NMR IN BIOMEDICINE
NMR Biomed. 2003;**16**:358–369
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/nbm.852

Review Article

Clinical experience with ^{13}C MRS *in vivo*

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Received 24 June 2003; Revised 28 August 2003; Accepted 1 September 2003

ABSTRACT: ^{13}C MRS was installed on a clinical scanner at 1.5 T in order to facilitate integrated MR examinations of human brain disorders. Using a simplified protocol, (1-^{13}C) glucose and/or (1-^{13}C) acetate were administered orally or by intravenous infusion. ^{13}C spectra of diagnostic quality were acquired in more than 100 consecutive studies. Novel ^{13}C neurochemical data contributed to the understanding of Alzheimer's, Canavan's, mitochondrial and hepatic encephalopathy, epilepsy and normal brain development. ^{13}C MRS uncovered hitherto unknown disorders of NAA-synthesis, glutamate neurotransmission, TCA-cycle and glycolysis. Despite low inherent signal-to-noise, natural abundance ^{13}C MRS showed diagnostic promise. ^{13}C MRS is feasible in a clinical setting, at reasonable cost in neonates, children, adults and elderly patients. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: glutamate; glutamine; neurotransmitter; hepatic; mitochondrial; encephalopathy; dementia; epilepsy; ketones; clinical diagnosis; ^{13}C

INTRODUCTION

The earliest steps taken with *in vivo* NMR spectroscopy, be they in intact frog muscle, isolated perfused rat heart, kidney or liver, or the first human studies[1] had one thing in common: they were all dynamic studies. They exploited the central theme of *in vivo* NMR, that of non-destructiveness, to make repeated measurements over the course of a perturbation in the same organ or subject. By generating data on metabolite flux, NMR largely overcame its patent absurdities—low sensitivity, high cost, poor access. Who, in the age of HPLC, GCMS, flourimetric analysis of nanomoles of literally thousands of important metabolic intermediates and signals, would pay thousands of dollars to measure the steady-state concentrations of a mere three or four metabolites in the millimolar range, in small numbers of subjects? However, as the ensuing 25 years have shown, even this seemingly trivial property of NMR has offered extremely interesting insights into human metabolism. Yet the 'pick-in's' have been sparse, usually because the best of the chemist's NMR tools, including the more sophisticated NMR sequences themselves, have severe restrictions, preventing *in vivo* application and limiting their use to body fluids or surgical biopsy specimens *in vitro*.

^{13}C MRS, also developed 30 years ago, held the promise of circumventing many problems by offering dynamic metabolic analysis.[2,3] It is the purpose of this Review to examine the hypothesis that dynamic analysis of metabolite flux rates *in vivo* is more powerful in clinical investigation than static analysis. In other words, that ^{13}C NMR after enrichment provides more useful diagnostic information than other steady-state concentration measurements using ^{1}H, ^{31}P, ^{23}Na etc., and thereby compensates for its poor sensitivity.

HISTORY OF CLINICAL ^{13}C MRS

Although the ground work for all ^{13}C MRS was established by chemists, physical chemists and physicists over many years, we owe almost all of the translational research which made it possible to perform simpler ^{13}C NMR experiments in whole-body NMR instruments at relatively low magnetic fields (1.5, 2.1, 4.1 T) to a small number of laboratories,[4–9] particularly at the University of Zurich, Switzerland and Yale University, USA. The move to higher fields has also proceeded only slowly, and has so far largely been confined to normal volunteers. Other papers review these development (Gruetter *et al.* and de Graaf *et al.*, in this volume), and provide a catalogue of the achievements necessary before any approach to the question of clinical utility of ^{13}C MRS could be made.

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Abbreviations used: AD, Alzheimer's disease; Ala, alanine; Asp, aspartate shuttle; DDS, dynamic difference spectroscopy; DMA, dynamic metabolic analysis; GCMS, gas chromatography mass spectrometry; Gln, glutamine; Glu, glutamate; GNT, glutamate neurotransmission; GS, glutamine synthesis; HE, hepatic encephalopathy; IND, investigational new drug; ITP, isotopomer analysis; Lac, lactate; NAA, *N*-acetyl aspartate; NOE, nuclear Overhauser effect; PAG, phosphate-activated glutaminase; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; R_f, radio frequency; TCA, tri-carboxylic acid.

CLINICAL ^{13}C MRS INVESTIGATIONS

Details are given in a number of publications.[10–19] The following topics will be considered: R_f coil design; proton-decoupling and safety; nuclear-Overhauser effects; peak assignments; quantitative analysis; ^{13}C substrate preparation; practicable i.v. infusion protocols; interpretation of metabolic models; experimental design and metabolic modeling to yield meaningful results; and clinical experience.

In this laboratory, our goal was to reproduce on a purely routine clinical MR scanner much of what had before been practicable only on research spectrometers. If this could be achieved in a tolerably short time scale, the many other benefits of the routine MR scanner (stability, FDA approval, clinical routine, combined first-class diagnostic imaging and routine diagnostic ^{1}H MRS) could then be integrated into a ^{13}C MRS examination to allow exploration of simple clinical questions not previously available to the NMR scientist.

LESSONS LEARNED FROM ^{13}C MRS ON A CLINICAL MR SCANNER

R_f coil design

After several fruitless designs, we have adopted that of Adriany and Gruetter;[20,21] Bluml and Harris (unpublished) successfully constructed several 'clones' for use in other centers. The half-head field of view (Fig. 1) spares the lens and eyes from possibly SAR-exceeding R_f power deposition during decoupling, and has proved acceptable for young and old alike. It was capable of acceptable MRI and localized ^{1}H MRS, the latter providing a simple means of quality assurance and quantitation[13,17] of subsequently enriched ^{13}C brain metabolites.

Figure 1. 'Half-head' dual tuned ^{13}C MR coil. A surface-coil design was chosen for ^{13}C MRS to maximize signal and avoid heat deposition over the eyes. By combining ^{1}H and ^{13}C resonators it is possible to obtain MRI for anatomy and 'localization' as well as good-quality ^{1}H MR spectra for diagnosis and quantification (see below). The subject lies comfortably with the occiput applied to the thinly padded plastic tray, protected from direct contact with the R_f coils (two proton and one ^{13}C). Tuning and matching is accomplished with the patients in position, but outside the magnet-bore, via capacitors mounted in the head-support. Additional padding keeps the patient's head in a constant position to facilitate later spectral data processing. Sedated infants can be examined in the same coil, or in a custom made 'mini-coil' (S. Bluml, unpublished data)

Proton-decoupling safety and NOE

Natural abundance ^{13}C spectra, with only 1.1% intrinsic enrichment, demonstrate the effectiveness of standard WALTZ-16[22] to decouple well within the brain. Bluml[17] demonstrated useful properties of even this simple experiment (Fig. 2) by determining altered mI, glutamate and glutamine concentrations in patients with hepatic encephalopathy, and confirming the extraordinary increase in NAA concentration long-known to characterize children with Canavan's disease. A novel (unpublished) finding in the same experiment, is that in hypoxic infant brain, glutamine rather than glutamate accumulates. This directly confirms the earlier and often controversial findings of short-*TE* ^{1}H MRS[23] and demands a re-thinking of the neuroscientists' dogma that it is toxic accumulation of glutamate which causes hypoxic neuronal injury.[24] Because NOE and proton decoupling *in vivo* and *in vitro* are similar, but not identical, a study of all the expected brain metabolites was undertaken using a series of phantoms. Correction factors were then applied for the quantification of clinical ^{13}C spectra (Table 1).[10,15,19]

Peak assignments

Broad-band excitation and proton-decoupling over the entire 4 kHz range results in excitation and assignment of all of five carbon atoms of glutamate and glutamine [Fig. 3(A)], and many other interesting brain metabolites besides. However, this is achieved at the expense of localization. The worst effect is the inclusion of extracerebral lipids and glycerol resonances and furthermore, it greatly complicates the clinical examination. Thus, for all of the studies performed to date, a rather lengthy 'prescan' to obtain the natural abundance ^{13}C spectrum of each patient was included [Fig. 3(B)]. Difference spectra were then constructed for each of the post-enrichment acquisitions (Fig. 3, upper panel). Such a procedure cannot be interrupted since it is very hard to ensure identical

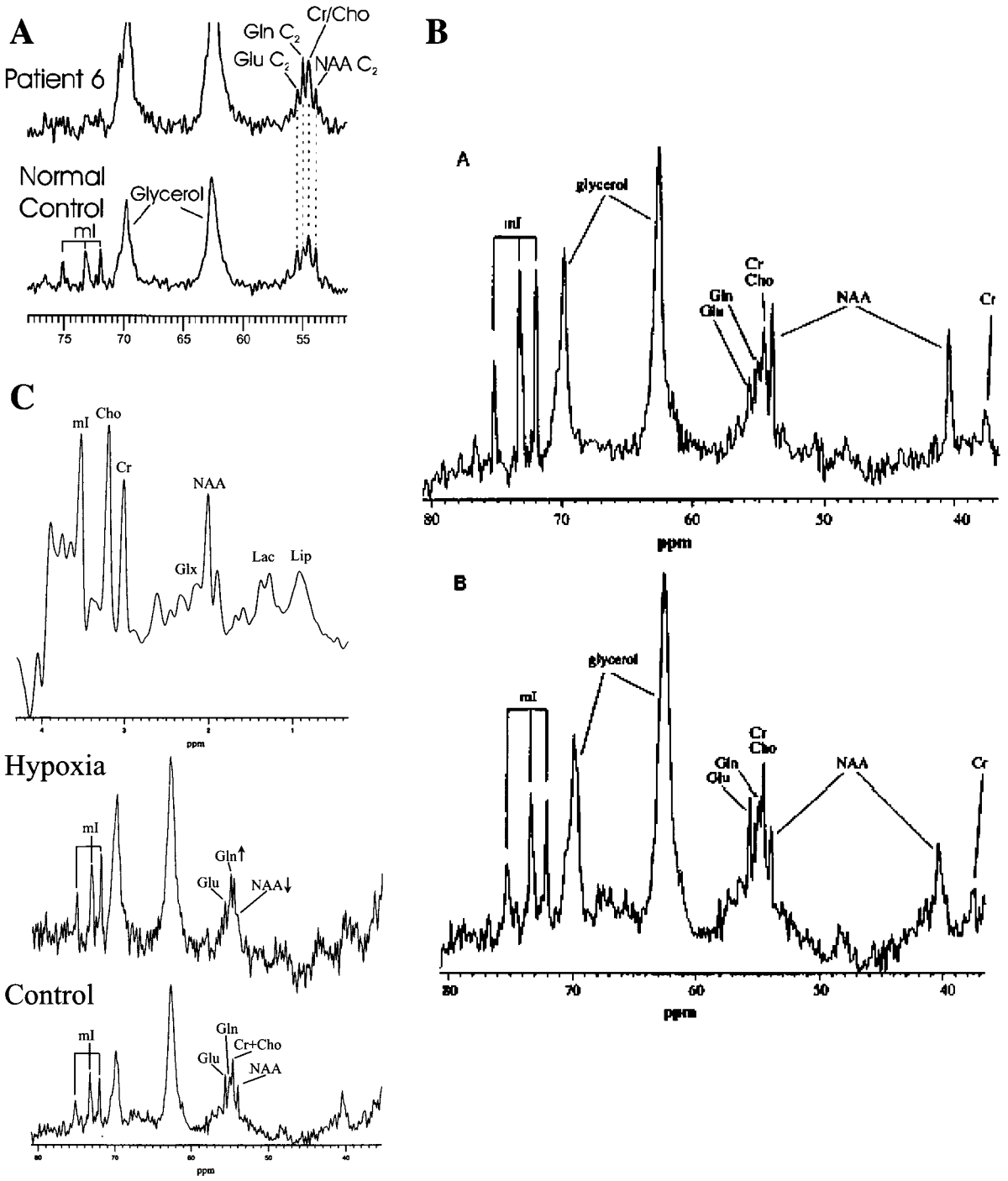

Figure 2. Proton-decoupled ^{13}C natural abundance spectra of clinical relevance. Despite a very low signal (only 1.1% of ^{12}C), ^{13}C at natural abundance can provide useful information from the diseased human brain. Natural abundance ^{13}C MRS acquisitions of 15–30 minutes duration are illustrated. (A) Hepatic encephalopathy (a common metabolic consequence of liver failure) is characterized by absent myoinositol, 3-fold excess of glutamine and (not appreciated in this spectrum) a 20% reduction in glutamate. Glycerol resonances seen in the patient and the control are not of diagnostic significance, as they arise from superficial triglycerides included in the non-localized excitation volume. (B) Canavan's disease (an inborn error of brain metabolism, aspartoacylase deficiency) characterized by accumulation of its substrate *N*-acetyl aspartate (NAA), also demonstrates 50% reduction in cerebral glutamate (Glu) concentration and 50% increase in myoinositol. (C) Hypoxic-encephalopathy, a common pediatric emergency, preferentially impacts neurons and has profound effects on cerebral energy metabolism. This is reflected in reduced NAA and increased lactate. Glx, the 'combined' resonances of glutamate and glutamine are increased in ^{1}H MR spectra (above). ^{13}C MRS confirms the reduced NAA, but also resolves glutamate from glutamine, revealing that it is glutamine which is markedly increased in this hypoxic brain, while glutamate is unchanged (below). Lactate, readily detected by ^{1}H MRS but at a concentration of only 2–3 mM in this patient, was not observed with natural abundance ^{13}C MRS

Table 1. ^{13}C metabolite concentrations and chemical shifts for human brain

Metabolite	Carbon	Chemical shift (ppm)	Carbon concentration (mmol/kg)
Glutamate	C_1[a]	175.3	
	C_2[a]	55.7	10
	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	5.6
	C_3[a]	27.2	
	C_4[a]	31.8	
	C_5	178.4	
NAA	C_1	174.3	
	C_2[a]	54.2	9.2
	C_3[a]	40.4	
	C_4	179.5	
	$C_{C=O}$	179.5	
Aspartate	C_2[a]	53.3	2.8
	C_3[a]	37.5	
Myo-inositol	$C_{1,3}$	72.1	6.0
	$C_{2,4,6}$	73.3	
	C_5	75.3	
Glucose	$C_{1,\alpha}$[a]	93.0	4.8–7.3
	$C_{1,\beta}$[a]	96.8	
	$C_{3\beta,5\beta}$	76.8	1.0
Taurine	C_2	48.5	
	C_3	36.4	
HCO_3^-	C[a]	161.0	20
(Phospho)creatine	C_3	37.8	10[b]
	C_4	54.7	
	$C_{C=N}$	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] After deducting Cho(1.5 mmol/kg); 11.5 mmol/kg after i.v. glucose.

patient positioning needed for accurate spectral subtractions.[10] Others have experimented with localization and the consequent narrower ^{13}C-excitation range,[6,7,9] thereby avoiding difference-spectroscopy. However, the localization scheme itself (ISIS) is a difference method and shares some of the disadvantages.

Quantitative analysis

The practical solution adopted in this laboratory is only one of many possibilities, constrained by the coil design (NMR signal from a surface coil is difficult to quantify), pulse-sequence and limits of patient tolerance. Nevertheless, it has been of value to have quantifiable data, even if the experimental range is rather broad.[15,17,18] Myoinositol, which appears in ^{1}H and ^{13}C MR spectra (Fig. 4) and is not enriched during several hours of (1-^{13}C) glucose infusion, provides a robust internal reference from which all ^{13}C signals can be derived in mmol/unit brain volume. It remains to be seen whether clinically useful results require this extra attention to detail. In clinical ^{1}H MRS, a handy convention of peak ratios, without quantitation, has proved every bit as useful as more demanding quantitative methods.[25]

^{13}C-substrate preparation

Enrichment of neurometabolic tools with ^{13}C is the key to the huge potential of ^{13}C MRS. To date we have explored in a clinical setting, only two: (1-^{13}C) glucose and (1-^{13}C) acetate. Both need to be prepared with care, sterile and refrigerated to avoid untoward reactions. Most investigators have felt that, as glucose and acetate are 'standard' dietary constituents, no more than usual care was necessary in use of these harmless stable isotopes. Our policy has been to gain FDA oversight by submission of an IND, to permit use in infants and sick adults. Furthermore, an FDA approved pharmacy was asked to prepare solutions on an individual named patient basis. Only two trivial and unrelated incidents (both minor self-limiting skin rashes) were reported in over 100 clinical uses. Nevertheless, we advocate extreme care as administration of hypertonic intravenous glucose itself carries a clear risk, which prevents unauthorized use.

Practicable infusion protocols for ^{13}C MRS in patients

The most important compromise which we adopted for our clinical trials (Table 2) was to leave the well-trodden path of the glucose–insulin clamp, developed by earlier investigators.[4] This has severely compromised our ability to compare results with other laboratories where steady-state models have proved most powerful in modeling brain function. Indeed, without steady state, much of our data becomes un-interpretable if seen in the light of a generation of ^{14}C flux-rate analysis.[26] Fortunately, with the more limited goal of finding clinically meaningful differences between patients and controls, our less rigorous approach has proved effective.[15,27]

The arguments against ^{13}C glucose–insulin clamp are three: (a) cost—a total of 40–50 g of (1-^{13}C) glucose is typically infused to achieve and maintain steady state; we were able to reduce the cost of (1-^{13}C) glucose by 80% in our simpler protocol; (b) FDA concerns—somatostatin, the hormone which is used to prevent reactive insulin release during the glucose clamp, is not approved for use in children; (c) duration of the study—while achieving steady state can take several hours, 120 min in the MR scanner is already excessive. In future, it is probable that ^{13}C administered to the patient outside the MR scanner, with brief data acquisition at a pre-determined time, will provide a practical solution.

We have found several protocols for ^{13}C administration to be essentially interchangeable.[11,18] Furthermore, insisting on patient-fasting overnight, a relic of earlier studies of liver metabolism and of the glucose clamp, may prove to be unnecessary for neuro-spectroscopy, bringing ^{13}C MRS into an out-patient test environment.

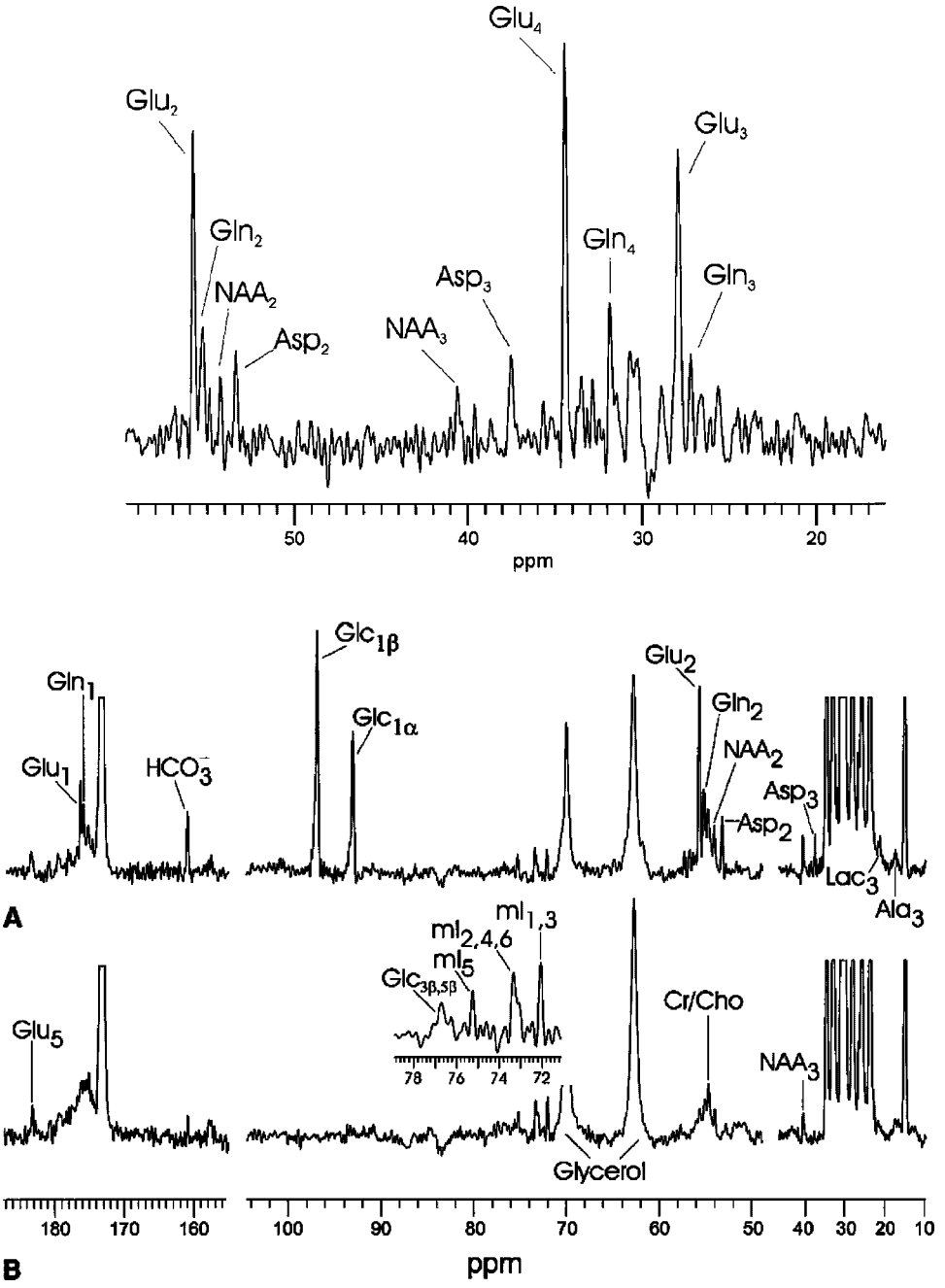

Figure 3. Enrichment of cerebral metabolites by infusion of (1-^{13}C)-glucose. Broad-band excitation after (1-^{13}C) glucose infusion permits detection of a larger number of ^{13}C resonances; these include the infused substrate itself (Glc 1α, 1β) and metabolites not previously detected in human brain (HCO_3, glutamate and glutamine C_1 and C_5) (A,B). However, because of the overlying glycerol–lipid resonances, difference spectroscopy is necessary for detection of the more important glutamate and glutamine C_3 and C_4 resonances (upper panel). In all more than 20 enriched metabolite-carbon atoms are identifiable in clinical ^{13}C spectra acquired at 1.5 T

^{13}C 'snapshot' spectroscopy

In an attempt to reduce time in the MR scanner, after prescans for MRI, ^{1}H MRS and natural abundance ^{13}C MRS (total time 35 min) the subject (a healthy female volunteer, fasted 6 h) was removed from the scanner. ^{13}C enriched substrates were given by mouth as a cocktail of (1-^{13}C) glucose (15 g, 99% enriched) and (1-^{13}C) sodium acetate (10 g, 98% enriched) in 50 ml of cranberry juice (pH = 6). The subject rested quietly for 2 h before being returned to the MR scanner for 20 min of ^{13}C MR acquisition. The selected spectral regions displayed illustrate a 'snapshot' of glial (C_5 glu + gln and HCO_3) and neuronal (C_2 glu, gln and aspartate) TCA cycle activity at

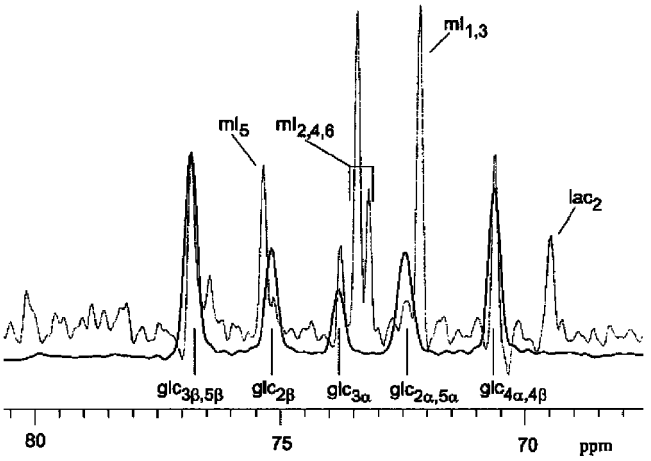

Figure 4. Use of *myo*-inositol as an internal references in quantitative *in vivo* ^{13}C MRS. Of the several metabolites observed readily in both ^{1}H and ^{13}C MR spectra, *myo*-inositol is best suited as a quantifiable internal reference. mI has a complex ^{13}C spectrum with three resolvable peaks [see inset, Fig. 3(B)], which do not overlap with, or become ^{13}C enriched from infused glucose. In a simplifying method (2), a 'pseudo-super-singlet' mI peak which could serve as a reference for peak heights of key cerebral intermediates in individual patients. The actual mI concentration in the appropriate brain region was determined from the localized ^{1}H MR spectrum previously acquired using PRESS or STEAM *TE* 30 ms in each patient

near-steady state (Schweinsburg, Harris, Bhattacharya, Zimmerman, Lin, unpublished data; spectrum was acquired on a GE LX 8.3 with stand-alone decoupler unit; Schweinsburg *et al.*, unpublished data).

Interpretation of metabolic models

While we did not set out to ignore prior metabolic models of brain metabolism, as described above, we limited their applicability by less rigorous enrichment procedures. Nevertheless, we have found it relatively easy to demonstrate pathology in ^{13}C brain spectra using a mixture of peak analysis, difference spectroscopy, relative enrichment rates, and task-specific modeling.[12,13,18]

CLINICAL FINDINGS

Table 3 describes the principal brain disorders which we selected for study with the two ^{13}C substrates at our disposal. Here we discuss our principal findings.

Use of ^{13}C MRS to determine the *in vivo* rate of NAA synthesis[12] (Fig. 5)

NAA concentration is remarkably uniform in normal humans and changes only slowly in a number of brain diseases. NAA is therefore believed to have a low turn-

Table 2. ^{13}C enrichment protocols

	Method		NMR reagent	Reference	Principal biomed. application(s)
1	Partial steady state	i.v.	(1-^{13}C) glucose	11	Diagnosis children and adult
2	Partial steady state	Oral		19	
3	Partial steady state	i.v.	(1-^{13}C) acetate	16	Glial flux rates for TCA
4	Partial steady state	Oral		19	
5	Partial steady state	Combined	^{13}C acetate plus ^{13}C glucose	28	Gln-Glu cycle; substrate selection in health and disease
6	'Snapshot'	Oral	^{13}C acetate plus ^{13}C glucose	Schweinsburg *et al.*, unpublished data	Unstable or uncooperative patients

Table 3. Clinical disorders examined with ^{13}C MRS after enrichment

Disease	Number of patients	Principal findings	Reference	Technique
Alzheimer's	3	TCA/GNT/NAA	19	DDS
Polyglucosan storage	3	TCA/GNT	—	DDS
Epilepsy ketogenic diet	3	GS/PC	19,29	ITP
Epilepsy (valproate)	1	GS	16	DMA
Hepatic encephalopathy	6	GS/TCA/PAG	15	DDS
Mitochondrial encephalopathy	3	Lac/Ala	13	DDS
Aspartate shuttle 'disorder'	1	Asp	13	DDS
Leukodystrophy (VWMD)	5	Lac/Ala	13	DDS
Canavans	5	NAA synth.	12	DMA
Prematurity	1	Glu/PDH	13	DMA
Hypoxia	1	TCA/GNT	13	DDS
Dietary fuel selection	11	GS/PC	30	ITP

DDS, dynamic difference spectroscopy; ITP, isotopomer analysis; DMA, dynamic metabolic analysis; TCA, tricarboxylic acid cycle; GNT, glutamate neurotransmission; NAA, *n*-acetyl aspartate; GS, glutamine synthesis; PC, pyruvate carboxylase; PAG, phosphate-activated glutaminase; Lac, lactate; Ala, alanine; Asp, aspartate shuttle; PDH, pyruvate dehydrogenase; VWMD, vanishing white matter disease.

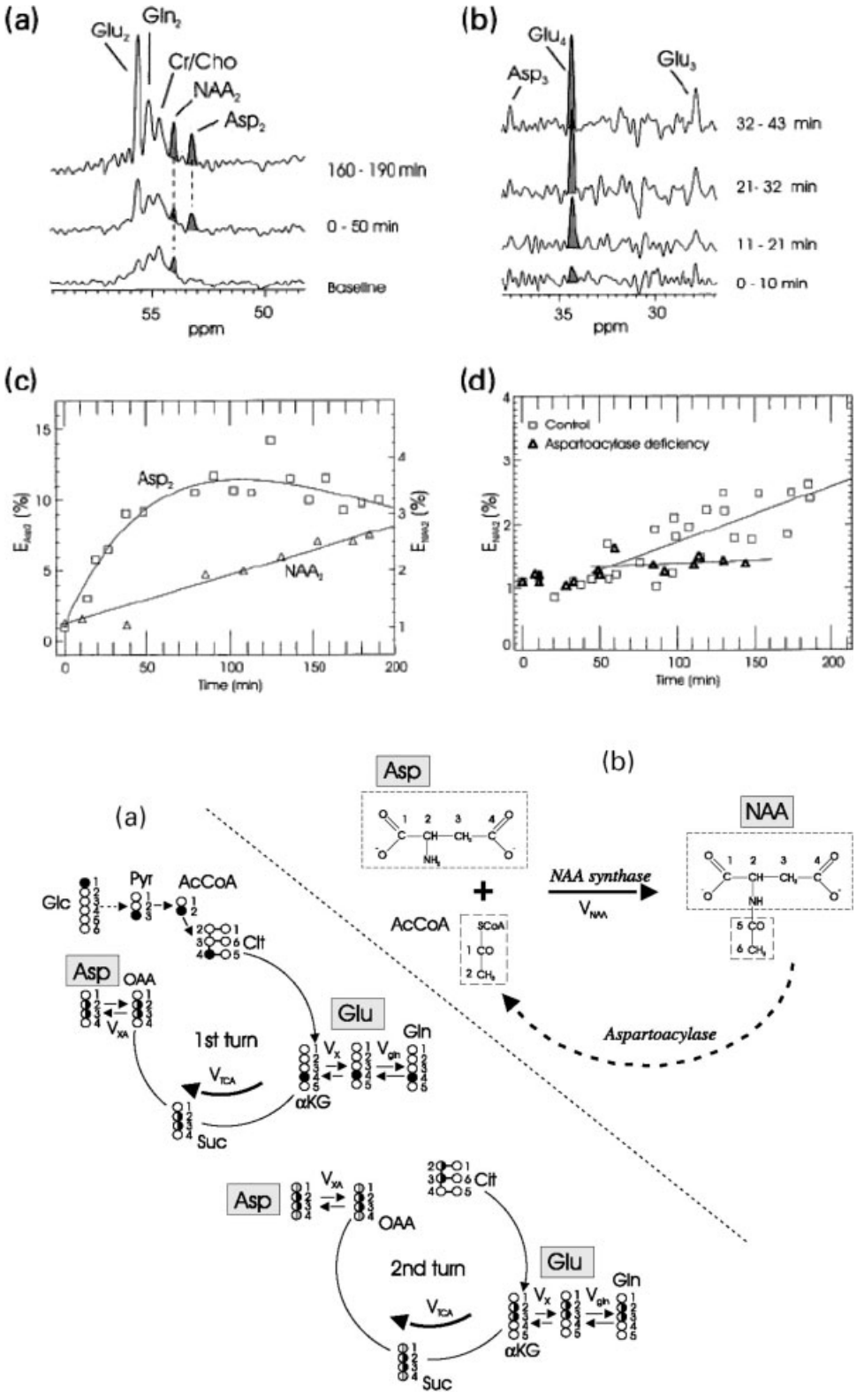

Figure 5. Use of ^{13}C MRS to determine the *in vivo* rate of NAA synthesis. NAA concentration is remarkably uniform in normal humans and changes only slowly in a number of brain diseases. NAA is therefore believed to have a low turnover rate in man. However, NAA synthesis has never been directly demonstrated and it was not previously known whether the rate could be modified. Using (1-^{13}C) glucose to enrich aspartate (a), one of the precursors of NAA, it was then possible to observe the appearance of ^{13}C in NAA (b,c). The calculated rate of NAA synthesis was indeed very low, and fell by a further 50% in patients with Canavan's disease (d). For full details, see Bluml *et al.*[16]

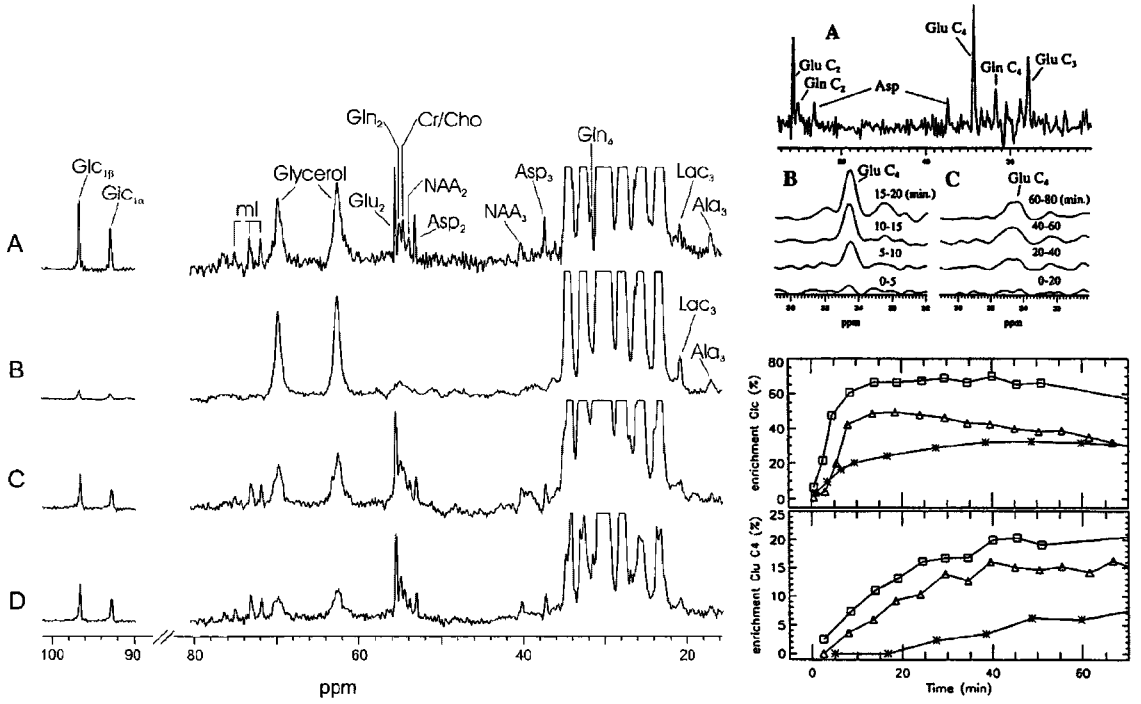

Figure 6. Mitochondrial brain disorders revealed by ^{13}C MRS. The spectra on the left were acquired from infants with (A) unidentified encephalopathy, (B,C) mitochondrial encephalopathy, and (D) control. Patient (A) demonstrated a higher Glc $C_{1\alpha,\beta}$ signal and increased incorporation of ^{13}C into aspartate C_2, C_3. Patient (B) had a terminal mitochondrial defect that demonstrated low entry of glucose into the brain with incorporation of ^{13}C in lactate and aspartate only. Patient (C) had a mitochondrial DNA defect (T3394C) but demonstrated an enrichment pattern of ^{13}C that was indistinguishable from pediatric control (D). The spectra on the right were acquired from a premature infant with normal childhood development. Spectra (A, right) are the result of summed spectra over a period of over 80 min after infusion. Spectra (B) and (C, right) demonstrate the delayed incorporation of ^{13}C in the newborn brain in the indicated time scales. The graphs (lower-right) clearly illustrate the delayed enrichment of Glc (top) and Glu C4 (bottom) in the newborn brain (*) when compared with adults and children (open square and triangle, respectively)

over rate in man. However, NAA synthesis has never been directly demonstrated and it was not previously known whether the rate could be modified. Using (1-^{13}C) glucose to enrich aspartate [Fig. 5(a)], one of the precursors of NAA, it was then possible to observe the appearance of ^{13}C in NAA [Fig. 5(b,c)]. The calculated rate of NAA-synthesis was indeed very low, and fell by a further 50% in patients with Canavan's Disease [Fig. 5(d), and lower panel].

Mitochondrial brain disorders revealed by ^{13}C MRS[13,29]

Because of the central role of mitochondria in the electron transport chain and the TCA-cycle (see Figs 5 and 9), (1-^{13}C) glucose infusion followed by ^{13}C MRS *in vivo* can be used as a test of mitochondrial dysfunction in infants with undiagnosed encephalopathy. Three examples are illustrated (Fig. 6): undiagnosed mitochondrial disorder with accumulation of ^{13}C aspartate, alanine and lactate, but apparently normal rates of TCA cycle and glutamine–glutamate cycling [Fig. 6(A)]; absent TCA- and glutamine–glutamate cycles in a child with proven mitochondrial encephalopathy; the continuation of non-mitochondrial cerebral glycolysis is suggested by the appearance of ^{13}C in lactate and alanine [Fig. 6(B)]; and mitochondrial DNA-defect which was without cerebral metabolic impact [Fig. 6(C)]. ^{13}C glucose entered metabolic pools at a rate comparable to that of age-matched pediatric controls [Fig. 6(D)], suggesting that there may be a dissociation between the standard gene-mapping tools (fibroblast, blood cells etc.) and the metabolic defects in the brain. For this reason, it may be valuable to perform *in vivo* ^{13}C MRS of the brain even in children in whom the gene defect has been fully characterized. Delayed mitochondrial maturation may be a normal feature of new-borns (Fig. 6: right hand panel). Thus, the rate at which (1-^{13}C) glucose was converted to C_4-glutamate appeared to be very slow in this premature infant, who then went on to normal childhood development. One known possibility is that pyruvate-dehydrogensase, the key mitochondrial enzyme which controls entry to the TCA cycle, had not yet reached its full activity in this premature infant. However, no excess ^{13}C lactate was observed, suggesting that, unlike pathological mitochondrial disorders, this represents a well-regulated reduction of the overall rate of brain metabolism.

Alzheimer's disease may represent a failure of glutamine–glutamate cycling and glutamate neurotransmission.[18] An important concept in ^{13}C MRS is that of the glutamine–glutamate cycle between normal neurons and astrocytes, several features of which can for the first time be directly probed in the human brain *in vivo*. By demonstrating a 1:1 stoiciometry between glucose oxidation and glutamate-glutamine cycling, Magistretti and colleagues[31] propose that the glutamate neurotransmitter rate can be determined directly. This is an important concept which has been tested in a small number of clinical settings. One such is the patient with Alzheimer's disease. Lin and colleages[18] demonstrated reduced C_4 glutamate formation from (1-^{13}C) glucose in a small number of AD, in whom ^{1}H MRS was used to also demonstrate the expected decrease in neuronal numbers. There was a linear correlation between the reduced C_4 glutamate and reduced NAA in these patients, as expected if glutamate–glutamine cycling and glutamate neurotransmitter rate was determined by the number of functioning neurons. A representative ^{13}C brain spectrum from an AD patients and an age-matched control is illustrated (Fig. 7).

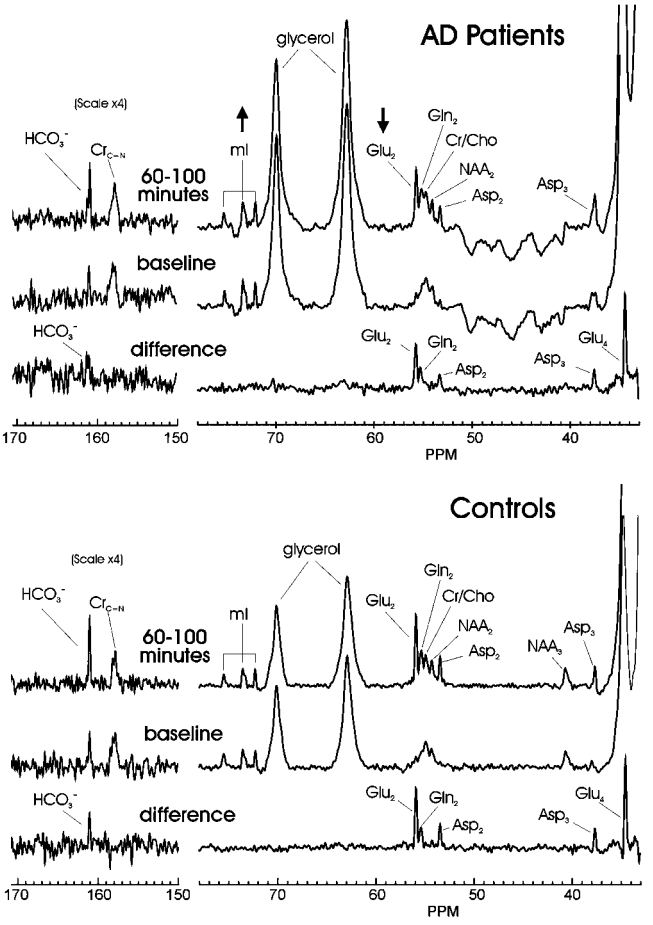

Figure 7. Alzheimer's disease may represent a failure of glutamine–glutamate cycling and glutamate neurotransmission. ^{13}C spectra acquired from patients with Alzheimer's disease (top) and age-matched controls (bottom). Within each set of spectra, the top spectrum is ^{13}C enriched spectra acquired 60–100 min after infusion. The middle spectrum is the baseline spectra acquired prior to infusion. The bottom spectra are the ^{13}C enriched metabolites created using the difference between baseline spectra from post-infusion spectra. A comparison of the patients and controls shows a significant reduction of Glu C_2 and HCO_3 in the Alzheimer's difference spectra and increased mI in the baseline and infused spectra

Hepatic encephalopathy and impaired consciousness[15]

Hepatic encephalopathy is a progressive but reversible neurological disorder providing an ideal setting to explore the role played by cerebral glutamate in normal consciousness, using ^{13}C MRS. HE results in a striking reduction is C_2 glutamate formation from (1-^{13}C) glucose (Fig. 8). Furthermore, the extent of the reduction is progressive with increasing severity of the clinical disorder. NAA is normal in this disease, so that the changes cannot be simply attributed to loss of neurons (contrast AD above). Instead, we postulate a reversible abnormality of glutamate neurotransmission at another step, possibly that of astrocyte glutamine synthesis. This result also provides an interesting test of the Magestretti hypothesis, since progressive cognitive impairment ending with loss of consciousness appears to be directly linked to failure of glutamate neurotransmission (Fig. 9).

^{13}C acetate can be used to probe cerebral metabolism in patients who are intolerant of glucose[16,19,31]

Children with epilepsy can gain great medical benefit with reduction in their seizures by transferring to a high-fat diet which is ketogenic. The mechanism of this fascinating effect is clearly 'metabolic' and slowly yielding to research, particularly with ^{1}H, ^{31}P and now ^{13}C MRS. However, to give ^{13}C glucose would be unethical, as seizures would undoubtedly be precipitated. Fortunately, an alternative ^{13}C probe, (1-^{13}C) acetate, is harmless in these patients and yields similar metabolic information concerning cerebral TCA cycle rate and the glutamate–glutamine cycle (Fig. 10). (1-^{13}C) acetate enters C_5 glutamate and glutamine, rather than C_2 and C_4. Furthermore, there is good evidence that acetate is more readily metabolized in astrocytes than in neurons. The rate of astrocyte TCA-cycle has been determined[16,29] and marked differences in epileptics on ketogenic diets have been described in studies using (1-^{13}C) acetate followed by ^{13}C MRS.[19]

CONCLUSIONS

(1) ^{13}C MRS is feasible on a clinical 1.5 T MR scanner.
(2) A limited range of diagnostic information, mostly concerning glutamate and glutamine cycling between

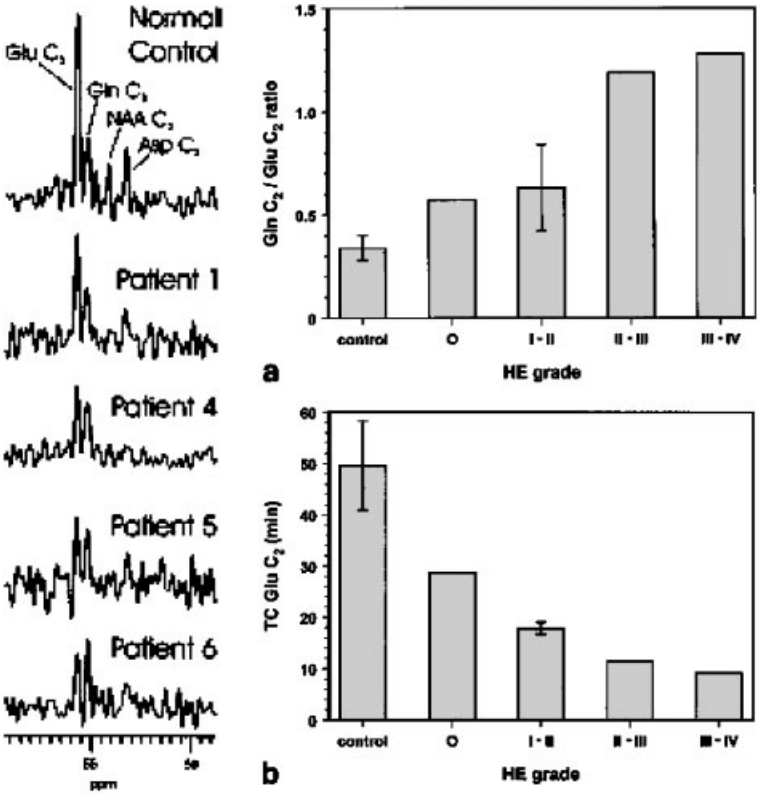

Figure 8. Hepatic encephalopathy and impaired consciousness. Cerebral difference spectra acquired from control and four patients with increasing severity of chronic hepatic encephalopathy are shown (left). Glutamate C_2 formation is severely reduced in the HE brain. In addition, aspartate C_2 and NAA C_2 are hardly enriched whereas glutamine C_2 labeling is unchanged. The graphs on the right (a) demonstrate that the reduction in Glu C_2 is significantly correlated with the severity of the disease (HE grade). The time constant (TC_{Glu2}) is inversely correlated with disease severity (b)

neurons and glia, can be obtained which shows its value in neurological research.

(3) Further developments are necessary if ^{13}C MRS is to be a diagnostic tool which matches the ease and versatility of ^{1}H MRI and ^{1}H MRS.

(4) ^{13}C MRS confirms the added power of dynamic as opposed to static 'imaging' of traditional ^{1}H and ^{31}P MRS.

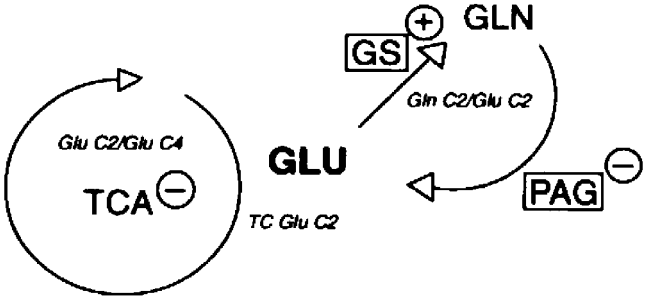

Figure 9. Hypothesized limitation of Glu–Gln cycle in HE. This model demonstrates the possibility of a reversible abnormality of glutamate neurotransmission at another step, possibly that of astrocyte glutamine synthesis. Reductions in the tricarboxcylic acid (TCA) cycle as well as changes in glutamine synthesis (GS) and phosphate-activated glutaminase (PAG) explain the results shown in Fig. 8

Acknowledgments

Work was funded by NARSAD, HMRI and the Rudi Schulte Research Institute, Santa Barbara. We thank Jean Devlin and Rudi Schulte for their encouragement. Technical developments were the achievement of Drs Else Danielsen, Stefan Bluml, Angel Moreno, Frederick Shic and Jung-Hee Hwang, whose previous published work forms the basis of our review. We thank Dr Paul Johnson of Huntington Memorial Hospital IRB, the FDA, Ms Darlette Luke of Fairview Hospital Pharmacy, Minneapolis, Maureen Duffy of Cambridge Isotopes Laboratory, and Dr Keiko Kanamori for critical discussions of simplified models in human brain. Kent Harris is a Young Investigator and Brian Ross a Distinguished Investigator of NARSAD. Alexander Lin is a Senior Research Scien-

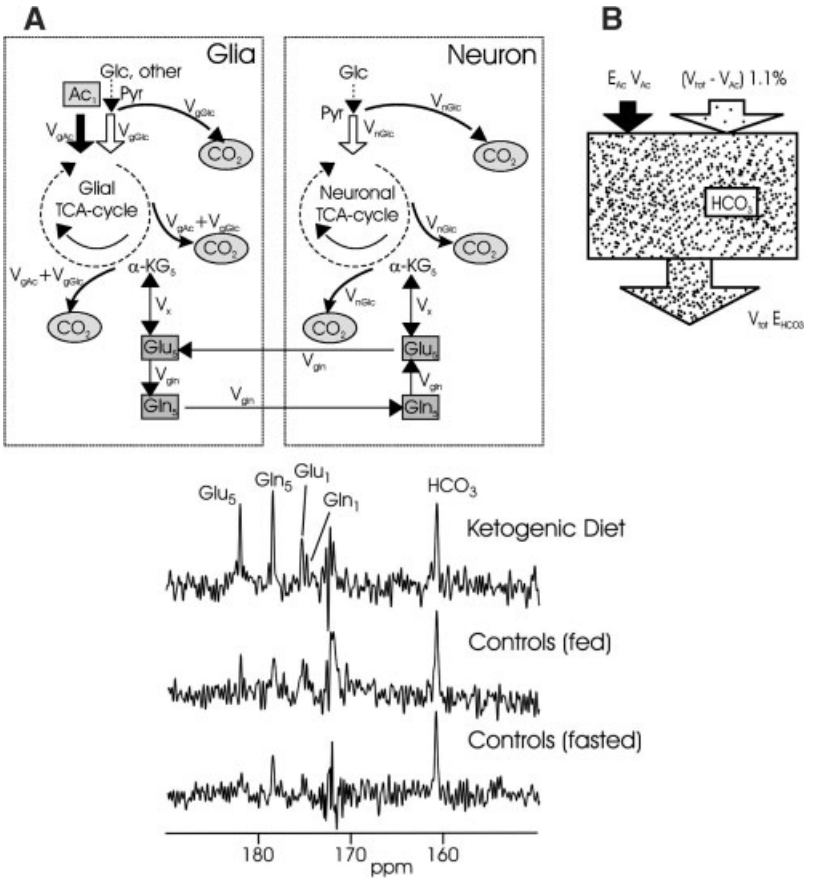

Figure 10. ^{13}C acetate can be used to probe cerebral metabolism in patients who are intolerant of glucose. (A) The transfer of ^{13}C label from 1-^{13}C acetate to the TCA cycle intermediates and exchange partners are illustrated. (B) The influx/outflux of from the bicarbonate pool at steady state is shown on the right. The bottom spectra demonstrate marked differences in epileptics on ketogenic diets and controls. Glc, glucose; Pyr, pyruvate; V_{gGlc}, glial glucose oxidation rate; V_{nGlc}, neuronal glucose oxidation rate; V_{gAc}, rate of acetate oxidation; CO_2, carbon dioxide; α-KG_5, alpha-ketoglutarate; V_x, neuronal/glial alpha-ketoglutarate exchange rate; V_{gln}, glutamine synthesis rate; E_{Ac}, ^{13}C fractional enrichment of acetate; V_{tot}, total rate of ^{12}C and ^{13}C label entering/exiting the bicarbonate (HCO_3^-) pool

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