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Impact of fasting on human brain acid-base homeostasis using natural abundance ^{13}C and ^{31}P MRS

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Abstract

Purpose—To use ^{13}C MRS and ^{31}P MRS to develop direct assay for regional $[\text{HCO}_3^-]$ in the human brain and to define brain pH and physiological response of $[\text{HCO}_3^-]$ to fasting.

Materials and Methods—Seven healthy subjects underwent MRS examinations on a 1.5T MRI scanner. Subjects were well fed with repeated examinations performed after 4 and 12 hours of fasting. Proton noise decoupling ^{13}C MRS were acquired using pulse and acquire acquisition while ^{31}P MRS were acquired using 2-D chemical shift imaging method with TR of 2s.

Results—Fasting brain bicarbonate concentrations (6.7 ± 2.5 mM for 12hr fasting, $P=0.002$ and 8.3 ± 2.1 mM for 4 hr fasting, $P=0.015$) are significantly reduced compared to fed state (11.6 ± 1.3 mM). However, no significant difference in brain pH is observed, confirming the critical role of pCO_2 in intracerebral pH homeostasis.

Conclusion—We demonstrated that the intracellular HCO_3^- in human brain is readily modified by diet but appears to have no measureable effect on cerebral pH. Natural abundance ^{13}C can provide useful information relevant to human brain pH homeostasis by giving information of HCO_3^- .

Keywords

Brain pH; ^{13}C MRS; ^{31}P MRS; Henderson-Hasselbalch equation

INTRODUCTION

Acid-base homeostasis remains a cornerstone of human acute medical care. Its maintenance is a vital function of living systems. Changes of human body pH in either direction can result in irreversible harm or death. Nowhere is pH homeostasis more critical than in the brain (1), where maintaining proper brain pH is important for neurotransmission processes. It is often assumed that fasting impacts intracellular brain pH, resulting in acidosis. Various intracranial pH monitoring devices are commonly used in clinical settings (2–3). Using such devices, it was found that brain pH falls in acute isocapnic metabolic acidosis and rises in acute isocapnic metabolic alkalosis (4–5). Alternatively, magnetic resonance spectroscopy (MRS) has been used successfully to measure concentrations of different brain metabolites and to study brain energy metabolism. While ^{31}P MRS can be used to measure changes in

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intracellular pH based on the chemical shift difference between inorganic phosphate (Pi) and phosphocreatine (PCr) (6–7), ^{13}C MRS can be used to measure CO_2 and bicarbonate (HCO_3^-) concentrations based on the pH-dependent equilibrium between CO_2 and HCO_3^- according to Henderson-Hasselbalch equation (8):

$$\text{pH} = \text{pK}_a + \log \left\{ \frac{[\text{HCO}_3^-]}{[\text{CO}_2]} \right\}$$

where pK_a is the acid dissociation constant of CO_2 , which is 6.15 in the Krebs-Henseleit buffer (9). The relationship shows at any given instance that in order to maintain constant pH, either $[\text{HCO}_3^-]$ or $[\text{CO}_2]$ need to adjust accordingly. Alternatively, realignment in CO_2 – HCO_3^- may be over-ridden by $[\text{H}^+]$. Counter-intuitively, the ketogenic diet, when causing a severe acidosis load, leaves human intracranial (white matter) pH constant (10) while bicarbonate administration causes brain acidosis in rat (11). However, results of human and rodent studies for example, in ketogenic diets, have been mixed. Pan et al (10) argue in favor of cerebral alkalization in brain of fasted humans, based upon elevated intracerebral lactate; working in diabetic ketoacidotic rats Al-Mudallal et al (12) saw no pH change and Glaser et al (13) observed acidification. The goals of this study are to use MRS to develop direct assay for regional $[\text{HCO}_3^-]$ in human brain and to define brain pH and physiological response of $[\text{HCO}_3^-]$ to fasting using ^{13}C MRS method. In addition, proton decoupled ^{31}P MRS will be used to define intracerebral pH during fasting in the same subjects. We anticipate that the combination of these two heteronuclear techniques, ^{13}C MRS and ^{31}P MRS could completely define variation in human brain pH in health and disease.

METHODS

The study was approved by the local institutional review board. Healthy subjects were recruited from the local community and all gave informed consent to participate in the study. ^{13}C and ^{31}P MRS studies were performed on seven subjects (5F/2M) with mean age of 29 ± 2 year with three subjects participating in both MRS studies. In total, 13 measurements ($3 \text{ measurements} \times 3 \text{ subjects plus } 2 \text{ measurements} \times 2 \text{ subjects}$) of ^{13}C MRS and 15 measurements ($3 \text{ measurements} \times 5 \text{ subjects}$) of ^{31}P MRS were available for analysis. Subjects were instructed to consume their normal low fat and high protein diet prior to the scheduled examinations. Repeat examinations were performed after 4 and 12 hours of fasting. One subject did not participated in the fed state ^{13}C MRS examination and one subject missed the 4 hours fasting ^{13}C MRS examination. The MRS examinations were performed in the posterior brain using a similar published protocol (14). The examinations were performed on a 1.5T GE MRI scanner equipped with multi-nuclear data acquisition and a standalone proton decoupling capability. The subjects lay supine on top of the modified head volume coil (15) where the posterior brain region was included in the field of view. The imaging portion of the protocol involved spoiled gradient-recall acquisition steady-state (SPGR) axial images with the following acquisition parameters: TE = 4.2 ms, TR = 175 ms, flip angle = 60° , field of view = 24cm, slice thickness = 2mm, with 1 mm gap. Prior to carbon and phosphorous data acquisitions, field homogeneity adjustment was performed using the automated single voxel proton spectroscopy (PRESS) approach from which the voxel of interest was prescribed from the SPGR images and included the entire sensitive volume of the coil, (typically 100–140 cc). The volume was shimmed to achieve full-width at half maximum of 20–30 Hz of the unsuppressed water signal. For ^{13}C MRS, proton-noise decoupling carbon MRS data was acquired using pulse and acquire acquisition with TR of 3sec in 6.5 minutes block for 60–70 minutes similar to published studies (16). The signal-to-noise ratio of natural abundance ^{13}C creatine plus phosphocreatine resonance exceeded 4:1.

Proton decoupled ^{31}P MRS was performed using a dual tuned half-head 1H - ^{31}P rf coil with 3 Watts decoupling power as previously described (17). Intracerebral pH was calculated from PCr to Pi chemical shift using (7). To avoid ^{31}P signal contamination from scalp muscle, 2-dimensional chemical shift imaging was used with the following parameters; pulse and acquire $8\times 8\times 1$ phase encoding steps with four excitations per step, TR of 3sec.

DATA ANALYSIS

MR spectra were analyzed using off-line processing software available from GE Healthcare (SAGE version 2007 software package). The processing of each spectrum involved zero filling to 16K; 5 Hz Gaussian line broadening, fourier transformation, and manual phasing. For ^{13}C MRS, chemical shift scaling of the processed spectra was set relative to the prominent lipid carbonyl resonance at 172ppm followed by summation of the 60–70 minutes MRS data sets acquired from each subject. A non-linear least-squares Levenberg-Marquardt fitting algorithm was used to fit the spectra and resulting peak areas were used to calculate bicarbonate concentration using total creatine concentration (tCr) of 11 mM as the internal concentration reference (18). For ^{31}P MRS, inspection of all resonances, relative peak ratio to gamma-ATP were compared between fed and fasted states and the phosphocreatine to inorganic phosphate chemical shift difference was used to compute brain pH using the formula described by Petroff and colleagues (7):

$$\text{pH}=6.75+\log \{(\delta-3.27)/(5.63-\delta)\}$$

where δ is chemical shift difference in part per million between phosphocreatine and inorganic phosphate resonances.

RESULTS

A representative summed natural abundance ^{13}C spectrum from 148 to 170 ppm chemical shift region from a fed (A) and 12 hour fasted (B) in a single healthy subject is shown in Figure 1. The resonances at 158 ppm assigned to total creatine (creatine plus phosphocreatine) and bicarbonate at 161 ppm are clearly visible in each spectrum after an acquisition time of 45–50 min. A significant reduction in the peak area assigned to ^{13}C bicarbonate, relative to that of ^{13}C total creatine after fasting was observed. (Table 1) examined after shorter (4 hours, $P=0.015$) or longer (12hours, $P=0.002$) periods of fasting (Figure 2).

A typical proton decoupled ^{31}P MRS spectrum from the same volunteer in fed and fasted state is displayed in Figure 3. There were no notable differences between the identity and relative peak areas of the ^{31}P resonances, PME, Pi, PDE, PCR, alpha, beta or gamma ATP. The chemical shift difference between Pi and PCr in fasted and fed states was unchanged. As shown in Table 1, intracerebral pH was not statistically different after fasting, with a small but not significant increase from pH 7.14 to 7.15 during 4 hr fasting ($P=0.39$) and to 7.18 during 12 hr fasting ($P=0.27$).

DISCUSSION

We demonstrated that the intracellular HCO_3^- in human brain is readily modified by diet but appears to have no measureable effect on cerebral pH; this is contrary to the predictions of (19–21). Estimated HCO_3^- concentrations in human brain, based on these novel natural abundance ^{13}C MRS observations closely match those from direct chemical assays in animal studies range, from 14 to 17 mM (21). While direct measurement for HCO_3^- concentration of human brain has not previously been available, its concentration can be calculated using

the Henderson-Hasselbalch equation based on acid-base equilibrium of carbonic acid and bicarbonate if brain pH and CO₂ concentrations are known. ³¹P MRS has been widely used to measure intracellular pH of tissue and organs in vivo. The approach relies on chemical shift different between inorganic phosphate (P_i) and prominent phosphorous compounds most usually phosphocreatine (PCr). Intracellular fluid pH has been reported in brain of several animal species ranging from 7.04 in rat (22) 7.13 in cat (23), and 7.05 in dog. We observed similar pH in fed healthy subjects to previous values reported from our laboratory (7.13 ± 0.02 vs 6.99 ± 0.01) (24). In addition it is also similar to pH in cat brain measurement using lipid soluble pH sensitive fluorescent indicator (25).

Natural abundance ¹³C can provide useful information relevant to human brain pH homeostasis by giving information of HCO₃⁻. This data, combined with ³¹P MRS assays of cytosolic intracellular pH, provides two of the three parameters of the Henderson-Hasselbalch equation. While it is still not possible with conventional ¹³C MRS, as used here, to measure brain CO₂ concentration in real time due to its very low concentration (1–2 mM) the estimated pCO₂ (Table 1) falls within the range determined invasively in surgical patients; that concentration falls in parallel with the determined reduction in brain bicarbonate (26).

In future, it may be possible to determine pCO₂ directly in human brain using hyperpolarized ¹³C MRS thereby obtaining a complete picture of the complex metabolic events maintaining pH homeostasis. Infusing a hyperpolarized labeled substrate ([1-¹³C] pyruvate) in rat hearts has been reported to demonstrate CO₂ and HCO₃⁻ (27). There are limitations to our technique: first, dedicated hardware is required for stable broadband excitation. Second, long total data acquisition time of 50 to 60 minutes is required. Third, relatively larger brain volume with no spatial localization is examined.

In conclusion, this conventional natural abundance ¹³C MRS in combination with proton decoupled ³¹P MRS technique appears to reliably record two of the desired parameters and to define brain pH in humans.

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Figure 1. Summation of 9 blocks of the 6.5 minutes acquisition of ^{13}C MRS from a volunteer acquired for 50–60 minutes with fed (left) and 12 hour fasted (right). Bicarbonate resonance at 161ppm is clearly visible and displays significantly lower peak amplitude in the 12 hour fasted compared to the fed condition while Cr + PCr resonance remains constant. Dotted lines demonstrate Cr + PCr resonance is unchanged with reduced bicarbonate resonance after 12 hr of fasting.

Figure 2.

Changes of bicarbonate to creatine ratio from five healthy subjects at fed, 4 and 12 hr fasting. Three subjects completed all three scanning conditions, fed, 4 and 12 hr of fasting. One subject did not participated in the fed state and one missed the 4 hr fasted appointment.

Figure 3.

Averaged proton decoupled ^{31}P MRS spectra (10 minutes acquisition time) from a normal volunteer acquired at fed (left) and at 12 hr of fasting (middle). The chemical shifts of the inorganic phosphate (Pi) and the phosphocreatine (PCr) resonances remain unchanged as shown (right).

Table 1

Effect of fasting on intracerebral metabolites in seven healthy volunteers.

Nutritional State	[Cr] mM	[HCO ₃] mM	pH	Calculated [CO ₂] mM
Fed	11.0	11.6 ± 1.3 (N=4)	7.14 ± 0.04 (N=5)	1.06
Fasted, 4 hr.	11.0	8.5 ± 2.5 (N=4)	7.15 ± 0.05 (N=5)	0.76
P (vs. fed)		0.015	0.39	
Fasted, 12 hr.	11.0	6.7 ± 2.5 (N=5)	7.18 ± 0.13 (N=5)	0.56
P (vs. fed)		0.002	0.27	

N= number of subjects examined