Marjo S. van der Knaap, MD • Jeroen van der Grond, PhD • Peter C. van Rijen, MD •
Joop A. J. Faber, PhD • Jaap Valk, MD, PhD • Kobus Willemse, MD, PhD

Age-dependent Changes in Localized Proton and Phosphorus MR Spectroscopy of the Brain[1]

After birth the human brain is subject to major maturational changes, which are associated with changes in the biochemical composition of the brain and brain metabolism. Magnetic resonance (MR) spectroscopy has special capabilities in the analysis of in vivo metabolism. Volume-selective proton and phosphorus MR spectroscopy of the brain was performed on a 1.5-T magnet in 41 healthy children aged 1 month to 16 years. With advancing age, phosphorus spectra revealed a decrease in the ratios of phosphomonoesters (PMEs) to β-adenosine triphosphate (ATP) and PMEs to phosphocreatine (PCr) and an increase in the ratios of phosphodiesters to β-ATP, PCr to β-ATP, and PCr to inorganic phosphate (Pi). No significant changes were observed in Pi/β-ATP and pH. No changes occurred after the age of 3 years. Proton spectroscopy revealed an increase in the ratios of *N*-acetylaspartate (NAA) to choline (Ch) and NAA to creatine (Cr) and a decrease in Ch/Cr with increasing age. The most rapid changes were noted during the first 3 years of life, but changes were still observed at the age of 16 years.

Index terms: Brain, growth and development • Brain, MR studies, 10.1214 • Magnetic resonance (MR), in infants and children • Magnetic resonance (MR), phosphorus studies • Magnetic resonance (MR), spectroscopy

Radiology 1990; 176:509–515

[1] From the Department of Child Neurology, University Hospital for Children, Wilhelmina Kinderziekenhuis, PO Box 85500, 3508 GA Utrecht, The Netherlands (M.S.v.d.K, K.W.); the Departments of Radiodiagnosis (J.v.d.G.) and Neurosurgery (P.C.v.R.), University Hospital of Utrecht; the Center of Biostatistics, University of Utrecht (J.A.J.F.); and the Free University Hospital, Amsterdam (J.V.). Received January 4, 1990; revision requested March 8; revision received April 9; accepted April 19. Supported by grant 002817090 from the Dutch Praeventiefonds. **Address reprint requests to** M.S.v.d.K.

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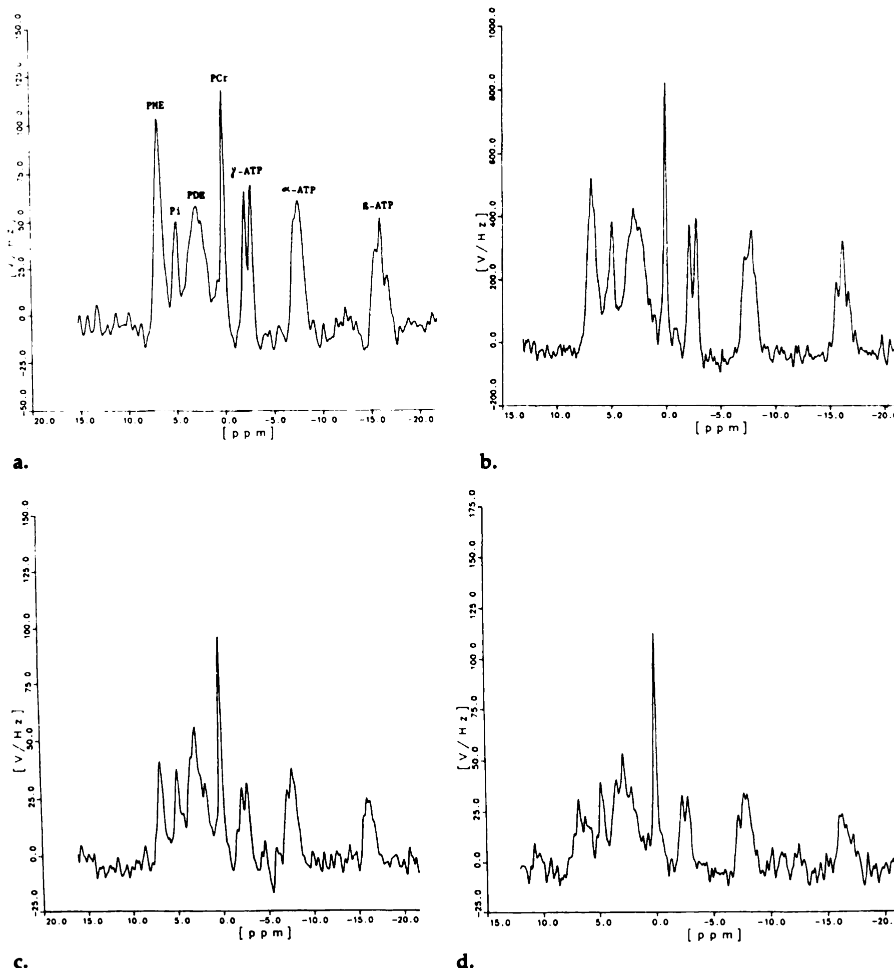

Figure 1. P-31 spectra of the brain obtained at the age of 1 month **(a)**, 4 months **(b)**, 2½ years **(c)**, and 15 years **(d)**. Spectral peaks are assigned to PME (6.5 ppm), Pi (4.9 ppm), PDE (2.6 ppm), PCr (0 ppm), γ-ATP (−2.6 ppm), α-ATP (−8.0 ppm), and β-ATP (−16.5 ppm). Numbers along y axis are in arbitrary units.

THE brain is subject to major changes not only before but also after birth, through the processes of neuronal organization (1–10), proliferation and differentiation of glial cells (11–14), and myelination (15–19). The maturational processes are associated with changes in biochemical composition of the brain and brain metabolism. Biochemical analysis is suitable for showing differences in brain composition at different ages but not for showing differences in in vivo metabolism. Magnetic resonance (MR) spectroscopy has special capabilities in the analysis of in vivo

Abbreviations: ATP = adenosine triphosphate, Ch = choline, Cr = creatine, ISIS = image-selected in vivo spectroscopy, NAA = *N*-acetylaspartate, PCr = phosphocreatine, PDE = phosphodiester, Pi = inorganic phosphate, PME = phosphomonoester, TE = echo time, TR = repetition time.

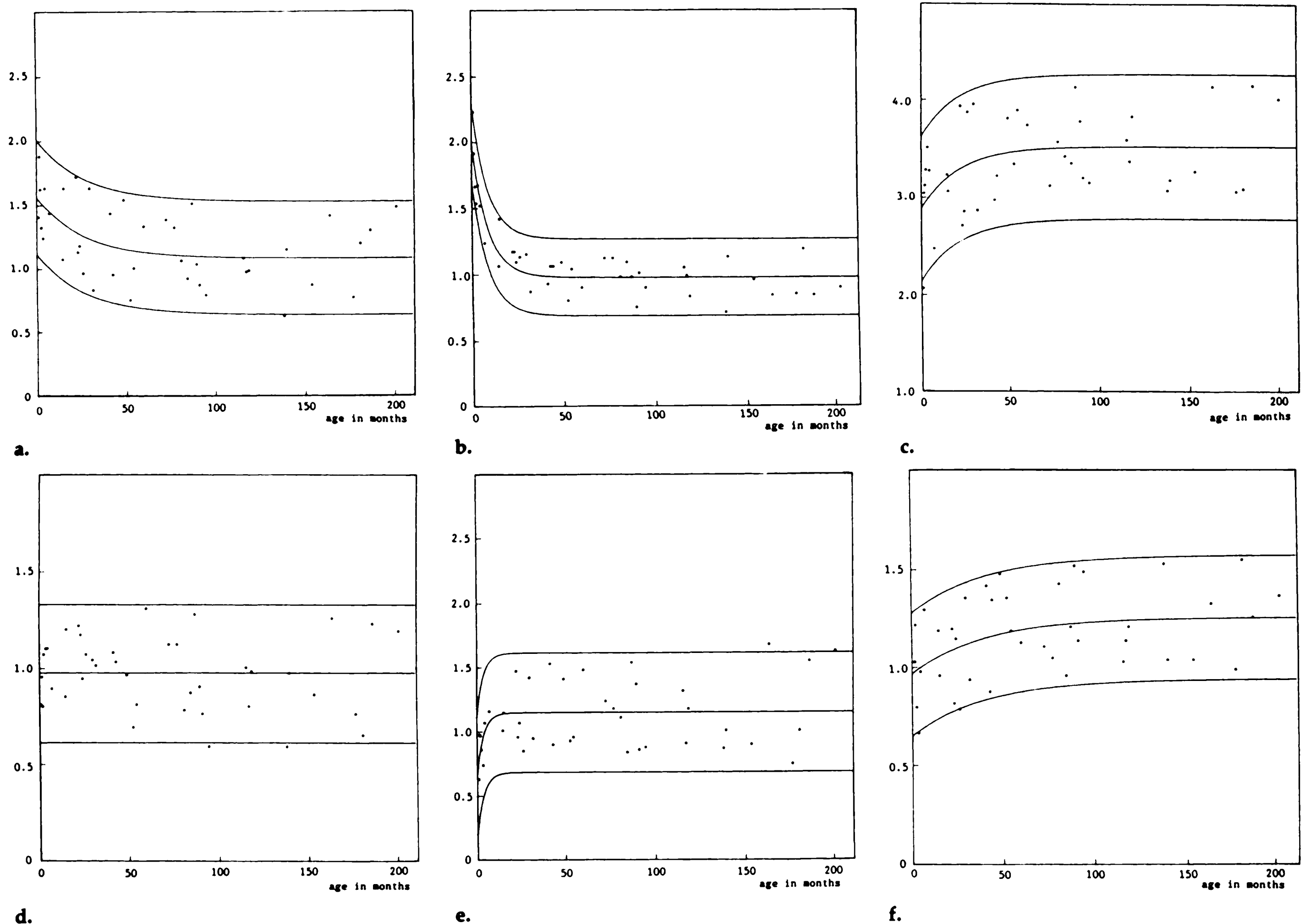

Figure 2. Regression lines and 2.5 and 97.5 percentile lines for PME/β-ATP **(a)**, PME/PCr **(b)**, PDE/β-ATP **(c)**, Pi/β-ATP **(d)**, PCr/β-ATP **(e)**, and PCr/Pi **(f)**.

brain metabolism. It has been shown repeatedly that phosphorus-31 MR spectra of the brain in neonates are different from those in adults (20–27). However, to our knowledge, no normal values have been presented for P-31 spectra of the brain at different ages and no data are available for proton (hydrogen-1) spectra of the brain in children of different ages.

Interest in child neurology implies interest in brain maturation and methods suitable for quantitative assessment of aspects of brain maturation. We began this study to examine maturational changes in H-1 and P-31 spectra of the brain from birth to the age of 16 years. A second aim of this still ongoing study was to acquire normal values to permit interpretation of the spectra of children with clinical signs of a delay of brain maturation or with other cerebral disorders.

MATERIALS AND METHODS

Children

Between October 1988 and July 1989, 41 children (19 boys, 22 girls) underwent H-1 and P-31 MR spectroscopy of the brain, with the informed consent of their parents. All children were healthy and had normal MR images of the brain. No children were investigated twice. Their ages ranged between 1 and 200 months, with a mean of 71 months. No sedation was used. Neonates and infants were investigated after feeding and tightly wrapped in a sheet. With older children, one of the parents accompanied the child in the MR apparatus to keep him or her quiet and cooperative. The whole investigation, including imaging and spectroscopy, could be completed in 1–1½ hours.

MR Spectroscopy

The investigations were performed on a 1.5-T unit (Gyroscan; Philips, Utrecht, The Netherlands) operating at 63.87 MHz for H-1 spectroscopy and at 25.86 MHz for P-31 spectroscopy. A Helmholtz saddle coil with a diameter of 30 cm was used for H-1 spectroscopy. For the P-31 studies, a head coil consisting of two 18-cm-diameter loops in a Helmholtz configuration was placed orthogonally inside the proton coil.

T1-weighted spin-echo images with a repetition time (TR) of 600 msec and an echo time (TE) of 30 msec were acquired in the transverse plane. These images were used to locate the volume of interest. The volume chosen was always in the paraventricular region. This volume contains predominantly white matter but also some deep gray matter from the basal nuclei. The mean size of the volume was 7 × 3 × 3 cm, with the long axis of the volume parallel to the axis of the lateral ventricle. The size of the volume was adapted to the size of the brain and varied from 6 × 3 × 3 cm in small children to 8 × 3 × 3 cm in teenagers. Local shimming of the magnetic field was performed by optimizing the H-1 signal from water. Usually a half-height line width of the water signal of 0.1 ppm was achieved. Starting with shim settings that from prior experience were known to result in a near-optimal water signal, additional shimming took 5–15 minutes.

P-31 spectroscopy of the selected volume was performed with the ISIS (image-selected in vivo spectroscopy) technique (28,29). The TR was 3,750 msec, the sample frequency 3,000 Hz, and the number of sample points 2,048. For each spectrum, 256 measurements were obtained. The averaged free induction decays were zero filled to 4,096 data points and processed with a convolution difference procedure (150 Hz) and exponential multi-

plication (8 Hz). After Fourier transformation, a linear phase correction was applied. The spectra were quantified by peak area measurements. This was accomplished by first drawing a straight baseline through the noise from both sides of the spectral region of interest. Peak areas were calculated by measuring the triangular areas under the peaks manually. To reduce variability introduced by quantification, all spectra were quantified twice and mean values were calculated. Brain tissue pH was derived from the difference in chemical shift between inorganic phosphate (Pi) and phosphocreatine (PCr), with use of the equation given by Petroff et al (30).

H-1 spectra were acquired with a selective 90°-180°-180° pulse sequence and a zero-crossing inversion recovery technique with a selective adiabatic inversion pulse for water suppression (31,32). The TR was 2,500 msec, the TE 272 msec, the sample frequency 1,000 Hz, and the number of sample points 512. For each spectrum 128 measurements were obtained. The average free induction decays were zero filled to 2,048 data points and processed by fast Fourier transformation and data shift accumulation (33) to suppress the residual water signal. No further gaussian or exponential multiplication was applied. After a straight baseline was drawn through the noise, the spectra were quantified by means of peak height measurements. In contrast to P-31 spectra, in H-1 spectra peaks were always sharp and reasonably well separated. (In our experience, area measurements on H-1 spectra do not show systematic, significant differences from peak height measurements, whereas peak height measurements clearly have the advantage of smaller variability.) Quantification was performed twice, and mean values were calculated.

Analysis of the data included calculation of ratios of spectral peaks and plotting of the values obtained against age. We calculated nonlinear regression equations of the type $y = a \times \exp(bt) + c$ or $a \times \exp(bt) + c \times \exp(dt)$, where y is the ratio of spectral peaks at age t and a, b, c, and d are parameters influencing the level of the curve and the extent of increase or decrease (34). The 2.5 and 97.5 percentile lines were computed.

RESULTS

Phosphorus Spectra

Figure 1 shows representative P-31 spectra of the brain in children of different ages. The most obvious change is observed in the phosphomonoester (PME) peak. Relative to adenosine triphosphate (ATP), Pi does not change, whereas the phosphodiesters (PDEs) and PCr increase. The following ratios were calculated: PME/β-ATP, Pi/β-ATP, PDE/β-ATP, PCr/β-ATP, PME/PCr, and PCr/Pi. The data points, regression lines, and 95% prediction intervals are shown in Figure 2. During the first 2-3 years of life, a decrease is observed in PME/β-ATP and an increase in PCr/β-ATP. Due to the opposing tendencies, PME/PCr undergoes a larger change. PDE/β-ATP increases during the first 2-3 years. After the age of 2-3 years, no changes are observed. No significant changes are found in Pi/β-ATP and in the pH. As calculated from the difference in chemical shift between PCr and Pi, we found a mean pH of 7.04, with a 95% confidence interval of 6.96-7.12.

Proton Spectra

Figure 3 shows the H-1 spectra corresponding to the P-31 spectra of Figure 1. Major changes are seen in the relative height of N-acetylaspartate (NAA), choline (Ch), and creatine (Cr). Ratios of NAA/Cr, NAA/Ch, and Ch/Cr were calculated. Data points, regression lines, and 2.5 and 97.5 percentile lines for these ratios are shown in Figure 4. In common with the P-31 spectra, the most rapid changes occur during the first 2 or 3 years of life; in contrast to the P-31 spectra, the ratios still undergo changes after that age, and evidently, adult values are not yet reached at the age of 16 years.

DISCUSSION

Data from Other Studies

The changes in P-31 spectra were noted before. In 1983, attention was drawn to a large peak in the spectra of newborn babies (20). This peak is now known as the PME peak. In 1984, similar, more extensive observations were made (21,22). In 1986, the first quantitative data for cerebral P-31 spectra of infants of different ages were presented (23). In 1989, Laptook et al (24) reported on repeated P-31 spectroscopy of the brain in an infant from birth till 8½ months of life. They found an increase in PCr/β-ATP and PCr/Pi with increasing age. In the same year, Azzopardi et al (25) published values for pH and different ratios of phosphorus-containing compounds for children with an age range of 26-42 postconceptional weeks. The investigated 30 infants were healthy, apart from prematurity. A significant increase with age was found for PCr/Pi, PCr/total phosphorus, and PDE/total phosphorus. A significant decrease with age was noted in PME/ATP, PME/total phosphorus, and Pi/total phosphorus. No significant change was found in ATP/total phosphorus, PCr/ATP, Pi/ATP, PDE/ATP, and pH. Also in 1989, Boesch et al (26) reported on the results of P-31 spectroscopy in 12 infants and children. Eight children were studied at about 40 weeks after conception; three children were investigated later in the 1st year of life, and one child was studied at the age of 6 years. A decrease in PME/PDE and an increase in PCr/β-ATP and α-ATP/β-ATP were seen with advancing age.

In conclusion, strictly speaking, normal values for P-31 spectroscopic studies of the brain were previously known only in infants with a postconceptional age of 26-42 weeks and not for older infants and children. No data were available about H-1 spectroscopy of the brain in children of different ages.

A disadvantage of the data of these previously performed studies is that all investigations were done with surface coils with the inherent suboptimal spatial localization and demarcation.

Present Data

We performed H-1 as well as P-31 spectroscopy in healthy children of different ages. We used volume-selective spectroscopy, with the volume always chosen in the same paraventricular area. As it is known that spectra are different in different areas of the brain (35,36), a volume-selective method with strict spatial demarcation and standard localization is preferable to surface coil techniques.

Important age-related changes were demonstrated in H-1 as well as P-31 spectra of the brain. At this point the explanation of the observed changes is still largely a matter of deduction and probability, but it is clear that they are in some way related to maturational processes in the brain.

Processes of Brain Maturation

Most neurons are formed before term birth, but at birth neuronal development is incomplete. After birth there is an important increase in the number and size of neuronal dendrites and in the number and complexity of axonal and dendritic connections (1-4). Associated with this elaboration of dendritic and axonal ramifications and the appearance of synaptic elements is a progressive

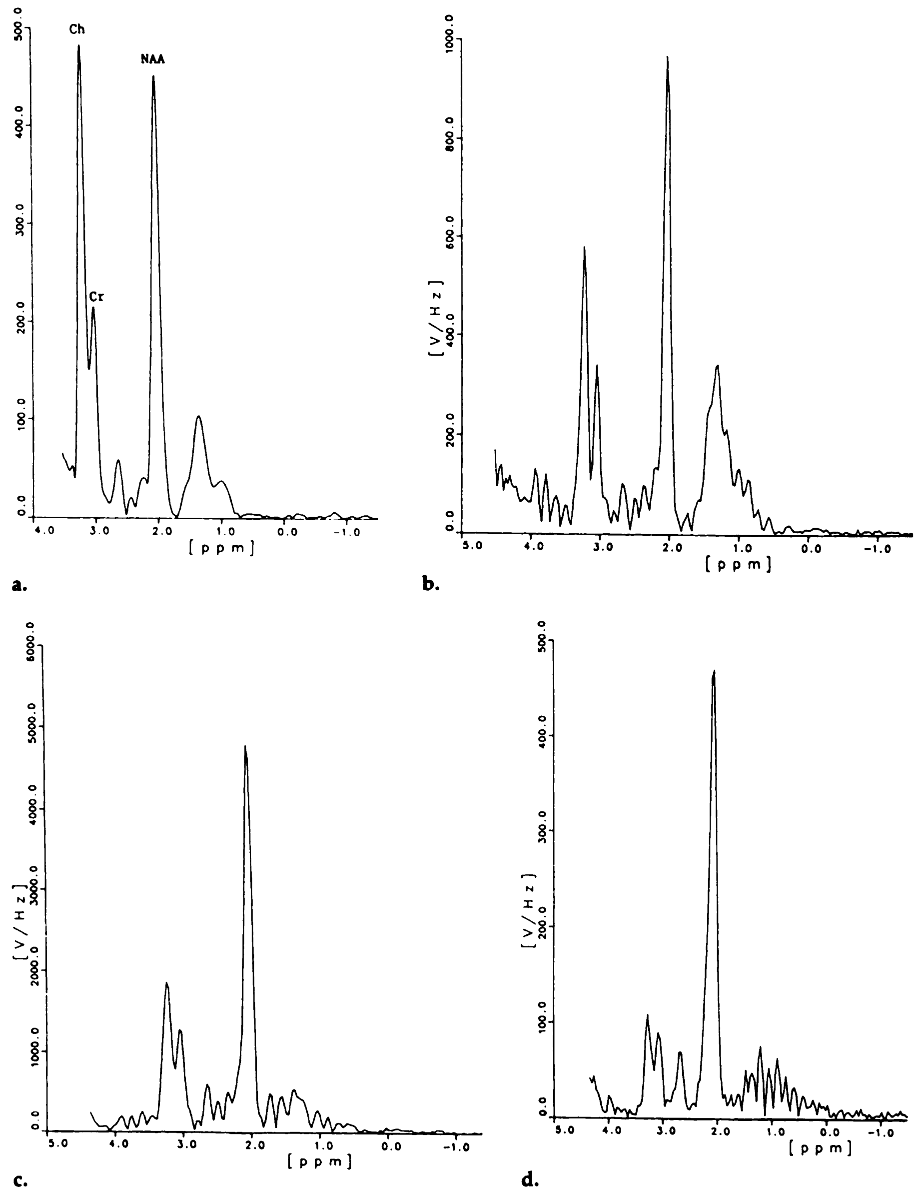

Figure 3. H-1 spectra of the brain obtained at the age of 1 month **(a)**, 4 months **(b)**, 2½ years **(c)**, and 15 years **(d)**. Spectral peaks are assigned to choline (3.2 ppm), creatine (3.0 ppm), and NAA (2.02 ppm). Numbers along y axis are in arbitrary units.

differentiation of neurons, axons, dendrites, and synapses (5,6). Neuronal organization is refined by so-called regressive events, consisting of selective elimination of neurons, neuronal processes, and synapses, in order to adjust the size and structure of the neuronal population to the functional needs of the particular part of the brain (4,7–10).

Proliferation of neuroglia occurs not only before but also after birth. Eventually neuroglia by far outnumber neurons in the brain (11–13). Glial differentiation is of major importance in the developing brain (14). Astroglia play complex nutritive and supportive roles. Oligodendroglia are involved in the formation of myelin.

The process of myelination of the brain mainly occurs after birth (15–19). Major events of brain maturation take place during the first few years of life, but complete maturity is not reached before early adulthood (13,15).

Phosphorus Spectra

In calculating ratios we chose β-ATP as reference, because studies have indicated that ATP does not change much with age (37–39). The β-ATP peak does not include any other phosphorus compounds known to be present in brain tissue in significant amounts and is used as a reference for the ATP concentration. We found evidence of a decrease in PME (Fig 2a) and an increase in PDE (Fig 2c) and PCr (Fig 2e) with age. Changes are only observed during the first 3 years of life (Fig 2).

The statement that the PME peak is created almost exclusively by phospholipid precursors (40) is a simplification of a complex situation. The main components of the PME peak are phosphoethanolamine, phosphocholine, and glycerol-3-phosphate. Minor components are phosphoserine and phosphoinositol. It is true that all PME components are involved in phospholipid anabolism. Phosphocholine is a precursor of phosphatidylcholine and sphingomyelin (41,42). Phosphoethanolamine is a precursor of phosphatidylethanolamine. Glycerol-3-phosphate is a precursor of phosphatidylethanolamine, phosphatidylcholine, and plasmalogens (41,42). However, phosphocholine and phosphoethanolamine are both compounds in the catabolism of sphingomyelin (41,42), and glycerol-3-phosphate is involved in the catabolism of phosphatidylethanolamine, phosphatidylcholine, and plasmalogens (41,42).

The PME peak is known to be elevated in areas of rapidly growing tissue and in cases of rapid membrane synthesis, such as in growing brain; it is probable that the elevation is caused by the increased presence of compounds meant for the production of membrane phospholipids.

Myelination constitutes a major portion of brain growth after birth (16,43–45). Myelin is a membranous structure, which is rich in lipids and phospholipids (18,46). At birth there is hardly any myelin in the brain; at about 2–3 years an advanced stage of myelination is reached (15,18). After the age of 2–3 years, myelination continues, mainly in the directly subcortical areas, at a low rate until early adulthood (15). The attainment of more or less adult PME values (Fig 2a) coincides with the attainment of almost complete myelination. It is therefore likely that the elevation of the PME peak at birth and during the first 2 or 3 years of life is related to the process of myelination.

Part of the elevation of the PME peak, however, is probably explained by the membrane synthesis associated with the proliferation and growth of glial cells. The synthesis of neuronal membranes is probably responsible for only a minor part of the elevation of the PME peak, as the increase in the total amount of neuronal membrane, caused by growth of axonal and dendritic ramifications, is relatively small (13).

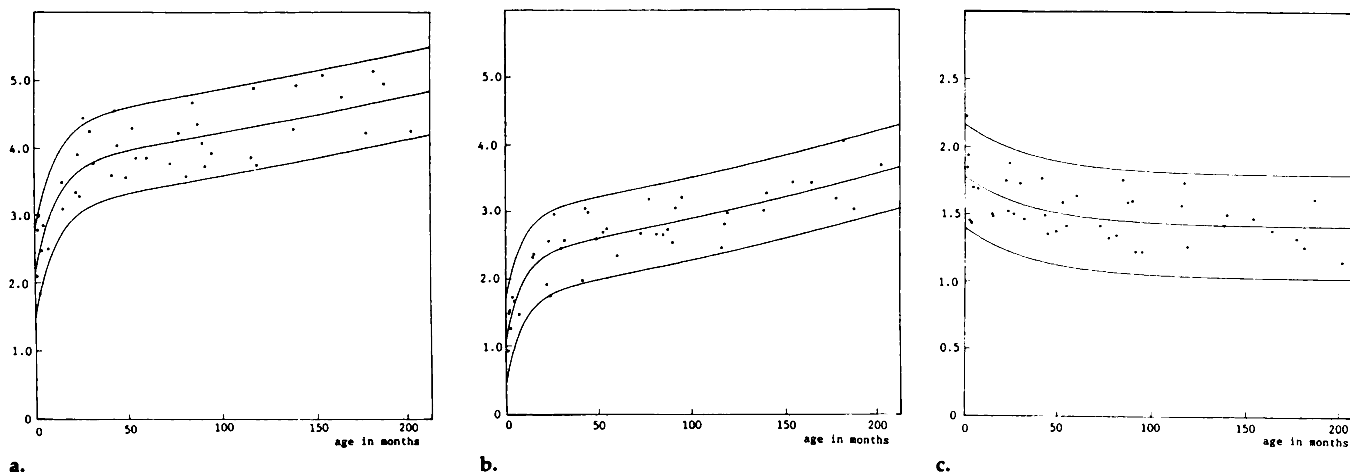

Figure 4. Regression lines and 2.5 and 97.5 percentile lines for NAA/Cr **(a)**, NAA/Ch **(b)**, and Ch/Cr **(c)**.

The PME/PCr ratio undergoes a larger change than any other ratio (Fig 2b). This large, easily detected change makes the ratio suitable for use as a parameter for brain maturation. However, PME and PCr are not in any way related to each other, and the ratio as such has more practical than theoretical value.

The PDE peak is relatively low at birth and increases until final values are reached at the age of 2–3 years (Fig 2c). PDEs mainly represent phospholipid breakdown products (40–42). Major PDE components are glycerophosphoethanolamine and glycerophosphocholine. Minor components are glycerophosphoserine and glycerophosphoinositol. These compounds are present in the catabolic pathways of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and plasmalogens (40,42).

At birth there is only a small amount of myelin in the brain and therefore little myelin turnover. With increasing myelin content, the myelin turnover increases and therewith the concentration of intermediary products of myelin phospholipid breakdown.

The PCr peak is relatively low at birth and increases thereafter (Fig 2e); the Pi peak does not undergo age-related changes (Fig 2d). It was stated before (25,27,47) that PCr/Pi and PCr/β-ATP are a measure for the phosphorylation potential and energy status of the examined tissue. PCr is a high-energy compound. Intracellular PCr acts via the creatine kinase reaction as a buffer to maintain a constant ATP level in the face of variable energy demands. Only when PCr is exhausted does ATP fall, leaving adenosine diphosphate and adenosine monophosphate. The increase in PCr/Pi and PCr/β-ATP (Fig 2e, 2f) may therefore imply an increase in energy reserve of infant brain tissue. However, as long as it is not known whether the concentrations of free creatine and total creatine change, one cannot be sure about an increase in energy reserve. To know whether the equilibrium of the creatine phosphokinase reaction undergoes a shift or remains constant, one must know the concentrations of not only PCr and Pi but also free or total creatine.

Proton Spectra

The choice of reference is less obvious with H-1 spectra than it is with P-31 spectra. It is stated that total creatine, the sum of PCr and free creatine, is of approximately constant concentration in the brain in different metabolic conditions (48). As such, the total creatine concentration would be suitable as standard for calculation of ratios. However, it is not known whether the total creatine concentration remains constant with changing age. Relative to creatine, NAA increases and choline decreases after birth (Fig 4a, 4b). At the age of 16 years, adult values are not yet reached (Fig 4).

Biochemical studies of animal brain have shown an increase in NAA concentration with increasing age (49). This is in conformity with the observed rise of the relative peak height of NAA with increasing age (Fig 4a, 4b). The "NAA" peak originates not only from the acetyl residue of NAA but also from other acetates (50). The real NAA concentration appears to be 30%–40% lower than that calculated from this NAA peak (50). It is not clear to what extent the changes in peak height are caused by changes in actual NAA concentration and to what extent the other acetyl compounds contribute.

The function of NAA is not known. It is located mainly in neurons (51). As there is no major increase in number of neurons after birth, the increase in NAA concentration must be related to neuronal maturation, such as increases in the number of axons, dendrites, and synaptic connections. Neuronal maturation is completed many years after birth (13,15).

Free choline and choline-containing compounds, including phosphocholine and glycerophosphocholine, are found in pathways of synthesis as well as breakdown of choline-containing phospholipids (40–42). Thus, changes in the choline peak (Fig 4b, 4c) are in a complex way related to changes in PME and PDE peaks of the P-31 spectrum.

The observed values for NAA/Ch suggest not a steady increase after the age of 3 years but rather a kind of plateau phase between the ages of 3 or 4 years and 12 years, with a more rapid increase thereafter (Fig 4b). The plateau phase may be a consequence of undersampling or may actually be present. More data are necessary for confirmation.

It is not known whether total creatine changes with age or remains constant, as no use was made of an external reference. Though the spectra (Fig 3) suggest no change or a decrease of total creatine, even an increase cannot be excluded. Thus it is not clear whether the creatinephosphokinase equilibrium changes with age.

General Remarks and Conclusion

Changes in spectra, as found in this study, must be interpreted with

caution. The T1s of metabolites detected in P-31 and H-1 spectra are relatively long (29,52,53), meaning that the spectra are obtained under conditions of partial saturation. Tanaka et al (54) analyzed the influence of T1 effects for compounds detectable at P-31 MR spectroscopy in subjects aged 1 month to 80 years. They found that the T1 of PME is relatively long in the infantile brain and that the T1 of PCr is relatively long in the infantile as well as the aged brain. No change in T1 was found for Pi, PDE, and ATP. This means that the elevation of PME in the infantile brain may be underestimated with partially saturated spectra and that the increase of PCr during the first few years of life may be in part explained by a decreasing T1. No data are available about age-dependent changes in T1 of compounds detectable with H-1 MR spectroscopy.

With unsedated children it is not possible to obtain fully relaxed spectra at all ages, because with a TR of 16–20 seconds the acquisition time would be a number of hours. Until now we have not been able to calculate multiplying factors for all different ages to correct for the effects of partial saturation.

The T2 of metabolites present in P-31 and H-1 spectra is relatively short (29,52). The ISIS technique avoids the use of spin echoes. It is therefore relatively insensitive to the T2 values of the visualized compounds. Only small losses caused by T2 relaxation processes occur during the short duration of the inversion pulses of the ISIS sequence. A disadvantage of the ISIS scheme is, however, that it is sensitive to contamination of the spectrum by signals originating from outside the selected volume and that special adjustments of the scheme are required to prevent this (28,29,55).

With the technique of H-1 spectroscopy we used, the TE is relatively long, considering the T2 of the visualized metabolites (52). As T2 of creatine is shortest, the loss in peak height of creatine is largest (52). No data are available about age-dependent changes in T2 of the compounds present in H-1 spectra, but it can be assumed that such changes occur.

In statistical analysis of the obtained data, nonlinear regression lines appeared to provide the best fit with the smallest residuals. The data did not show a completely normal distribution, but the residuals of the regression line (ie, the observed values corrected for a nonlinear trend) show a positive kurtosis. This applies to all calculated ratios. As a consequence, calculation of 95% prediction intervals based on normal distribution is not reliable. Use of distribution free methods based on ordered residuals gives the shown 2.5 and 97.5 percentile lines, which form a good estimation of reference values. However, from a statistical point of view the sample size (41 children) is rather small. With a study population of 41 children the confidence that the calculated lines represent exactly the 2.5 and 97.5 percentile lines for all normal children is approximately 65%. To reach a confidence of 90% 140 children are required.

In conclusion, major age-related changes have been demonstrated in P-31 as well as H-1 spectra of the brain. This study has made it clear that in the absence of normal values for different ages, P-31 and H-1 spectra of the brain in children cannot be appraised. ■

Acknowledgments: We thank the children and their parents, without whose help and effort this study would have been impossible. We thank Lenie de Vries for her secretarial help in preparing this article.

References

1. Conel J. The postnatal development of the human cerebral cortex. Cambridge: Harvard University Press, 1939.
2. Huttenlocher PR. Synaptic and dendritic development and mental defect. In: Buchwald NA, Brazier MAB, eds. Brain mechanisms in mental retardation. New York: Academic Press, 1975; 123–140.
3. Becker LE, Armstrong DL, Chau F, Wood MM. Dendritic development in human occipital cortical neurons. Dev Brain Res 1984; 13:117–124.
4. Huttenlocher PR, De Courten C, Garey LJ, Van der Loos H. Synaptogenesis in human visual cortex: evidence for synapse elimination during normal development. Neurosci Lett 1982; 33:247–252.
5. Purpura DP. Dendritic differentiation in human cerebral cortex: normal and aberrant development patterns. In: Kreutzberg GW, ed. Advances in neurology. New York: Raven, 1975; 91–116.
6. Marin-Padilla M. Prenatal and early postnatal ontogenesis of the human motor cortex: a Golgi study. I. The sequential development of cortical layers. Brain Res 1970; 23:167–183.
7. Purves D, Lichtman JW. Elimination of synapses in the developing nervous system. Science 1980; 210:153–157.
8. Hamburger V, Oppenheim RW. Naturally occurring neuronal death in vertebrates. Neurosci Comment 1982; 1:39–42.
9. Rakic P, Riley KP. Overproduction and elimination of retinal axons in fetal Rhesus monkey. Science 1983; 219:1441–1444.
10. Cowan WM, Fawcett JW, O'Leary DDM, Stanfield BB. Regressive events in neurogenesis. Science 1984; 225:1258–1265.
11. Dobbing J, Sands J. Quantitative growth and development of human brain. Arch Dis Child 1973; 48:757–767.
12. Pope A. Neuroglia: quantitative aspects. In: Schoffeniels E, Franck G, Tower DB, Hertz L, eds. Dynamic properties of glial cells. New York: Pergamon, 1978; 13–20.
13. Volpe JJ. Neurology of the newborn. Philadelphia: Saunders, 1987; 33–68.
14. Skoff RP. Neuroglia: a reevaluation of their origin and development. Pathol Res Pract 1980; 168:279–300.
15. Yakovlev PI, Lecours AR. The myelogenetic cycles of regional maturation of the brain. In: Minkowski A, ed. Regional development of the brain in early life. Oxford: Blackwell, 1967; 3–70.
16. Brody BA, Kinney HC, Kloman AS, Giles FH. Sequence of central nervous system myelination in human infancy: an autopsy study of myelination. J Neuropathol Exp Neurol 1987; 46:283–301.
17. Barkovich AJ, Kjos BO, Jackson DE, Norman D. Normal maturation of the neonatal and infant brain: MR imaging at 1.5 T. Radiology 1988; 166:173–180.
18. Valk J, van der Knaap MS. Magnetic resonance of myelin, myelination and myelin disorders. Heidelberg, Federal Republic of Germany: Springer-Verlag 1989; 13–15, 26–65.
19. van der Knaap MS, Valk J. MR imaging of the various stages of normal myelination during the first year of life. Neuroradiology 1990; 31:459–470.
20. Cady EB, Costello AM, Dawson MJ, et al. Non-invasive investigation of cerebral metabolism in newborn infants by phosphorus nuclear magnetic resonance spectroscopy. Lancet 1985; 1:1059–1062.
21. Younkin DP, Delivoria-Papadopoulos M, Leonard JC, et al. Unique aspects of human newborn cerebral metabolism evaluated with phosphorus nuclear magnetic resonance spectroscopy. Ann Neurol 1984; 16:581–586.
22. Hope PL, Costello AM, Cady EB, et al. Cerebral energy metabolism studied with phosphorus NMR spectroscopy in normal and birth-asphyxiated infants. Lancet 1984; 2:366–369.
23. Hamilton PA,Hope PL, Cady EB, Delpy DT, Wyatt JS, Reynolds EOR. Impaired energy metabolism in brain of newborn infants with increased cerebral echodensities. Lancet 1986; 1:1242–1246.
24. Laptook AR, Corbett RJT, Uany R, Mize C, Mendelsohn D, Nunnal RL. Use of 31P magnetic resonance spectroscopy to characterize evolving brain damage after perinatal asphyxia. Neurology 1989; 39:709–712.
25. Azzopardi S, Wyatt JS, Hamilton PA, et al. Phosphorus metabolites and intracellular pH in the brains of normal and small for gestational age infants investigated by magnetic resonance spectroscopy. Pediatr Res 1989; 25:440–444.
26. Boesch C, Gruetter R, Martin E, Duc G, Wüthrich K. Variations in the in vivo P-31 MR spectra of the developing human brain during postnatal life. Radiology 1989; 172:197–199.
27. Lawson B, Anday E, Guillet R, Wagerle LC, Chance B, Deliveria-Papadopoulos M. Brain oxidative phosphorylation following alteration in head position in preterm and term neonates. Pediatr Res 1987; 22:302–305.
28. Ordidge RJ, Conelly A, Lohman JAB. Image-selected in vivo spectroscopy (ISIS): a new technique for spatially selective NMR spectroscopy. J Magn Reson 1986; 66:283–294.
29. Luyten PR, Groen JP, Vermeulen JWAH, den Hollander JA. Experimental approaches to image localized human 31P NMR spectroscopy. Magn Reson Med 1989; 11:1–21.

30. Petroff OAC, Prichard JW, Behar KL, Alger JR, den Hollander JA, Shulman RG. Cerebral intracellular pH by 31P nuclear magnetic resonance spectroscopy. Neurology 1985; 35:781–788.
31. Ordidge RJ, Bendall MR, Gordon RE, Conelly A. Volume selection for in-vivo biological spectroscopy. Magn Reson Biol Med 1985; 387–397.
32. Bottomley PA. Spatial localization in NMR spectroscopy in vivo. Ann NY Acad Sci 1987; 508:333–348.
33. Roth K, Kimber BJ, Feeney J. Data shift accumulation and alternate delay accumulation techniques for overcoming dynamic range problems. J Magn Reson 1980; 41:302–309.
34. Draper N, Smith H. Applied regression analysis. 2nd ed. New York: Wiley, 1981; 458–529.
35. Hanstock CC, Rothman DL, Prichard JW, Jue T, Shulman RG. Spatially localized 1H NMR spectra of metabolites in the human brain. Proc Natl Acad Sci USA 1988; 85:1821–1825.
36. Hubesch B, Sappey-Marinier D, Deicken R, Seidenwurm P, Weiner MW. Regional differences of phosphorus metabolites in the human brain (abstr). In: Book of abstracts: Society of Magnetic Resonance in Medicine. Vol 1. Berkley, Calif: Society of Magnetic Resonance in Medicine, 1989; 447.
37. Tofts PS, Wray S. Changes in brain phosphorus metabolites during the postnatal development of the rat. J Physiol 1985; 359:417–429.
38. Samson FE, Balfour WM, Dahl NA. Rate of cerebral ATP utilization in rats. Am J Physiol 1960; 198:213–218.
39. Mandel P, Edel-Harth S. Free nucleotides in the rat brain during postnatal development. J Neurochem 1966; 13:591–595.
40. Pettegrew JW, Kopp SJ, Minshew NJ, Glonek T, Feliksik JM, Tow JP, Cohen MM. 31P nuclear magnetic resonance studies of phosphoglyceride metabolism in developing and degenerating brain: preliminary observations. J Neuropathol Exp Neurol 1987; 46:419–430.
41. Dawson RMC. Enzymatic pathways of phospholipid metabolism in the nervous system. In: Eichberg J, ed. Phospholipids in nervous tissues. New York: Wiley, 1985; 45–78.
42. Porcellati G, Arienti G. Metabolism of phosphoglycerides. In: Lajtha A, ed. Handbook of neurochemistry. Vol 3. Metabolism in the nervous system. New York: Plenum, 1983; 133–161.
43. Gilles FH, Shankle S, Dooling EC. Myelinated tracts: growth patterns. In: Gilles FH, Leviton A, Dooling EC, eds. The developing human brain. Boston: Wright, 1983; 118–183.
44. Norton WT. Formation, structure and biochemistry of myelin. In: Siegel GJ, Albers RW, Agranoff BV, Katzman R, eds. Basic neurochemistry. 3rd ed. Boston: Little, Brown, 1981; 63–92.
45. Norton WT, Poduslo SE. Myelination in rat brain: changes in myelin composition during brain maturation. J Neurochem 1973; 21:759–773.
46. Norton WT, Cammer W. Isolation and characterization of myelin. In: Morell P, ed. Myelin. New York: Plenum, 1984; 147–195.
47. Chance B, Leigh JS, Clark BJ, Maris J, Kent J, Smith D. Control of oxidative metabolism and oxygen delivery in human skeletal muscle: a steady state analysis of the work/energy cost transfer function. Proc Natl Acad Sci USA 1985; 82:8384–8388.
48. Siesjö BK, Folbergrová J, MacMillan V. The effect of hypercapnia upon intracellular pH in the brain, evaluated by the bicarbonate-carbonic acid method and from the creatine phosphokinase equilibrium. J Neurochem 1971; 19:2483–2495.
49. Tallan HH. Studies on the distribution of N-acetyl-L-aspartic acid in brain. J Biol Chem 1957; 224:41–45.
50. Hanstock CC, Rothman DL, Howseman A, et al. In vivo determination of NAA concentration in the human brain using the proton aspartyl resonance (abstr). In: Book of abstracts: Society of Magnetic Resonance in Medicine. Vol 1. Berkeley, Calif: Society of Magnetic Resonance in Medicine, 1989; 442.
51. Nadler JV, Cooper JR. N-acetyl-L-aspartic acid content of human neural tumours and bovine peripheral nervous tissues. J Neurochem 1982; 19:313–319.
52. Frahm J, Bruhn H, Gyngell ML, Merboldt KD, Hänicke W, Sauter R. Localized proton NMR spectroscopy in the different regions of the human brain in vivo: relaxation times and concentrations of cerebral metabolites. Magn Reson Med 1989; 11:47–63.
53. Galloway GJ, Field J, Rose SE, et al. In vivo high-resolution volume-selected proton spectroscopy and T1 measurements in the dog brain. Magn Reson Med 1989; 9:288–295.
54. Tanaka C, Higuchi T, Naruse S, et al. 31P-MRS changes associated with development of human brain (abstr). In: Book of abstracts: Society of Magnetic Resonance in Medicine. Vol 1. Berkeley, Calif: Society of Magnetic Resonance in Medicine, 1989; 462.
55. Lawry TJ, Karczmar GS, Weiner MW, Matson GB. Computer simulation of MRS localization techniques: an analysis of ISIS. Magn Reson Med 1989; 9:299–314.