8 mmol/L before loading and 2.0 mmol/L after 20 min). CSF lactate was not controlled during the diet. Serum lactate and pyruvate

Table 2. Activities of mitochondrial enzymes in muscle tissue

	Patient	Control Range
Cytochrome c oxidase	97	73-284 *
Succinate: cyt c oxidore- ductase	7.0	4.1-16 *
NADH: Q1 oxidoreductase	4.7	4.7-19 *
Pyruvate dehydrogenase complex	1.2	2.8-6.2 *
Citrate synthetase	55	48-146 *
Carnitine, total	1.0	2.7-4.6 **
Carnitine, nonesterified	0.9	2.2-4.2 **
Creatine, total	2.6	> 15 **
* In mU/mg homogenate prot	ein [2].	

\*\* In µmoles/gm wet weight [2].

did not change significantly during the diet (Table 1). Serum cholesterol was 211 mg/dl before the diet and increased to 232 and 242 mg/dl during the diet (normal: 120-246 mg/dl).

## Morphologic Studies

Histochemical studies of gastrocnemius muscle tissue at the age of  $3\frac{1}{2}$  years disclosed no abnormalities. Ultrastructural studies of muscle, skin, and lymphocytes proved normal; sural nerve study demonstrated slight signs of demyelination and some loss of unmyelinated axons.

# **Biochemical Studies**

Enzyme activities and carnitine content were determined as described previously [15] in total homogenates prepared from frozen gastrocnemius muscle. The enzyme complexes of the respiratory chain, cytochrome c oxidase, succinate: cytochrome c oxidoreductase, NADH: Q1 oxidoreductase and the mitochondrial reference enzyme citrate synthetase had normal activities (Table 2); however, the activity of the PDHc [16] was diminished to 43% of the lowest control value. Immunochemical investigation using anti-bovine heart PDHc antiserum did not reveal a specific decrease in one of the proteins composing the complex after SDS-polyacrylamide gel electrophoresis [16]. Diminished values of muscle carnitine were found, but the significance is obscure because the reference compound creatine also had a very low concentration. Serum carnitine level was normal (43  $\mu$ mol/L; normal: 35-55  $\mu$ mol/L).

# Neuroradiologic Investigations

Figure 1 demonstrates the time course of the disease with respect to neuroradiologic investigations and diet.

Computed Tomography. CT was performed at  $2\frac{1}{2}$  years of age (Siemens DRH scanner,  $512 \times 512$  matrix), and demonstrated bilateral, nearly symmetric, hypodense areas in the lenticular nucleus as well as in the right head of the caudate nucleus without pathologic enhancement on contrast medium.

Magnetic Resonance Imaging. MRI was performed with a 1.5 T Magnetom (Siemens; Figs 2A,2B). T<sub>1</sub>-weighted, multi-slice, single spin-echo technique was used in sagittal, axial, and coronal orientation with TR 600 msec, TE 15 msec, and  $256 \times 256$  matrix. Axial and coronal T<sub>2</sub>-weighted, multi-slice, double-echo sequences were obtained (TR: 2,400 msec, TE: 120 msec).

MRI at 3 years, 3 months of age (MRI 1, Figs 1,2A,2B) demonstrated bilateral defects of the putamina and caudate head on the right hemisphere, defects being hypointensive on  $T_1$ - and hyperintensive on  $T_2$ -weighted images. In addition to these results, MRI at 4 years, 2 months of age (MRI 2, Figs 1,2A,2B) disclosed an increase in volume of the left head of the caudate nucleus with abnormal signal intensities (decreased in  $T_1$ - and increased in  $T_2$ -weighted images).

At 4 years, 8 months of age, (MRI 3, Figs 1,2A,2B), the extent of the abnormal areas was unchanged, the left head of the caudate nucleus had a decrease in volume and a hyperintense area on the  $T_1$ -weighted images which was masked on the  $T_2$ -weighted images.

At 5 years, 4 months of age (MRI 4, Figs 1,2A,2B), there were no additional tissue defects. This finding was unchanged 10 months later



Figure 3. MR volume selective proton spectroscopy with TE 135 msec of the left caudate head at 4 years, 8 months of age (MRVS1) demonstrating normal peaks for choline (Ch), creatine/phosphocreatine (Cr/PCr), and N-acetyl-aspartate (NAA), and an abnormal lactate (Lac) peak; at 6 years, 2 months of age (MRVS3) demonstrating no lactate; at age  $6\frac{1}{2}$  years (MRVS4, right caudate head) disclosing a small lactate peak.

(MRI 5, Figs 1,2A,2B). At  $6\frac{1}{2}$  years of age, the right caudate head again disclosed abnormal signal intensities (MRI 6, Figs 1,2A,2B).

Magnetic Resonance Volume Selective Proton Spectroscopy. Volume selection was achieved with a spin-echo technique, 2 spectra with 256 acquisitions, TR 1,500 msec and TE 135 and 270 msec were obtained to ensure the identification of lactate. The voxel  $(2 \times 2 \times 2 \text{ cm})$  was situated in the left head of the caudate nucleus for MRVS 1-3 and in the right head for MRVS 4. In addition to normal peaks of *N*-acetyl-aspartate (NAA), creatine/phosphocreatine and choline, there was a peak of lactate at 4 years, 8 months of age. Lactate could no longer be demonstrated at 5 years, 4 months of age and at 6 years, 2 months, but could be seen again at the age of  $6\frac{1}{2}$  years (Fig 3). This latter MRVS control was performed because ketonemia could no longer be detected due to discontinuation of the diet.

# Discussion

Our patient presented with leg-dominated spasticity, dystonia, and dysarthria, beginning at the age of 2 years. Mental development was normal. Neurologic symptoms were slowly progressive during the first 2 years. Since the age of about 41/2 years, the child stabilized, with the institution of a diet with increased, saturated short-chain fatty acids. This diet was chosen to replace, in part, pyruvate oxidation by oxidation of ketone bodies [17] because no specific enzyme defect within the PDHc could be verified which could have been more specifically influenced by thiamine or lipoic acid [18,19] and because a 6-month trial of orally administered thiamine (500 mg daily) failed to produce a clinical response (MRI even disclosed progression of lesions). Animal studies demonstrated better utilization of ketone bodies to replace pyruvate oxidation in brain of younger animals when compared to that of mature ones [20]. There is evidence of positive effects in clinical studies [17].

Cerebral CT and especially MRI disclosed bilateral lesions of the putamina and pallidum, as well as progressive lesions of the head of the caudate nucleus affected initially on the right side, then on the left, and then again on the right. Clinically, the child initially exhibited a predominantly left-sided spastic tetraplegia which gradually became symmetric; the second lesion of the right caudate head did not produce a significant clinical correlate. The findings of elevated CSF lactate, slightly elevated serum lactate and pyruvate, and especially diminished activity of the PDHc complex in muscle tissue all support a defect in pyruvate oxidizing metabolism. These data together with the clinical findings and course and neuroradiologic abnormalities strongly suggest the diagnosis of Leigh disease. The metabolic defect concerning energy metabolism of the brain could further be supported by the detection of lactate in the lesion demonstrated by MRVS volume selective proton spectroscopy which provides an additional tool for noninvasive biochemical tissue analysis. Lactate concentration in brain tissue is normally below 1 mM and is not clearly detectable by proton spectroscopy [21]. Our patient demonstrated a distinct lactate peak at the first spectroscopic examination which was performed at the onset of the diet after a chronically progressive disease course over  $2\frac{1}{2}$  years. The lactate peak disappeared completely at the second and third MRVS 6 and 16 months after the onset of the diet. It reappeared at age  $6\frac{1}{2}$  years when there was evidence of a new lesion which coincided with discontinuation of the diet. There is still little knowledge about in vivo brain lactate in pathologic conditions. One study reported that it could still be detected 4 days after acute stroke in an adult [21]. There was no evidence of lactate 4 days after neonatal asphyxia [22]. Detre et al. reported elevated brain lactate, particularly in basal ganglia lesions, in an 11-month-old child with Leigh disease. Grodd et al. demonstrated a clear lactate peak in basal ganglia lesions of 2 other patients with Leigh disease, 3 and 18 months after acute neurologic deterioration [24].

The impact of the diet is debatable; Leigh disease may have an episodic course where acute deterioration may be followed by spontaneous recovery; however, it is noteworthy that after a long period of clinical deterioration and progressive tissue destruction – demonstrated by cranial MRI – clinical stabilization and normalization of the spectrum coincided with the application of a diet and reappeared when the diet was discontinued. The disappearance of the lactate peak while this patient was on the diet may be attributed to the successful diminution of pyruvate oxidation in brain tissue by an increase in the oxidation of ketone bodies. Further case studies will be required to validate these findings. The authors thank Mrs. Vera Rühl-Ullrich for planning the diet.

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