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Novel Peak Assignments of *in Vivo* ^{13}C MRS in Human Brain at 1.5 T

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^{13}C MRS studies at natural abundance and after intravenous 1-^{13}C glucose infusion were performed on a 1.5-T clinical scanner in four subjects. Localization to the occipital cortex was achieved by a surface coil. In natural abundance spectra glucose $C_{3\beta,5\beta}$, myo-inositol, glutamate $C_{1,2,5}$, glutamine $C_{1,2,5}$, *N*-acetyl-aspartate $C_{1\text{-}4,C=O}$, creatine CH_2, CH_3, and $C_{C=N}$, taurine $C_{2,3}$, bicarbonate HCO_3^- were identified. After glucose infusion ^{13}C enrichment of glucose $C_{1\alpha,1\beta}$, glutamate $C_{1\text{-}4}$, glutamine $C_{1\text{-}4}$, aspartate $C_{2,3}$, *N*-acetyl-aspartate $C_{2,3}$, lactate C_3, alanine C_3, and HCO_3^- were observed. The observation of ^{13}C enrichment of resonances resonating at >150 ppm is an extension of previously published studies and will provide a more precise determination of metabolic rates and substrate decarboxylation in human brain. © 2000 Academic Press

Key Words: **proton decoupled ^{13}C MRS; glutamate; glutamine; glucose infusion; tricarboxylic acid cycle.**

INTRODUCTION

Natural abundance ^{13}C MRS and ^{13}C MRS after intravenous 1-^{13}C-labeled glucose (Glc) infusion are established techniques for the noninvasive analysis of human brain metabolism. Recently we demonstrated that, by using a novel coil design and broadband proton decoupling, quantitation of cerebral glutamate C_2 (Glu_2, the subscript number indicates a particular position in the molecule), and glutamine C_2 (Gln_2) in humans is feasible on a routine 1.5-T clinical MR scanner (*1*). These experiments were carried out using an excitation bandwidth of 4 kHz (5 kHz receiver bandwidth) which corresponds to a chemical shift range of >200 ppm at 1.5 T. Within this large excitation bandwidth all metabolite resonances, in particular the resonances of $Glu_{2,3,4}$ and $Gln_{2,3,4}$ between 20 and 60 ppm and $Glu_{1,5}$ and $Gln_{1,5}$ between 170 and 185 ppm, can be observed simultaneously. In their pioneering work, Rothman *et al.*, Mason *et al.*, Gruetter *et al.*, and others (*2–10*) demonstrated that once intravenously infused 1-^{13}C glucose passes the blood–brain barrier it is readily metabolized and accumulation of label from glucose can be observed in glutamate, glutamine, aspartate (Asp), γ-amino butyric acid (GABA), and lactate (Lac). These experiments in humans did not allow the observation of signals resonating at >150 ppm, and ^{13}C enrichment in particular of Glu_1 and Gln_1, as demonstrated in tissue slices and extracts of animal brain (*11, 12*), was not reported.

The present study was designed to determine peak assignments in natural abundance ^{13}C MRS and in ^{13}C MRS after 1-^{13}C glucose infusion. We observed accumulation of label in Glu_1 and Gln_1, as well as in bicarbonate (HCO_3^-) simultaneously with ^{13}C enrichment of $Glu_{2,3,4}$, $Gln_{2,3,4}$, $Asp_{2,3}$, and Lac_3. This potentially will provide a more precise simultaneous determination of the tricarboxylic acid (TCA) cycle flux rate, glutamine synthesis rates, malate-aspartate shuttle exchange rate, lactate metabolism, α-ketoglutarate/glutamate exchange rates, and substrate decarboxylation. Because this technique was demonstrated on a routine clinical MR scanner, hitherto unrecognized metabolic abnormalities as well as new methods of treatment monitoring *in vivo* might emerge when applied in pathology.

MATERIAL AND METHODS

Subjects. This report presents data acquired in four human subjects. Two healthy adult controls (male, 27 years; female, 29 years) and one child with a severe myelination disorder (male, 4 years) were studied with natural abundance ^{13}C MRS and with ^{13}C MRS after intravenous glucose infusion. The healthy male control was fed while the other two subjects were fasted for more than 6 h prior to the examination. Additionally, four natural abundance ^{13}C examinations were carried out in a child diagnosed with Canavan's disease, 13 months of age at the time of the first exam, over a period of 9 months. The two children were sedated with oral chloral hydrate (75 mg/kg body wt). The protocol was approved by FDA for use in children. Huntington Memorial Hospital IRB permission and informed parental consent were obtained prior to all examinations.

Infusion protocol. The infusion protocol was similar to that developed by DeFronzo *et al.* (*13*) but omitted a formal glucose clamp. The use of somatostatin was considered unnecessary for the present investigation. A total of 600 mg/kg body wt 1-^{13}C glucose (Cambridge Isotope Laboratories, Andover, MA), 20% weight per volume, was infused in two steps: an initial bolus of 500 mg/kg (99% enriched) over 5 min to quickly raise the fractional enrichment of blood glucose was followed by a maintenance dose of 100 mg/kg (70% enriched) over 45 min. Natural abundance baseline spectra (up to ≈30 min acquisition time) were acquired before glucose infusion

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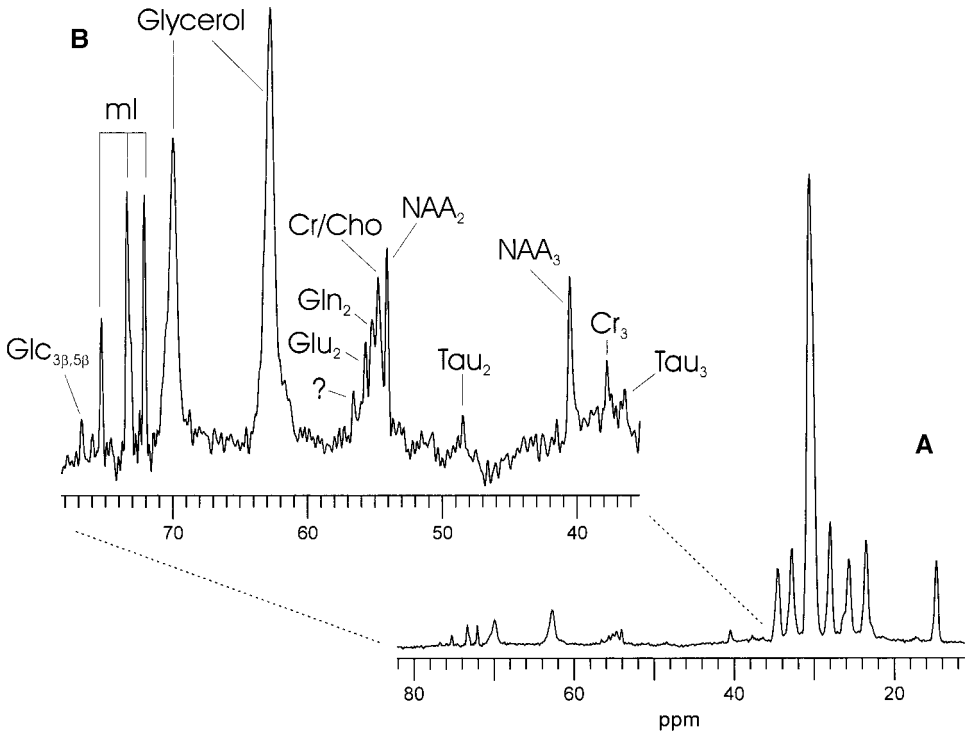

FIG. 1. Sum of four natural abundance ^{13}C MRS examinations in a patient with Canavan's disease (A). The total acquisition time was 140 min. The frequency range >85 ppm of this spectrum is shown in Fig. 2A. The upper trace is an expansion to allow a more detailed inspection of the region between 35 and 85 ppm (B).

start and spectra were acquired up to 200 min after start of glucose infusion.

MRS acquisition and data processing. Proton decoupled ^{13}C MRS ({^{1}H}-^{13}C MRS) was performed on a General Electric Signa 1.5-T clinical MR scanner equipped with a second channel for decoupling. The coil used and its performance are described in detail in Ref. (*1*). Localization with this coil arrangement is achieved by the ^{13}C surface coil being mounted on a headrest adjacent to the occipital region of the brain containing mostly grey matter. {^{1}H}-^{13}C spectra were obtained by a simple rectangular pulse and acquire experiment with an excitation bandwidth of 4 kHz (250 ppm chemical shift range). The flip angle was calibrated by minimizing the lipid signal $(CH_2)_n$ at ≈30 ppm from the skull adjacent to ^{13}C coil by a spatial saturation pulse. Due to the very short T_1 time of lipids this RF power corresponds closely with a 90° pulse. For the actual acquisition, which did not include outer volume suppression, the pulse amplitude was scaled to realize a 45° flip angle at this location. The position of the saturation band varied as pediatric and adult subjects have different skull thicknesses. Therefore the pulse angle in the center of the coil varied in our experiments. Decoupling was achieved using the WALTZ-16 (*14, 15*) scheme, with a decoupling bandwidth of 640 Hz, during the 0.2 s acquisition period. The local specific absorption rate with this coil arrangement is within FDA limits (*1, 16*). The center frequency of the decoupler was set to 2.7 ppm. Receiver bandwidth was 5 kHz with 1024 complex data points sampled. The ^{13}C transmitter frequency was set to 90 ppm and the repetition time TR was 1 s. Peak assignments are based on Refs. (*9, 11, 12, 17*) and on experiments on model solutions of NAA, Glu, Gln, phosphorylcholine (PC), Cr, phosphocreatine (PCr), mI, Asp, and bicarbonate (HCO_3^-) measured either individually or in combination. Spectra were processed off-line on a Sun SPARCstation 2 using the SA/GE software package provided by General Electric. All spectra were zero filled to 8192 data points, Fourier transformed, and zero- and first-order phase corrected. The baseline was corrected by the SA/GE sinc deconvolution algorithm; a 2-Hz lorentzian to gaussian lineshape transformation was applied to improve spectral resolution. Difference spectra were obtained by subtracting the baseline spectrum from spectra acquired during infusion. Due to shifts in the resonance frequency over time spectra needed to be shifted left or right on the frequency axis relative to the baseline acquisition to achieve optimum subtraction results. The criteria for the subtraction were to minimize the residual signal from the dominating lipid peaks.

RESULTS

Peak identification in natural abundance ^{13}C MRS. A natural abundance spectrum from one of the pediatric patients (Canavan disease), demonstrating the full chemical shift range

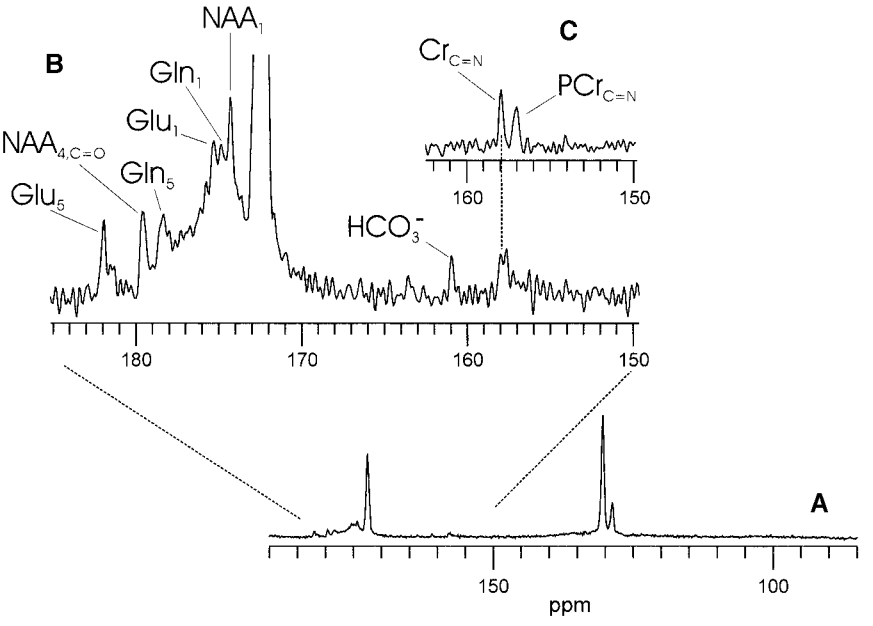

FIG. 2. Continuation of the natural abundance spectrum (Fig. 1A) from the patient with Canavan's disease (A). The middle trace is expanded to highlight the resonances hitherto not observed in humans, between 150 and 185 ppm (B). The upper trace is a spectrum acquired from a model solution of equal amounts of Cr and PCr at pH 7 (C) demonstrating that the resonance observed *in vivo* at ≈158 ppm is consistent with $Cr_{C=N}$ but not with $PCr_{C=N}$.

available with ^{13}C MRS at 1.5-T field strength, is shown in Figs. 1 and 2 (trace A in both figures). The sugars $Glc_{3\beta,5\beta}$ at 76.8, mI_5 75.3, $mI_{2,4,6}$ at 73.3, and $mI_{1,3}$ at 72.1 ppm are readily detectable. The glycerol peaks originating from lipids from the skull can be observed at 62.9 and 70.0 ppm. An as yet unassigned peak, also observed in the spectra from the adults (not shown), is detectable at 56.5 ppm. Glu_2 at 55.7 ppm, Gln_2 at 55.2 ppm, Cr CH_2 at 54.7 ppm with a small contribution from Cho CH_3, and NAA_2 at 54.2 ppm are well resolved. In the pediatric subject peaks at 48.5 and 36.5 consistent with taurine $C_{2,3}$ ($Tau_{2,3}$) are observable. There is no evidence for these resonances in the spectrum from either of the adult subjects (not shown). The significance of taurine in this pediatric patient is beyond the scope of the present report. NAA_3, with possible small contributions from ethanolamines (CH_2NH_2), is detected at 40.5 ppm. The resonance at a chemical shift of 37.8 ppm is assigned to creatine CH_3 (Cr_3). In Canavan's disease Glu is decreased while mI and NAA are increased (*1*). The increased concentration of NAA allows the unambiguous identification of $NAA_4/NAA_{C=O}$ at 179.5 ppm and of NAA_1 at 174.3 ppm in Fig. 2B. The resonance at 182.0 ppm is assigned as Glu_5. The resonance at 178.4 ppm is consistent with Gln_5. Glu_1 and PCr_1/Cr_1 all resonate at 175.3 ppm, while Gln_1 resonates at 174.8 ppm. In all examinations reported here, at 161.0 ppm the resonance from HCO_3^- is detectable while the more complex appearing signal at 157.9 ppm was tentatively assigned to free $Cr_{C=N}$. $PCr_{C=N}$ present in the human brain at the same concentration as free Cr was not observed (see model solution, inset, Fig. 2C).

Peak identification in ^{13}C MRS after intravenous 1-^{13}C-labeled glucose infusion. 1-^{13}C glucose infusion resulted in an increased number of ^{13}C MRS observable metabolites in all subjects. As expected $Glc_{1\alpha,\beta}$ is detectable at 93.0 and 96.8 ppm, respectively (not shown). Figure 3 shows a difference spectrum acquired between 75 and 145 min after infusion start from the occipital lobe of a healthy adult. In addition to those resonances identified in natural abundance ^{13}C MRS, labeling

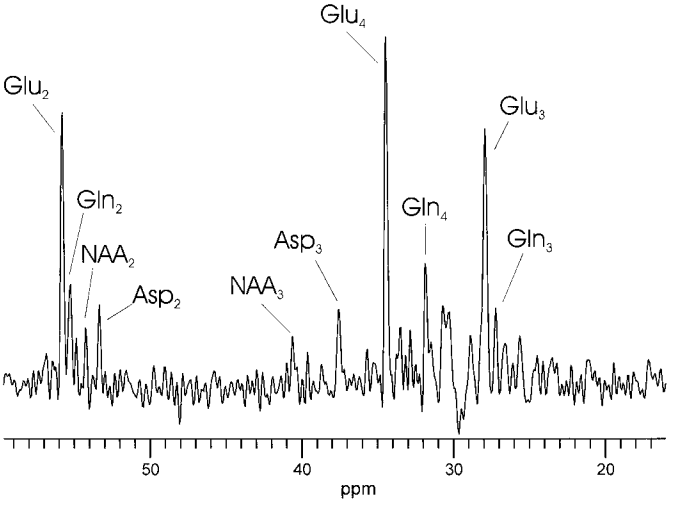

FIG. 3. *In vivo* ^{13}C MRS after intravenous 1-^{13}C glucose infusion. Shown is a difference spectrum acquired between 75 and 145 min after infusion start from the occipital lobe of a healthy adult. The baseline spectrum (not shown) was acquired in 30 min.

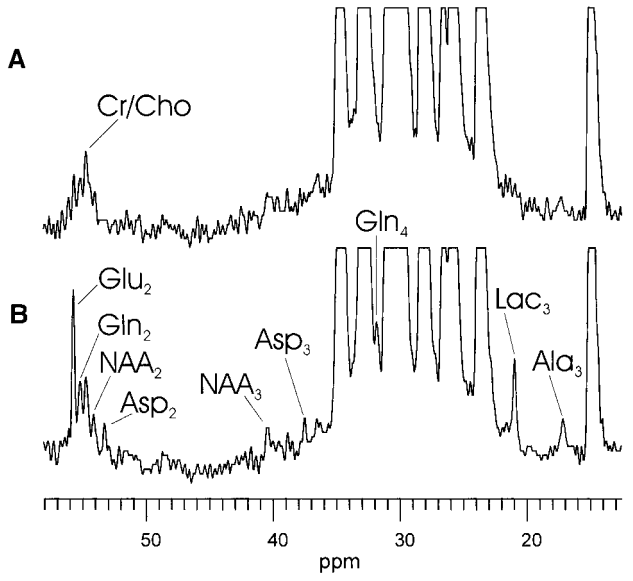

FIG. 4. Baseline scan, acquisition time 20 min (A), and a spectrum acquired between 70 and 110 min after infusion start from the occipital lobe of a patient with severe myelination disorder (B).

of $Glu_{2,3,4}$, at 55.7, 27.9, and 34.4 ppm, $Gln_{2,3,4}$, at 55.2, 27.2, and 31.8 ppm, and $Asp_{2,3}$ at 53.3 and 37.5 ppm can be identified. A small but significant enrichment of the $NAA_{2,3}$ resonances at 54.2 and 40.5 ppm was detected. ^{13}C enrichment of lactate C_3 (Lac_3) at 21.0 ppm and alanine C_3 (Ala_3) at 17.2 ppm, both not detectable in the natural abundance spectrum (Fig. 4A), was evident in a spectrum acquired between 70 and 110 min after infusion start in the child with the myelination disorder (Fig. 4B). Enrichment of these resonances was also observed in the adult control subjects, however, to a much smaller extent. Figure 5 shows a baseline spectrum (A) and a spectrum acquired from the fasted control 140–180 min after infusion start (B) aligned with spectra from model solutions of NAA, Glu, Gln, Cr (C), and Asp, Cr (D). The chemical shifts of the ^{13}C-enriched resonances, 175.3 and 174.8 ppm, are consistent with Glu_1 and Gln_1. ^{13}C enrichment of these resonances as well as labeling of the HCO_{3^-} resonance at 161.0 ppm was observed in all three subjects after ≈50 min. The peak assignments in natural abundance ^{13}C MRS and in ^{13}C MRS after 1-^{13}C-labeled glucose infusion are summarized in Table 1.

DISCUSSION

The present study confirms that natural abundance ^{13}C MRS and ^{13}C MRS after intravenous 1-^{13}C-labeled glucose infusion are feasible on a 1.5-T clinical scanner. The results are consistent with the uptake, glycolysis, and oxidation of 1-^{13}C glucose by the normal human brain. In the patient with Canavan's disease ^{13}C MRS confirmed earlier demonstrations by ^{1}H MRS of elevated NAA, but also identified a reduction of cerebral glutamate, a finding which may extend our understanding of the neurological impact of the defect in NAA biosynthesis (*1*). In another patient with abnormal myelination, infusion with 1-^{13}C glucose resulted in an excess ^{13}C accumulation in lactate and alanine, a result which points to a possible defect in glycolysis and/or glucose oxidation in the tricarboxylic acid cycle. Detailed clinical studies have commenced to further explore the potential of this technique in human brain disorders.

Carbon resonances within a bandwidth of ≈200 ppm were detected by using a simple pulse and acquire sequence. This chemical shift range includes in particular all carbon resonances of the amino acids glutamate and glutamine, some of which have not previously been reported *in vivo* in humans. The assignments of the various resonances and their chemical shifts are summarized (Table 1).

^{13}C MR spectra from the occipital lobe were acquired without any further localization other than the use of a surface coil. The spectra are dominated by lipid signals that originate in the scalp adjacent to the coil. In our approach it was therefore necessary to acquire a baseline scan in order to determine, after subtraction analysis, enrichments of $Glu_{3,4}$ and $Gln_{3,4}$. The subtraction analysis unavoidably results in some loss of *S*/*N* and suffers from hardware instabilities over examination periods which may exceed 2 h. One approach that allows us to obtain additional spatial localization of ^{13}C spectra while enabling the acquisition of information over the whole spectral range of 200 ppm is ^{13}C chemical shift imaging (*18*). Localized direct ^{13}C detection using ISIS (*19*) was introduced by Gruetter *et al.* (*7, 20*) on a 2.1-T experimental system. However, the acquisition time required to achieve sufficient *S*/*N* for reliable peak assignment of natural abundance spectra reported in these papers is too long for a clinical application of ^{13}C MRS. To overcome the problem of the intrinsic low *S*/*N* of ^{13}C MRS other investigators used proton observed ^{13}C editing methods (*6, 10*) and monitored the incorporation of ^{13}C label into Glu_4 in small regions of interest at high time resolution. Gruetter *et al.* (*16*) implemented a proton localized but carbon detected technique on a 4-T system which also allowed localization of small volumes with a high time resolution. However, we used a simple direct carbon detection pulse and acquire experiment for three reasons: (i) No changes in the hardware configuration after MR imaging and standard proton spectroscopy, as for techniques utilizing inverse detection, were necessary on our system. (ii) A wide excitation bandwidth was easily achieved. (iii) In contrast to methods utilizing ^{1}H–^{13}C *J*-couplings, there were no restrictions in observable resonances.

In experiments using tissue slices and studies in animal brain extracts Badar-Goffer *et al.* and Preece and Cerdan (*11, 12*) reported ^{13}C incorporation into the positions $C_{2\text{-}4}$, and C_1 of glutamate and glutamine. The present study demonstrates that ^{13}C enrichment of resonances consistent with Glu_1 and Gln_1 can be observed *in vivo* in humans. A careful comparison of *in vivo* spectra with *in vitro* phantom experiments shows that Glu_1

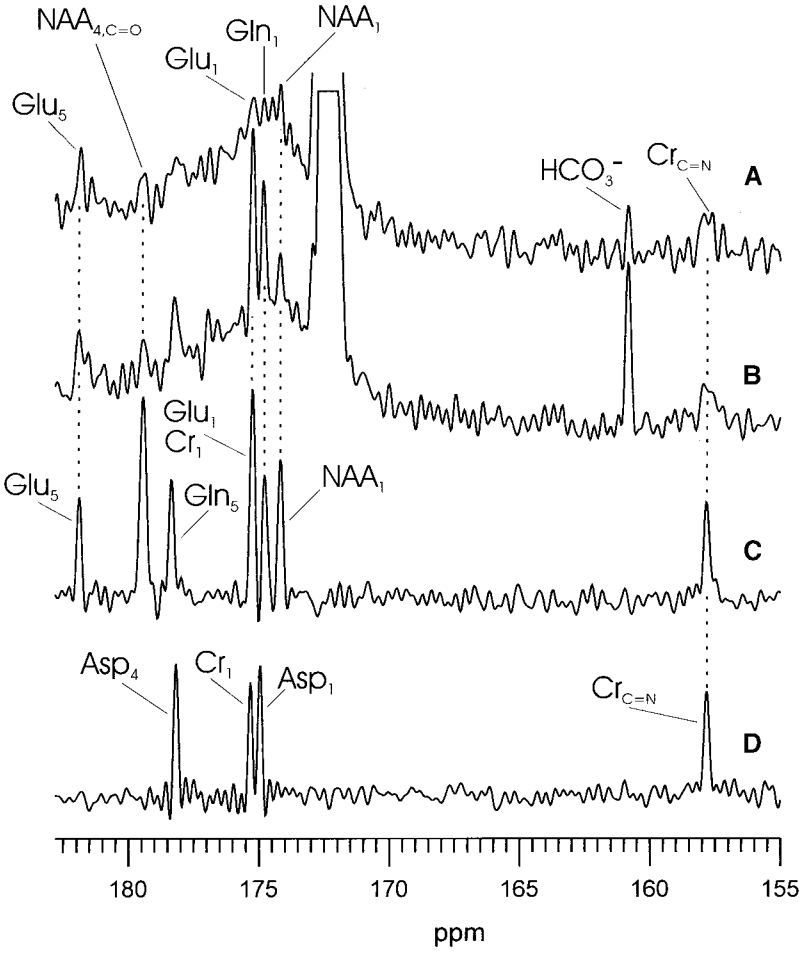

FIG. 5. A baseline spectrum, acquired in 25 min (A), and the spectrum acquired between 140 and 180 min after infusion start from an fasted adult subject (B) are shown. These spectra are aligned with spectra from model solutions of NAA, Glu, Gln, and Cr (C) and Asp and Cr (D).

and Gln_1 accumulate label but not Asp_1 which resonates at a similar chemical shift (see Fig. 5). We also observed, in all three subjects, labeling of the HCO_3^- resonance ≈50 min after start of infusion. These observations represent an extension of previous studies (*7–9*) on humans where enrichment of Glu_1, Gln_1, and HCO_3^- was not detected. Labeling of Asp_2 and Asp_3 was observed in all subjects. We also observed significant labeling of $NAA_{2,3}$ as reported by Gruetter *et al.* (*9*), but we were unable to observe enrichment of GABA at the present stage. This is likely due to the lower resolution and lower *S*/*N* at 1.5 T when compared to 4 T. The peak at 157.9 ppm tentatively assigned to $Cr_{C=N}$ appears to have two components. ^{13}C MRS carried out in a phantom with equal amounts of Cr and PCr demonstrated that the C=N resonances of both molecules can readily be separated but that their chemical shift difference *in vitro* does not account for the splitting observed *in vivo* (Fig. 2C). When ^{13}C MRS in skeletal muscle, where PCr exceeds Cr concentration by far, was compared with the Cr/PCr model solution spectrum, a dominant peak at the chemical shift of $PCr_{C=N}$ but not $Cr_{C=N}$ was observed (unpublished observation from this laboratory). It appears to be unlikely that the blurry signal at 157.9 ppm is caused by PCr/Cr signal from overlaying muscle with a different susceptibility since the Cr/PCr resonance at 54.7 ppm would have been equally affected. However, further investigations are necessary before a firm assignment of the complex signal in this part of the ^{13}C spectrum can be made.

Localization by surface coil alone leaves also some uncertainty about the origin of the MR signals, in particular about partial volumes of gray and white matter. This, in addition to the limited time resolution for some metabolites, may complicate the metabolic interpretation of the observed dynamics. Therefore, in order to identify anticipated changes of metabolism in diseases effecting mainly white matter (e.g., leukodystrophies), localized ^{13}C MRS may be required in future studies.

CONCLUSIONS

The information content of our data confirms much that has previously been established in human volunteers by means of

TABLE 1

Chemical Shifts of Resonances Detected with Natural Abundance ^{13}C MRS and with ^{13}C MRS after Intravenous 1-^{13}C-Labeled Glucose Infusion in Human Subjects at 1.5 T

Metabolite	Carbon	Chemical shift (ppm)
Glutamate	C_1[a]	175.3
	C_2[a]	55.7
	C_3[a]	27.9
	C_4[a]	34.4
	C_5	182.0
Glutamine	C_1[a]	174.8
	C_2[a]	55.2
	C_3[a]	27.2
	C_4[a]	31.8
	C_5	178.4
NAA	C_1	174.3
	C_2[a]	54.2
	C_3[a]	40.4
	C_4	179.5
	$C_{C=O}$	179.5
Aspartate	C_2[a]	53.3
	C_3[a]	37.5
myo-Inositol	$C_{1,3}$	72.1
	$C_{2,4,6}$	73.3
	C_5	75.3
Glucose	$C_{1,\alpha}$[a]	93.0
	$C_{1,\beta}$[a]	96.8
	$C_{3\beta,5\beta}$	76.8
Taurine[b]	C_2	48.5
	C_3	36.4
HCO_3^-	C[a]	161.0
(Phospho)creatine	C_3	37.8
	C_4	54.7
	$C_{C=N}$[c]	157.9
Lactate	C_3[a]	21.0
Alanine	C_3[a]	17.2

[a] Resonances accumulating label after 1-^{13}C glucose infusion.
[b] Not detected in adult subjects.
[c] Tentatively assigned to Cr but not PCr. See Fig. 2 and text for details.

^{13}C glucose infusion, and it indicates that it will be applicable to patients in routine clinical MR scanners. This preliminary report also suggests that broadband proton decoupled ^{13}C MRS in combination with glucose infusion allows a more detailed definition of the fates of 1-^{13}C glucose carbons, including the accumulation in Glu_1, Gln_1, and HCO_3^-, than previously available. This potentially will provide a more precise simultaneous determination of the TCA cycle flux rate, glutamine synthesis rates, malate–aspartate shuttle exchange rate, lactate metabolism, α-ketoglutarate/glutamate exchange rates, and substrate decarboxylation in normal and diseased human brain. Clinical studies using this assay are currently in progress (*21*) and will be reported separately.

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