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In Vivo Quantitation of Cerebral Metabolite Concentrations Using Natural Abundance ^{13}C MRS at 1.5 T

Stefan Blüml

Huntington Medical Research Institutes, 660 South Fair Oaks Avenue, Pasadena, California 91105; and Rudi Schulte Research Institute, Santa Barbara, California

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A method for the quantitation of cerebral metabolites on a clinical MR scanner by natural abundance ^{13}C MRS *in vivo* is described. Proton-decoupled spectra were acquired with a power deposition within FDA guidelines using a novel coil design. *myo*-Inositol, quantified by a separate proton MRS, and readily detectable in ^{13}C MRS, was used as an internal reference. Normal concentrations, measured in four control subjects, age 7 months to 12 years, were glutamate 9.9 ± 0.7, glutamine 5.6 ± 1.0, and NAA 8.8 ± 2.8 mmol/kg. In a patient diagnosed with Canavan disease, examined four times, glutamate was reduced to 46% of normal, 4.6 ± 0.5 mmol/kg. NAA was increased by 50% to 13.2 ± 1.6 mmol/kg in ^{13}C MRS, consistent with the 41% increase to 12.3 ± 1.1 from control 8.7 ± 1.1 mmol/kg assayed by ^{1}H MRS. Limited concentration of glutamate may impact on glutamatergic neurons and excitatory neurotransmission in Canavan disease. Quantitation of cerebral glutamate in human brain may have clinical value in human neuropathologies in which glutamate is believed to play a central role.

Key Words: **proton-decoupled ^{13}C MRS; glutamate; glutamine; *N*-acetylaspartate; Canavan disease.**

INTRODUCTION

The determination of *in vivo* cerebral glutamate (Glu) and glutamine (Gln) levels using proton magnetic resonance spectroscopy (^{1}H MRS) is compromised by the complex spectral appearance of glutamate/glutamine due to *J* coupling. Further, other metabolites such as the resonances of *N*-acetylaspartate (NAA) may contribute to the signal between 2.0 and 2.5 ppm, rendering the quantitation of glutamate/glutamine difficult. Earlier studies demonstrated the potential of natural abundance *in vivo* ^{13}C MRS for direct determination of cerebral metabolites at 1.5, 2.1, and 4 T (*1–5*). We find that on a clinical 1.5-T MR scanner glutamate and glutamine can be separated from each other and from other metabolites and natural abundance ^{13}C MRS provides enough signal-to-noise to quantify glutamate, glutamine, NAA, and creatine + choline (Cr/Cho) within exam times not exceeding 1 h. Proton decoupling is an essential requirement for achieving optimum sensitivity and resolution for ^{13}C MRS. However, relatively long and intense irradiation of radiofrequency (RF) power renders it difficult to achieve homogeneous decoupling in humans within FDA guidelines. Recently, the concept of a half-volume coil arrangement using two ^{1}H surface coils operating in quadrature mode and one ^{13}C surface coil has been successfully tested on a 4-T system (*6*). Since the power requirements at 1.5 T are favorable compared to those at 4 T this concept was adapted and a similar coil for a field strength of 1.5 T was built (*7*).

MATERIAL AND METHODS

Magnetic resonance imaging (MRI), proton-decoupled ^{13}C MRS ({^{1}H}–^{13}C MRS), and routine proton MRS (^{1}H MRS) were performed on a General Electrics Signa 1.5-T clinical scanner equipped with a second channel for decoupling. The ^{13}C coil consisted of a two-loop coil with a diameter of 10 cm made from 3/16-inch copper tubing. ^{1}H coils were made from 1/4-inch copper tubing and had diameters of 14 cm. The ^{1}H coils were mounted on a frame to form a quadrature field in the area adjacent to the ^{13}C coil. The frame was designed to fit a human head comfortably. The power deposition during decoupling was estimated by using a calibrated oscilloscope measuring the peak-to-peak voltage.

The proton coil homogeneity was determined with MRI (gradient echo (GRE), repetition time TR = 800 ms, echo time TE = 17 ms, flip angle $\alpha = 30°$, or fast spin echo (FSE), TR = 3.7 s, effective echo time TE_{eff} = 95 ms) in volunteers and patients. Nonlocalized {^{1}H}–^{13}C spectra were obtained by a simple pulse and acquire experiment with an excitation bandwidth of 4 kHz. Decoupling was achieved using the WALTZ-16 (*8, 9*) schema, decoupling bandwidth 400–500 Hz, during the 0.2-s acquisition period. The frequency of the decoupler was set to 2.7 ppm to cover the range from lipid CH_2 and CH_3 protons at 0.9 and 1.2 ppm up to the main brain metabolites NAA, Cr, Cho, and *myo*-inositol (mI) between 2.0 and 4.4 ppm. The receiver bandwidth was 5 kHz with 1024 complex data points sampled. Repetition time TR was 1 s. Proton spectra using the standard GE bird cage head coil were obtained from an occipital location containing mostly gray

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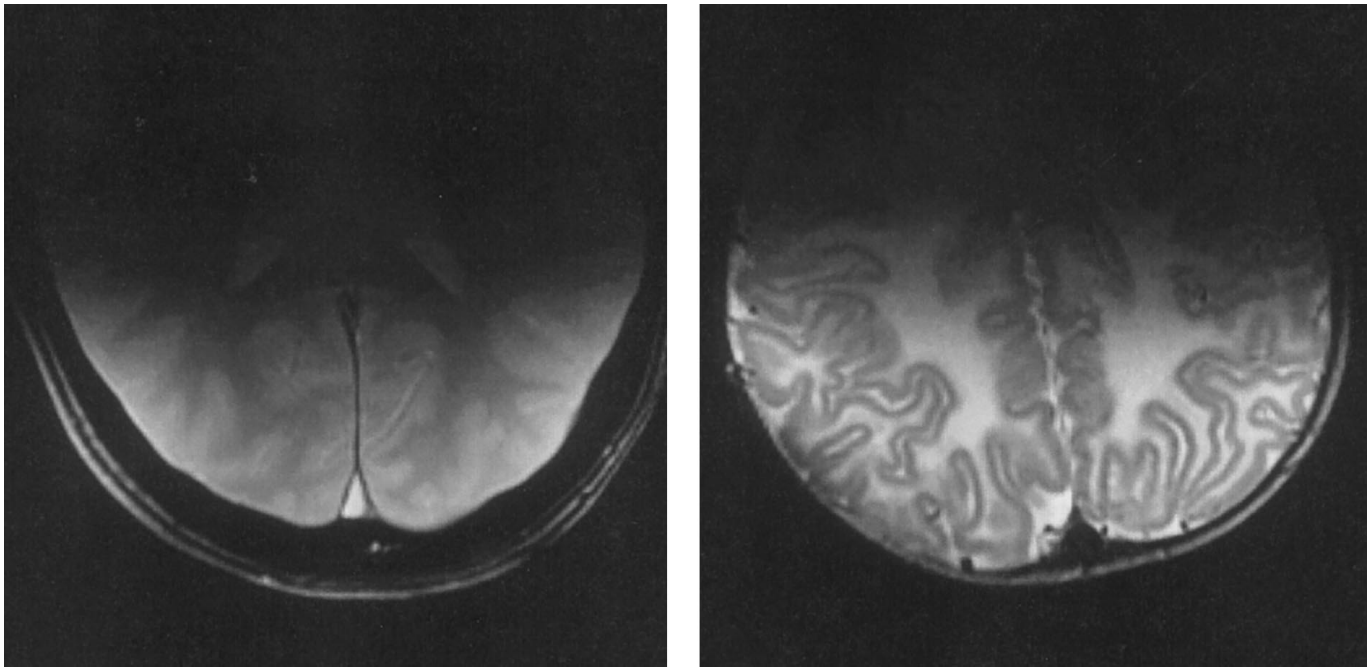

FIG. 1. Gradient echo MRI, TR = 800 ms, TE = 17 ms, $\alpha = 30°$, of a control (left) and a fast spin echo MRI, TR = 3.7 s, TE_{eff} = 95 ms, of the Canavan disease patient (right). Signal up to the frontal region of the brain (not shown on this gray scale) was observed, demonstrating the excellent RF homogeneity available for ^{1}H decoupling with this semi-volume coil.

matter in all subjects. This area coincides with the sensitive volume of the ^{13}C coil. These spectra were quantified as explained in detail in (*10–12*). Solutions of NAA, Glu, Gln, phosphorylcholine (PC), Cr, and mI were studied by $\{^{1}H\}$–^{13}C MRS either individually or combined (i) to obtain chemical shift references and (ii) to determine reference peak ratios of metabolites of known concentration using experimental conditions as *in vivo*. Spectra were processed off-line on a Sun SPARCstation 2 using the SA/GE software package provided by GE. All spectra were zero filled to 4096 data points, Fourier transformed, and zero and first order phase corrected. The baseline was corrected by the SA/GE sinc deconvolution algorithm; no line broadening was applied. After this user-independent data manipulation the peak areas of metabolites were obtained from fitting Gaussian lines to mI and to the Glu, Gln, Cr + Cho, and NAA resonances between 53.5 and 56.5 ppm, manually defining appropriate baselines for each peak. Peak ratios relative to mI were calculated and compared with the ratios obtained *in vitro*. The signal intensity of mI in the ^{13}C spectrum corresponds with the concentration of mI assayed in the same subject by ^{1}H MRS. Using mI as an internal reference the concentrations of Glu, Gln, NAA, and Cr + Cho in mmol/kg brain tissue were estimated.

Four $\{^{1}H\}$–^{13}C MRS measurements over a period of 9 months were carried out in a pediatric patient, 13 months of age at the time of the first exam. The diagnosis of Canavan disease in this patient was established from NAA excretion. The patient was on a diuretic treatment with a dose of 62.5 mg acetazolamide daily and an additional dose of 62.5 mg every third day, begun at age 8 months. Four patients with unrelated diseases aged 7 months, 3 years (two patients), and 12 years served as controls. A complete $\{^{1}H\}$–^{13}C MRS using the half-volume coil lasted less than 1 h in each case. Additional time necessary for obtaining quantitative proton data, including changing coils and patient repositioning, did not exceed 30 min. Pediatric patients were sedated with oral chloral hydrate (75 mg/kg body wt). Huntington Memorial Hospital IRB permission and informed parental consent were obtained prior to all examinations.

RESULTS

The power measured at the coil, necessary for achieving homogeneous decoupling, was <15 W. With a duty cycle of 20% the average transmitted power is 3 W. When taking into account that the RF coils cover more than 1 liter volume and a 80% efficiency for this coil design (*6*), the total average specific absorption rate (SAR) dissipated into the sensitive volume of the ^{1}H coils is less than 2.4 W/kg, which is well within FDA guidelines. After Adriany and Gruetter's (*6*) calculations the local specific absorption with this coil arrangement does not exceed 3.3 W/kg.

Figure 1 shows a gradient echo MRI of a control subject and a fast spin echo MRI of the Canavan disease patient.

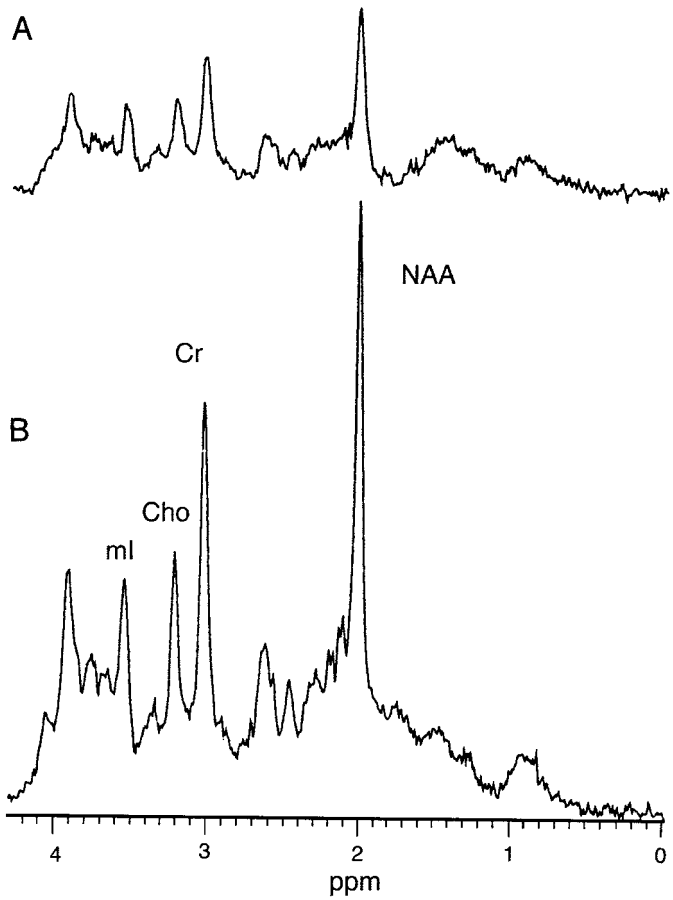

FIG. 2. Localized STEAM spectra (TR = 1.5 s, TE = 30 ms) acquired in the occipital lobe with a standard GE head coil (A) and with the half-volume coil (B). An at least threefold increase in signal-to-noise was achieved.

Signal up to the frontal region of the brain (not shown on this gray scale) was observed, demonstrating the excellent RF homogeneity available for ^{1}H decoupling with this semi-volume coil. Threefold signal-to-noise increase of the proton coils achieved in the occipital brain region was demonstrated by a comparison of proton MRS using the half-volume coil with a spectrum acquired with the standard GE bird cage head coil in a control (Fig. 2). The average spectrum of exams 3 and 4 performed at ages 1.8 and 1.9 years in Canavan disease and the average spectrum of the two 3-year-old subjects are shown in Fig. 3. *myo*-Inositol at 72.1 ($C_{1,3}$), 73.3 ($C_{2,4,6}$), and 75.3 (C_5) ppm can be readily identified. Note that Glu (C_2) at 55.7 ppm is apparently decreased in Canavan disease when compared with controls. Peaks at 54.7 and 55.2 ppm are consistent with Cr (C_2)/Cho (CH_3) and Gln (C_2). At 40.5 and 54.2 ppm NAA C_3 and C_2 resonances were observed in each spectrum. Both NAA and mI resonances appear to be elevated in Canavan disease. Glycerol peaks at 62.9 and 69.9 ppm are well decoupled. The resonance at a chemical shift of 37.8 ppm was assigned with Cr (C_3). Individual spectra were quantified as explained under Material and Methods. The quantitation of {^{1}H}–^{13}C spectra is demonstrated in a spectrum (exam 4) acquired in Canavan disease (Fig. 4A). Metabolite peak areas were fitted and subtracted from the spectrum (Fig. 4B). From the fitted curves (Fig. 4C) the peak ratios of Glu, Gln, and NAA relative to mI were calculated and compared with the peak ratios obtained from a model solution containing equal concentrations of NAA, Cr, mI, glutamate, and glutamine (Fig. 4D). Absolute concentrations in mmol/kg brain tissue were estimated using [mI] quantified with ^{1}H MRS in the occipital region of the brain as an internal reference. Mean glutamate concentration in controls was 9.9 ± 0.7 mmol/kg while mean glutamine was 5.6 ± 1.0 mmol/kg. Both results are in excellent agreement with *in vitro* data (*13*) and a previous *in vivo* ^{13}C MRS estimation where glutamate and glutamine were calculated from ^{13}C-labeled glucose infusion experiments (*3*). [NAA] assayed by {^{1}H}–^{13}C MRS and that assayed by ^{1}H MRS were statistically indistinguishable ($p > 0.3$, paired t test). [Cr + Cho] assayed by ^{13}C MRS was overestimated ($p < 0.01$). In Canavan disease a striking 54% reduction of glutamate to 4.6 ± 0.5 mmol/kg was measured. Also in Canavan disease an increase in [NAA] of 50% was estimated from the ^{13}C data which is consistent with the 41% increase of NAA measured by proton MRS. Mean glutamine in Canavan disease was indistinguishable from controls, and mI (from proton MRS) was elevated by 55%. Details are given in Table 1.

DISCUSSION

Proton-decoupled natural abundance ^{13}C MRS in the human brain was carried out in pediatric controls and in a child diagnosed with Canavan disease. Mean glutamate in Canavan disease was reduced by 54% to 4.6 ± 0.5 mmol/kg vs control 9.9 ± 0.7 mmol/kg. Using a novel coil design these experiments were performed safely within the FDA guidelines for power deposition in a clinical environment. With proton MRS at clinical field strengths the quantitation of glutamate is compromised by the complex spectral appearance of glutamate due to *J* coupling in the proton spectrum and by overlapping other metabolites. This study therefore attempted to quantify glutamate by means of proton-decoupled ^{13}C MRS measuring the C_2 glutamate resonating at 55.7 ppm. Quantitation of glutamate was achieved by using the readily detectable mI, which can be quantified accurately in a separate quantitative proton MRS exam, as an internal reference. The cerebral glutamate concentration in controls, even though no account was taken of possible differences in T1 saturation, agreed rather well with literature data (*3, 13*). Contamination from skeletal muscle, blood, or adipose tissue did not materially alter the results because of the comparatively low concentration of Glu (<1.6 mmol/kg) in

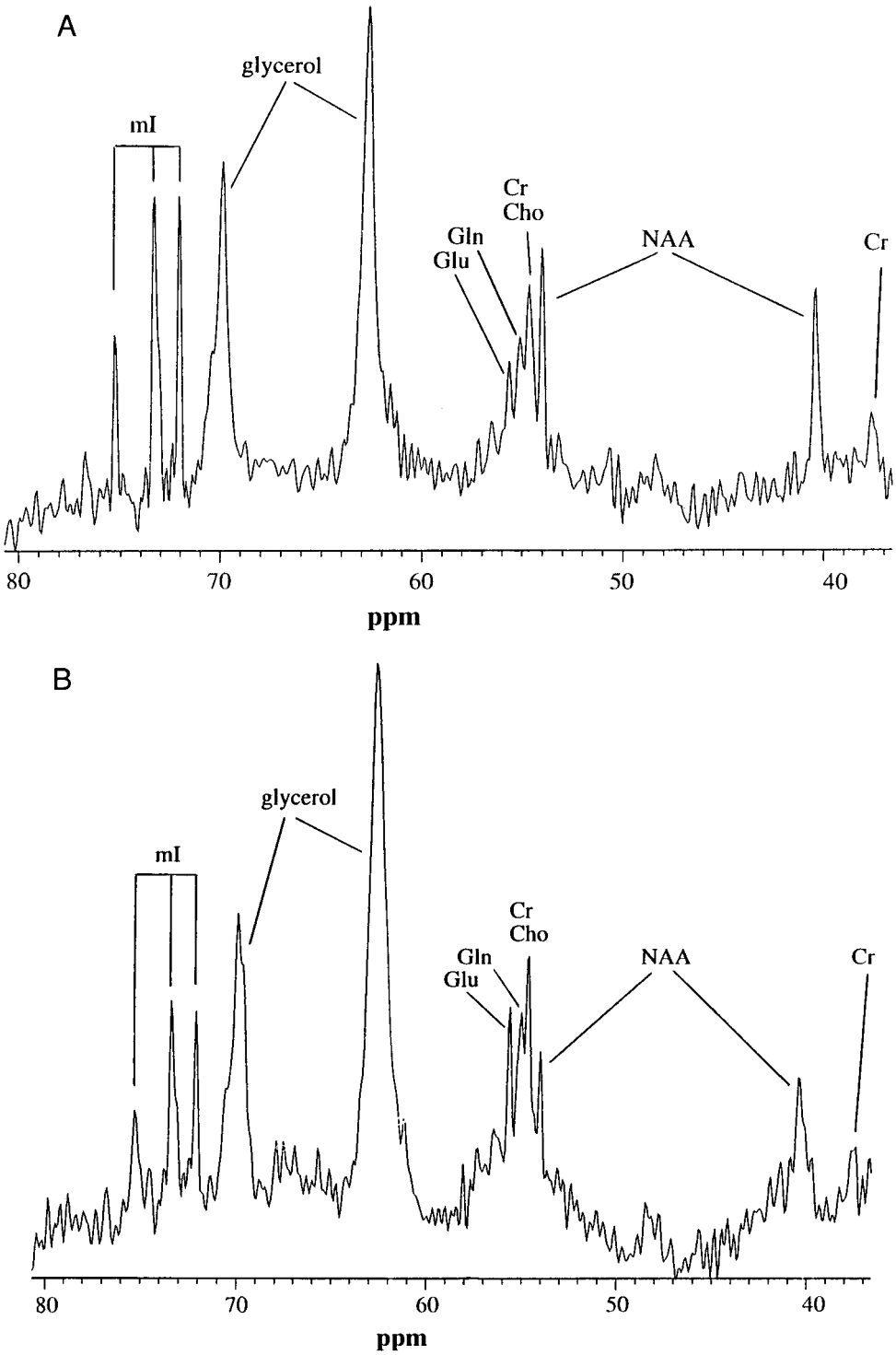

FIG. 3. Shown are *in vivo* $\{^{1}H\}-^{13}C$ spectra from Canavan disease (A) acquired at age 1.7 and 1.8 years (averaged spectrum from two exams) and the averaged spectrum from the two 3-year-old controls (B). Peak assignments are based on phantom studies and on (*4*). Note the apparent reduction of glutamate and the increase of NAA and mI in Canavan disease.

those tissue types (*14*). Quantitation of cerebral glutamine is compromised by its lower *in vivo* concentration (≈half of glutamate) and therefore larger relative errors and by a significant overlap with the larger resonance of Cr + Cho. This may in part explain the larger scatter of [Gln].

Cr + Cho and NAA were measured both by quantitative

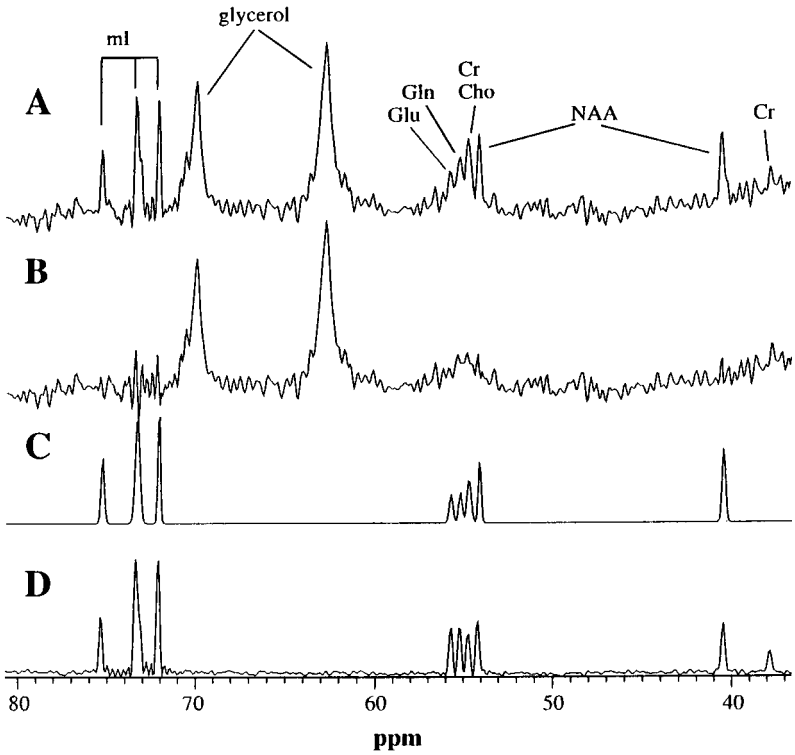

FIG. 4. The processing of {^{1}H}–^{13}C spectra is demonstrated in a spectrum (exam 4) acquired in Canavan disease (A). Metabolite peak areas were fitted and subtracted from the spectrum (B). From the fitted curves (C) the peak ratios of Glu, Gln, and NAA relative to mI were calculated and compared with the peak ratios obtained from a model solution containing equal concentrations of NAA, Cr, mI, glutamate, and glutamine (D). Absolute concentrations in mmol/kg brain tissue were calculated using [mI] quantified with ^{1}H MRS in the occipital region of the brain as an internal reference.

^{1}H MRS and by ^{13}C MRS. Even though the methods are not totally independent since mI from ^{1}H MRS was used as an internal reference the consistency of the data can be checked. Contamination from overlying skeletal muscle with a significantly higher Cr concentration (≈30 mmol/kg) may explain the statistically significant difference between [Cr + Cho] from proton and from ^{13}C MRS in a paired *t* test ($p < 0.01$). NAA concentrations measured with the two assays were indistinguishable ($p > 0.3$). The quantitation of Glu, Gln, Cr + Cho, and NAA is compromised by an underlying broad resonance (see Figs. 3 and 4) and the therefore somehow subjective definition of baselines for the determination of the metabolite peak areas. However, when spectra were reprocessed variations in the estimated concentrations were less than 15% for each metabolite. NAA C_3 at 40.5 ppm was not used for the NAA quantitation because of significant variations observed in the baseline in some spectra, probably due to proximity to the large lipid resonances originating from skull lipids using this nonlocalized technique. Further, other metabolites such as ethanolamines may contribute to the signal at 40.5 ppm. It can not be excluded that the relative proportions of gray and white matter are different in ^{13}C and ^{1}H MRS, even though quantitative ^{1}H MRS was carried out in the occipital cortex within the sensitive volume of the ^{13}C coil. However, in those subjects where additional ^{1}H MRS measurements were carried out in parietal white matter, differences between [mI] in white matter and occipital gray matter were less than 10%. This is consistent with differences in [mI] measured in normal brain (≈10% (*11*)). Therefore within the accuracy of this method partial volume effects do not alter the results.

In Canavan disease the deficiency of aspartoacylase (EC 3.5.1.15) results in demyelination with megalocephaly, blindness, spasticity, and death within the first few years of life (*15*). The determination of the impact of aspartoacylase deficiency and the elevated NAA concentration on other metabolites such as the neurotransmitter and brain osmolyte glutamate may elucidate the understanding of the pathophysiology of Canavan disease. A striking reduction of glutamate to 46% of normal was observed. A possible explanation for this finding is as follows: Reduced aspartoacylase activity results in an elevation of NAA:

$$N\text{-acetylaspartate} \not\rightarrow \text{aspartate} + \text{acetate}. \qquad [1]$$

TABLE 1
Estimated Cerebral Concentration of Glutamate, Glutamine, Creatine + Choline, and NAA in Canavan Disease and in Controls (in mmol/kg)

	Age (years)	Glu ^{13}C	Gln ^{13}C	Cr + Cho ^{13}C	Cr + Cho ^{1}H	NAA ^{13}C	NAA ^{1}H	mI ^{1}H
				Canavan Disease				
Exam 1	1.1	4.1	3.2	12.6	7.9	12.1	11.5	12.0
Exam 2	1.3	4.4	3.3	9.5	6.4	13.2	11.7	11.9
Exam 3	1.8	4.7	5.6	11.3	10.9	15.5	13.9	12.0
Exam 4	1.9	5.3	7.1	15.6	8.5	12.0	12.4	12.7
Average	1.5 ± 0.3	4.6 ± 0.5	4.8 ± 1.9	12.3 ± 2.6	8.4 ± 1.9	13.2 ± 1.6	12.3 ± 1.1	12.6 ± 0.9
				Controls				
1	0.6	9.8	5.7	9.8	9.2	7.5	8.5	8.5
2	3	9.4	5.2	11.4	8.3	9.1	7.2	6.1
3	3	10.9	7.0	14.1	9.6	12.6	9.3	9.6
4	12	9.5	4.6	11.7	10.2	6.0	9.8	8.3
Average	4.6 ± 5.0	9.9 ± 0.7	5.6 ± 1.0	11.9 ± 2.0	9.3 ± 0.8	8.8 ± 2.8	8.7 ± 1.1	8.1 ± 1.5

Note. mI assayed by quantitative ^{1}H MRS was used as an internal reference.

Sequestration of aspartate in NAA results in a reduction of available free aspartate. Glutamate, a metabolic precursor, is therefore expected to be depleted by conversion into aspartate by oxoglutarate amino transferase in Canavan disease. The only source of aspartate is diet, or synthesis from its metabolic precursor, glutamate, which is converted into aspartate by oxoglutarate amino transferase,

$$\text{aspartate} + \text{oxoglutarate} \leftarrow \text{glutamate} + \text{oxaloacetate}, \quad [2]$$

a ubiquitous enzyme present in high activity in normal brain.

The magnitude of a potential aspartate deficit was estimated to be about six times the free aspartate content of the brain by observing plasma NAA concentrations and excretion rates in Canavan disease patients by several groups (*15–19*). A possible role for $\{^{1}H\}$–^{13}C MRS could be the monitoring of cerebral glutamate concentrations during treatments which attempt to restore normal cerebral aspartate/glutamate in human neuropathologies in which glutamate is believed to play a central role.

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