

Cerebral Lactate and *N*-Acetyl-Aspartate/Choline Ratios in Asphyxiated Full-Term Neonates Demonstrated *In Vivo* Using Proton Magnetic Resonance Spectroscopy

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ABSTRACT. The purpose of this study was to test the hypothesis that a high lactate signal and a low *N*-acetyl-aspartate/choline ratio in neonates with postasphyxial encephalopathy indicated a high chance of an adverse outcome *in vivo* when proton magnetic resonance spectroscopy was used. Twenty-one full-term asphyxiated neonates were examined at a mean postnatal age of 7.1 d. Five patients died, and five survivors had handicaps. Eleven of the 16 survivors (seven without handicaps and four with handicaps) had a second examination at 3 mo of age. After magnetic resonance imaging, spectra were obtained at 1.5 tesla. A 20-mm-thick slice was selected through the basal ganglia. After optimizing the B-0 field, we used a double spin-echo pulse sequence (90-180-180°) with a time to repeat of 2000 ms and a time to echo of 272 ms. Two-dimensional spectroscopic imaging was performed by 32 × 32 phase encoding steps in two directions in a 225-mm field of view, resulting in 1-mL volumes, followed by computerized processing. Neuromotor development was examined at 6 wk, 3 mo, and every 3 mo thereafter. Lactate resonances were seen only in the five patients with grade 3 postasphyxial encephalopathy. Lactate was distributed diffusely ($n = 4$), or localized in areas of infarction ($n = 1$). *N*-acetyl-aspartate/choline ratios were significantly lower in the patients with an adverse outcome than in the survivors without handicaps, both neonatally ($p < 0.005$, Wilcoxon's rank sum test) and at 3 mo ($p < 0.05$). In conclusion, the presence of cerebral lactate and a low *N*-acetyl-aspartate/choline ratio demonstrated *in vivo* using proton magnetic resonance spectroscopy in full-term neonates with postasphyxial encephalopathy indicate a poor outcome. (*Pediatr Res* 35: 148-151, 1994)

Abbreviations

Cho, choline

¹H-MRS, proton magnetic resonance spectroscopy

¹H-MRSI, proton magnetic resonance spectroscopic imaging

MRI, magnetic resonance imaging

NAA, *N*-acetyl-aspartate

PRESS, point-resolved spectroscopy

TE, time to echo

TR, time to repeat

PAE, postasphyxial encephalopathy

MR, magnetic resonance

With ¹H-MRS, lactate resonances and a decrease of the NAA/Cho ratio could be demonstrated *in vivo* in adults with unilateral hypoxic-ischemic brain lesions and subsequent handicaps days to months after the insult (1-6). ¹H-MRSI showed the presence of lactate to be confined to the area of infarction (2). Also, in infants and children with near drowning, cerebral lactate has been demonstrated *in vivo* in those with permanent handicap (7). In neonates with perinatal asphyxia, only one group described findings of ¹H-MRS (8, 9). In the neonates with adverse outcome, a lower NAA/Cho ratio was found than in neonates with a normal neuromotor development, but lactate resonances could not be demonstrated in any of their neonates (8).

In accordance with the findings in adults with brain ischemia and in infants with near drowning, we hypothesized that the presence of a high lactate resonance and a low NAA/Cho ratio in the cerebrum of the newborns with PAE indicated a high chance of an unfavorable outcome.

PATIENTS AND METHODS

In 1992, 21 full-term neonates were examined who had been admitted immediately after birth to our neonatal intensive care unit because of perinatal asphyxia. These full-term infants had postconceptional ages of 39 to 42.3 wk and birth weights of 2430 to 4475 g. All had a well-documented history of perinatal asphyxia, which was indicated by pH values of umbilical blood samples below 7.10, 5-min Apgar scores below 6, and the need for resuscitation by positive pressure ventilation after birth followed by PAE. PAE was graded as grade 1 in 2 neonates, grade 2 in 14 neonates, and grade 3 in 5 neonates, according to the criteria of Sarnat and Sarnat (10). Ethical approval was granted by the Ethical Committee of the Wilhelmina Children's Hospital and of the Utrecht University Hospital.

All patients were transported from the Wilhelmina Children's Hospital to the MR unit of the Utrecht University Hospital in a transport incubator. Additional sedation was not given to any of the neonates. The patients tested at 3 mo of age were sedated with an intramuscular injection of 2 mg/kg pethidine, 0.5 mg/kg chlorpromazine, and 0.5 mg/kg promethazine. Heart rate, respiration, and transcutaneous oxygen saturation were monitored continuously during the procedure and for at least 12 h thereafter in the sedated patients. Average age at test was 7.1 d (range, 1 to 20 d) for the neonates and 94 d (range, 81 to 101 d) for the 3-mo-old infants.

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Studies were performed on a commercial 1.5 tesla whole-body system (Gyrosan S15, Philips Medical Systems, Best, The Netherlands) with a Helmholtz head coil of 30 cm diameter. Standard spectroscopy acquisition software was used. First, sagittal T1 (TR = 450 ms, TE = 30 ms) and transversal T1 (TR = 2500 ms, inversion time using inversion recovery pulse sequence = 800 ms, TE = 30 ms) and T2 (spin echo, TR = 3000 ms, TE = 50, 150 ms) images were made with a field of view of 225 × 225 mm, a slice thickness of 5 mm, and slice factor of 1.2. Thereafter, a PRESS sequence was used for volume selection, with a slice thickness of 20 mm and a maximum width and length, carefully avoiding contact with the bone of the skull (Fig. 1). Local optimizing the B-0 field was performed by optimizing the proton signal from water to a half-height line width of 0.1 ppm.

First, a ¹H-MR spectrum of the whole PRESS volume was acquired using a double-water elimination Fourier transform for water suppression, followed by a PRESS double spin-echo sequence (90-180-180°) (2). Relevant parameters were a TR of 2000 ms and an TE of 272 ms; 64 signals were averaged; 512 time points were taken; and the sampling band width was 1000 Hz. The data were processed by applying Lorenz-Gauss windowing in the time domain (exponential narrowing 4 Hz and gaussian broadening 6 Hz) for noise reduction and spectral resolution enhancement, followed by zero-filling to 2048 data points. Ad-

ditional water suppression was applied by the data-shift-accumulation method (11). The ratio of the peak heights to baseline of NAA and Cho was calculated.

Second, volume-selective two-dimensional spectroscopic imaging was performed with 32 phase encoding steps in two directions, resulting in volumes with a nominal size of 1 mL. Spectroscopic images were created by integration over selected spectral regions with use of special software (12). The spectroscopic data were processed identically as the single volume experiments to obtain metabolic maps. However, no zero-filling was applied in the time domain, but zero-filling to 128 × 128 was applied in two-dimensional K space.

With the use of this pulse sequence, resonances of NAA at 2.02 ppm, Cho at 3.25 ppm, creatine and phosphocreatine at 3.0 ppm, and—when present—lactate at 1.33 ppm could easily be identified. Lactate resonances were demonstrated by the typical doublet with a J-coupling of 7 Hz and the resonance occurring at the point of chemical shift for lactate (1.33 ppm), ruling out the possibility that mobile lipids gave rise to these resonances.

RESULTS

Neurodevelopmental outcome. Five patients died, all because of extensive cerebral damage, which was diagnosed clinically and by cerebral ultrasonography, EEG, and evoked potentials on which supportive treatment was withdrawn. Five others showed abnormalities at neurodevelopment: four had spastic quadriplegia, and one had psychomotor retardation. In addition, mental retardation was found in two infants, and one had severe seizures at 4 mo of age.

Magnetic resonance. MRI showed multiple cerebral infarcts in one patient who died, and abnormal areas in the basal ganglia were noted in another. Hematomas around the cerebellum were seen in two grade 2 patients. Neonatal MR images of the other patients did not demonstrate any abnormalities.

MR images obtained when the infants were 3 mo of age showed dilated ventricles, delayed myelination, and cortical atrophy in the five patients with handicaps.

Magnetic resonance spectroscopy. Lactate resonances with a height of at least 50% of the NAA resonance could be demonstrated only in the PRESS volume in all five patients with severe PAE, ages 1 to 6 d (Fig. 2A). A normal spectrum of a 2-wk-old control neonate illustrates the absence of lactate (Fig. 2B).

Spectroscopic imaging showed a diffuse signal of lactate extending over the region of the thalami/basal ganglia and the surrounding brain parenchyma in four patients with grade 3 PAE (Fig. 3). Ventricles could not be identified in the spectroscopic image in these patients, probably because of the reduction in ventricular size caused by edema. One patient showed a lactate signal in areas of localized infarction.

The mean NAA/Cho ratios (±SD) of the PRESS volume were 0.98 ± 0.13, 0.79 ± 0.09, and 0.72 ± 0.14 for neonates with normal outcomes, handicaps, and death, respectively (Fig. 4). The differences between neonates with a normal and an adverse outcome (*i.e.* handicaps or death) were significant ($p < 0.005$, Wilcoxon's rank sum test). The NAA/Cho ratios at 3 mo were 1.68 ± 0.33 and 1.23 ± 0.25 for infants without and with handicaps, respectively. These differences are also significant ($p < 0.05$).

DISCUSSION

Lactate resonances with a height of at least 50% of the NAA resonance could be demonstrated in all 5 patients with grade 3 PAE but in none of the 16 patients with less severe degrees of PAE. Small peaks at or around 1.33 ppm could be seen in some infants, but because these resonances could not be differentiated from background noise, they could not be regarded as indicative of high lactate concentrations. All patients had normal blood gas values and normal blood lactate values and were normoglycemic at the time of ¹H-MRSI, so it is unlikely that lactate was trans-

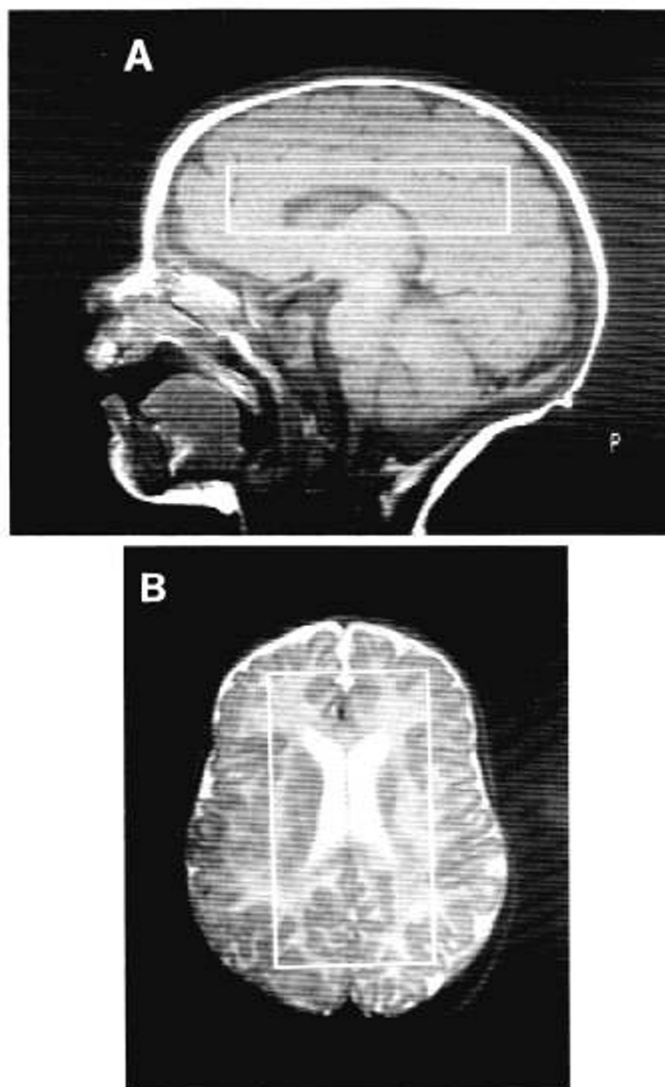


Fig. 1. Position of the PRESS volume in the cerebrum. A slice thickness of 20 mm is chosen, and contact with the skull is avoided. Sagittal T1 (A) and transversal T2 sections (B) through the brain.

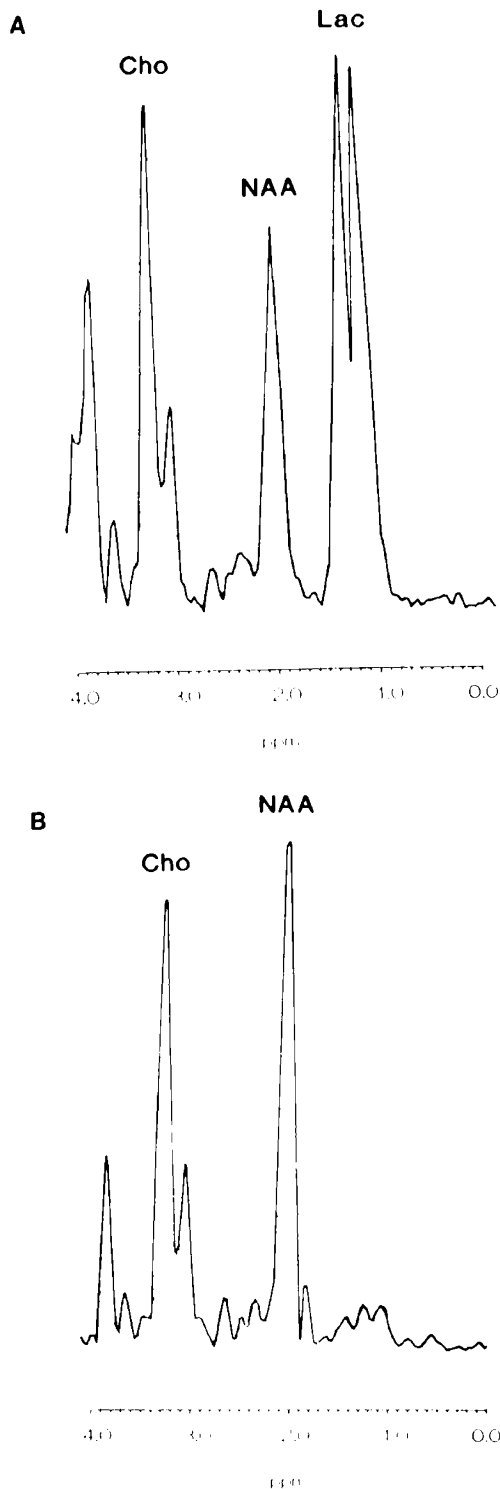


Fig. 2. ^1H -MR spectrum of the PRESS volume of a neonate with grade 3 PAE (A) and the spectrum of a neonate with a normal outcome (B). Spectra are presented of the PRESS volume of a neonate with grade 3 PAE (A) and a 2-wk-old neonate with grade 1 PAE and a normal outcome (B). The ^1H -MR spectra of the PRESS volume demonstrate resonances of Cho at 3.25 ppm, total creatine at 3.0 ppm, NAA at 2.02 ppm, and lactate (Lac) at 1.33 ppm (only in A).

ferred from the blood into the brain. Lactate levels in cerebrospinal fluid were not measured at the time of ^1H -MRSI. The lactate resonance could be caused either by lactate produced in the parenchyma by cerebral hypoperfusion; ischemia in the border zones of infarcted areas, the so-called ischemic penumbra; or phagocytic glial cell activity with anaerobic glycolysis (2).

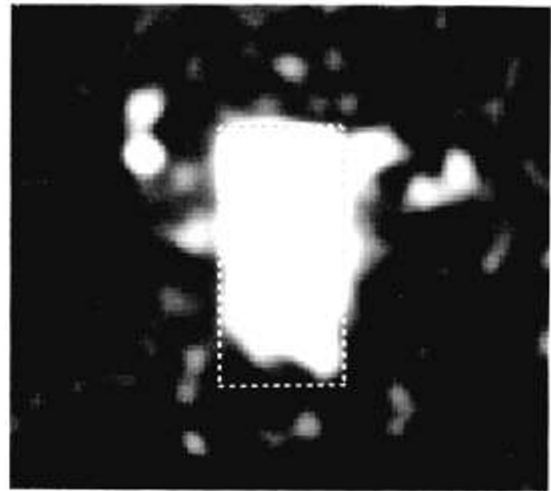


Fig. 3. ^1H -MRSI demonstrates lactate to be distributed evenly over the PRESS volume of a neonate with grade 3 PAE. Borders of the PRESS volume are indicated and are similar to those in Figure 1B. Ventricles cannot be identified, most likely because of ventricular compression by cerebral edema. White spots outside the PRESS volume are caused by artifacts.

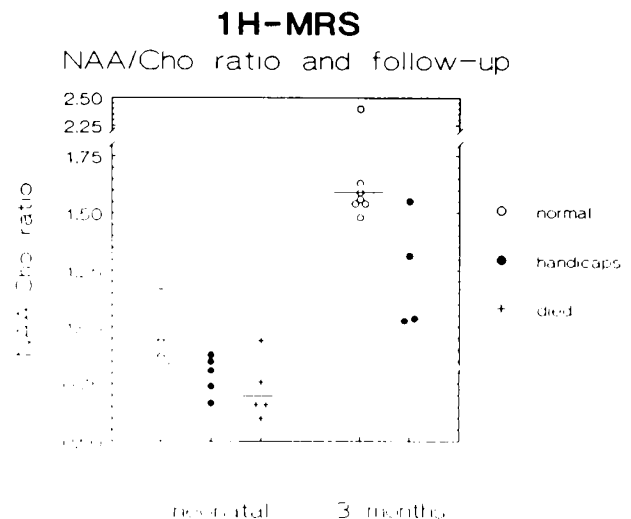


Fig. 4. NAA/Cho ratios are plotted according to postnatal age (neonatal, 3 mo) and outcome. Differences between neonates with a normal and adverse outcome (handicaps or death) are significant both in the neonatal period ($p < 0.005$, Wilcoxon's rank sum test) as well as at 3 mo ($p < 0.05$).

Although some gliosis was present in histologic analysis at post-mortem examination, it is unlikely that the relatively small number of phagocytotic cells created the high lactate resonances. With our methods, it was not possible to differentiate among the possibilities of lactate trapped in an infarcted, hypoperfused area or continuous lactate production in the ischemic penumbra.

All patients were treated with antiepileptic drugs and showed no clinical convulsions. From June 1992 onward, cerebral electric activity was monitored with the so-called cerebral function monitor, a one-channel compressed EEG device (13). Continuous seizure activity was not demonstrated in any of our patients, so it is unlikely that prolonged seizures caused the high lactate resonance.

Our results are in contrast to the findings of Peden *et al.* (8), who did not demonstrate any lactate, although perhaps one of their patients (case 8) could have shown a small lactate resonance. It is possible that their patients were not as severely affected, and the fact that none of their patients died support this theory.

The lactate resonance was localized in the whole PRESS volume in four newborns, including thalamic areas and basal ganglia. Among other areas, these brain areas are sensitive to anoxia, as is shown in rhesus monkeys and human newborns with severe perinatal asphyxia (14–19).

All five patients with PAE 3 died in the neonatal period. Postmortem examination was performed in four of them and revealed extensive diffuse neuronal necrosis in three patients and localized neuronal necrosis in the patient with the multiple infarcts. The areas of necrosis on histologic analysis corresponded to the areas of high lactate signal on MRI. The presence of lactate in areas of hypoxic-ischemic neuronal cell death is compatible with the presence of lactate in adults with ischemic stroke and in children with near drowning (1–7). The absence of lactate did not predict a normal outcome because five neonates without clear lactate resonances proved to have handicaps. It is possible that lactate had been present before MRS was performed. Also, they had prolonged hypoxia, which is known to affect cortical areas primarily (17, 19). With the present ¹H-MRS technique, however, cortical areas cannot be examined because of susceptibility effects, *i.e.* interference from skin and bone of the skull.

Because NAA/Cho ratios have previously been reported to be of predictive value (8, 9), they were calculated from the ¹H-MR spectra obtained. Peak values were chosen to avoid the influence of different peak widths in individual measurements. So far, normal NAA/Cho ratios in neonates have not yet been obtained in our institute. Normal values at the end of the first month of life are above 0.80 (20). The NAA/Cho ratios between neonates with a normal or adverse outcome were significantly different, confirming the findings of Peden, *et al.* (8, 9).

One patient who died and who had a high lactate peak had a NAA/Cho ratio of 0.94, which is within the normal range. This patient, however, was tested at a very early age after the birth asphyxia. As demonstrated in animals and human adults, NAA/Cho ratios decline hours to days after significant cerebral hypoxia-ischemia (5, 21). It is likely that the NAA/Cho ratio in this patient would have been lower if the patient had been tested at a later age. Unfortunately, because of the severity of the neonate's illness, the examination could not be repeated.

Patients with a normal outcome, as well as patients with handicaps, showed an increase in the NAA/Cho ratios in the first 3 mo of life just as normal infants do (20). The increase of the NAA/Cho ratio most likely reflects neuronal maturation. Nevertheless, the differences in ratios remained significant, possibly indicating permanent loss of neurones.

Testing time was limited because of the severity of illness in the neonates and the duration of sedation in the 3-mo-old infants, which lasted approximately 1.5 h. Thus, we were unable to prolong our ¹H-MRSI, which is necessary for precise concentration measurements of NAA, Cho, creatine and phosphocreatine, and lactate. Hüppi *et al.* (22) have reported NAA, Cho, and creatine and phosphocreatine concentrations in neonatal brain, but because they used short time-echo sequences, which produce different resonance heights, their findings cannot be used for calculation of metabolite concentrations in our patients.

Although neonatal MR images were useful for identifying tentorial hemorrhages, they were of limited value in cerebral ischemia in the immediate postasphyxial period, possibly because of the high water content of the neonatal brain. Parenchymal abnormalities could be found in only 2 of the 10 patients with an adverse outcome. Others using similar MR scanners and scanning sequences have the same experience (23), although one preliminary study with high-field (2.35 T) spectroscopy claims

usefulness of MRI in prognosis after full-term perinatal asphyxia (24).

This report confirms the findings of others that demonstrate that a decrease of the NAA/Cho ratio indicates a risk of an adverse neurodevelopmental outcome. To the best of our knowledge, this report is the first to demonstrate *in vivo* the presence and distribution of cerebral lactate in full-term neonates with PAE, indicating a hypoxic-ischemic cerebral insult.

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