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In Vivo Detection of ^{13}C Isotopomer Turnover in the Human Brain by Sequential Infusion of ^{13}C Labeled Substrates

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Abstract

This study demonstrates the feasibility of simultaneously detecting human brain metabolites labeled by two substrates infused in a sequential order. In vivo ^{13}C spectra of carboxylic/amide carbons were acquired only during the infusion of the second substrate. This approach allowed dynamic detection of ^{13}C labeling from two substrates with considerably different labeling patterns. $[2-^{13}\text{C}]\text{glucose}$ and $[\text{U}-^{13}\text{C}_6]\text{glucose}$ were used to generate singlet and doublet signals of the same carboxylic/amide carbon atom, respectively. Because of the large one-bond ^{13}C - ^{13}C homonuclear J coupling between a carboxylic/amide carbon and an aliphatic carbon (~50 Hz), the singlet and doublet signals of the same carboxylic/amide carbon were well distinguished. The results demonstrated that different ^{13}C isotopomer patterns could be simultaneously and distinctly measured in vivo in a clinical setting at 3 Tesla.

Keywords

In vivo ^{13}C MRS; Carboxylic/amide spectral region; Sequential infusion

INTRODUCTION

^{13}C magnetic resonance spectroscopy (MRS) allows investigators to monitor the incorporation of ^{13}C from ^{13}C labeled precursors into various metabolites, such as certain amino acids. In all reported in vivo ^{13}C MRS studies of human subjects, only one ^{13}C labeled substrate per experiment was administered to monitor the relevant metabolic pathways. Studies have noted that glucose is mainly metabolized in neurons, and that acetate is predominantly metabolized in glia. Therefore, ^{13}C labeled glucose has been used to study metabolism dominated by neurons (1–10), and ^{13}C labeled acetate has been used to detect metabolic events originated from glia (11–15 and references therein). Some investigators have used two substrates in order to more reliably measure metabolic pathways of interest. For example, a recent study compared brain mitochondrial metabolism between young and old healthy human subjects by infusing $[1-^{13}\text{C}]\text{glucose}$ in one experiment and $[2-^{13}\text{C}]\text{acetate}$

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to the same subject in a separate experiment (16). Co-administration of two substrates in the same session and under the same pathophysiological conditions would be desirable. However, reliable separation of contributions to ^{13}C signals from two or more substrates is difficult if all make contribution to the observed ^{13}C signals at the same time (17). In the above example, both $[1-^{13}\text{C}]\text{glucose}$ and $[2-^{13}\text{C}]\text{acetate}$ would have contributed to the same glutamate and glutamine aliphatic singlet and multiplet signals in the 20–60 ppm spectral region if the two ^{13}C -labeled substrates had been administered during the same session.

We previously developed a new method for low power ^{13}C MRS. Instead of detecting aliphatic ^{13}C signals in the 20–60 ppm spectral region, which requires relatively high power coherent proton decoupling, we used very low power stochastic methods for broadband decoupling of protons coupled to carboxylic/amide carbons (8, 9, 15 and references therein). In addition to the low power advantage carboxylic/amide carbons are located at the end of the carbon skeleton of amino acids. As such, they can form either a singlet if its neighbor carbon is ^{12}C , or a doublet if its neighbor carbon is ^{13}C , leading to natural simplification of their isotopomer patterns. When ^{13}C -labeled glucose is administered the glutamate and glutamine C4-C5 moiety and the aspartate C3-C4 moiety are labeled by glucose C1-C2 and/or C5-C6, regardless of the number of the turns of the tricarboxylic acid (TCA) cycle. As a result, when the input is $[1,2-^{13}\text{C}_2]\text{acetylCoA}$ glutamate C5, glutamine C5 and aspartate C4 can only form doublet signals (18). Vice versa, when the input is $[2-^{13}\text{C}]\text{acetylCoA}$ glutamate C5, glutamine C5 and aspartate C4 can only form singlet signals (18). This unique feature of the carboxylic/amide carbons makes it possible to simultaneously and unambiguously detect the labeling of glutamate, glutamine and aspartate by two substrates with the second substrate (e.g., acetate, ethanol, β -hydroxybutyrate or lactate) producing singlets only as demonstrated recently using isoflurane anesthetized rats and an 11.7 Tesla spectrometer (18).

Applying a similar strategy to studying human subjects would be very desirable. For example, it would make it possible to simultaneously measure the labeling of brain aminoacids by two different ^{13}C -labeled substrates with very low RF power deposition in a single experiment under the same pathophysiological conditions. There are, however, several potential confounding effects. Hepatic gluconeogenesis may produce glucose with different labeling patterns. If significant, it may lead to undesired ^{13}C isotopomers for a specific labeling pathway. In our animal studies we found no singlets at glutamate C5 when only $[\text{U}-^{13}\text{C}_6]\text{glucose}$ is administered over a prolonged period indicating negligible contribution from gluconeogenesis. Lactate is also significantly labeled by infused glucose (3). At field strengths accessible to most clinical investigations (3 Tesla) lactate C1 and glutamate C5 isotopomers may interfere with each other due to either poor shimming or subject movement.

In this study we report, for the first time, in vivo measurement of ^{13}C isotopomer turnover in the carboxylic/amide region of human brain using sequential infusion of $[2-^{13}\text{C}]\text{glucose}$ and $[\text{U}-^{13}\text{C}_6]\text{glucose}$. The purpose of the study is twofold: first to assess potential spectral and hepatic interferences with ^{13}C isotopomer MRS in the human brain; second to demonstrate a novel method for measuring glucose metabolism in human brain by detecting both its wash-in and the wash-out time courses simultaneously based on different isotopomer patterns. Infusion of the first substrate was begun while the subject remained outside the magnet, and without any spectral data acquisition. Before switching to the second substrate, the subject was positioned into the magnet. Data acquisition began when the infusion was switched from the first to the second substrate. The time course spectra of dynamically decreased labeling originating from the first substrate, and those of dynamically increased labeling originating from the second substrate were detected simultaneously while the second substrate was being infused. This infusion arrangement allowed the detection of maximum

signal intensity of the metabolites originating from the substrate that was infused first while minimizing patient discomfort.

METHODS

In vivo ^{13}C MRS data were acquired on a 3.0 Tesla (T) clinical scanner (GE Healthcare, Milwaukee, USA) A stand-alone proton decoupler (GE Healthcare, Milwaukee, USA) provided continuous bi-level stochastic waveform for Nuclear Overhauser Enhancement (NOE) and for proton decoupling: low level for NOE during relaxation delay and high level for proton decoupling during ^{13}C data acquisition (9). The stochastic waveform consisted of a sequence of repeated short rectangular pulses. The amplitude of the repetition pulse unit (1.2 ms) was kept constant, but its phase was randomly assigned to either 0° or 180° (19). A home-built RF coil consisting of a single circular ^{13}C coil and a proton quadrature surface coil was used as described previously (9). The proton carrier frequency was centered at the water signal. After taking into consideration the cable loss and coil loss, the RF power deposited in the human head was 25 W for proton decoupling and 0.78 W for NOE. The time-average power deposition inside the human head was 2.0 W ($25 \times 0.05 + 0.78 \times 0.95$). The static magnetic field homogeneity was optimized using the FASTMAP method implemented on the GE 3.0 Tesla scanner. First- and second-order shimming was performed on a voxel of $5 \times 5 \times 5 \text{ cm}^3$ cube in the occipital lobe. Typical water linewidth from the occipital voxel was 7–8 Hz.

Healthy human subjects (4 female and 3 male healthy volunteers, age = 26 – 43 years) were recruited in compliance with procedures approved by the Institutional Review Board of the National Institute of Mental Health (NIMH). Subjects fasted at least 12 hours before ^{13}C MRS infusion studies. Two antecubital veins were cannulated, one for infusing ^{13}C labeled glucose solution and the other for withdrawing blood to monitor glucose levels using a SureStepFlexx Glucose Meter (LifeScan, Inc, Milpitas, CA, USA). During glucose infusion, blood samples were withdrawn approximately every 10 minutes to measure blood glucose levels. Heart rate was monitored using MAGNITUDE (Invivo Corp., Orlando, FL, USA) which is MR-compatible. Glucose solutions were infused using a MR-compatible infusion pump, Continuum (MedRad, Indianola, PA).

Two different infusion protocols were used. In the first protocol ($n=3$), i.v. infusion began with $[\text{U-}^{13}\text{C}]\text{glucose}$ (20% w/w) for ~75 minutes followed by infusion of $[\text{2-}^{13}\text{C}]\text{glucose}$ (20% w/w) for an additional 75 minutes. In the second protocol ($n=2$), the order of the infused solutions was reversed. In each protocol, the infusion of the first solution began with a bolus infusion rate of 900 ml/hr followed by an exponential decay to the rate of 100 ml/hr at the 15th minute of infusion. The subsequent infusion rate was adjusted to keep glucose levels at 130–160 mg/dL. The second solution was infused without an initial bolus infusion and the infusion rate was adjusted to maintain constant glucose levels. The experiment began with the infusion of the first glucose solution while the subject remained outside the magnet. Approximately 20 minutes before switching to the second solution, the subject was positioned into the magnet. Imaging, localization and B_0 field shimming were performed before infusion of the first solution had ended. When infusion of the second solution began, proton decoupled ^{13}C spectra were acquired (one spectrum every 8.5 minutes) for the duration of the second infusion. A total of 60–80 g of $[\text{U-}^{13}\text{C}_6]\text{glucose}$ and $[\text{2-}^{13}\text{C}]\text{glucose}$ was typically used for a full time course study. In vivo ^{13}C MRS spectra were acquired using the GE FID CSI pulse sequence without the phase encoding gradients. A 500 μs rectangular pulse was used to create a nominal flip angle of approximately 45° . TR was four seconds with a data sampling time of 205 ms (SW = 5 kHz, number of data points = 1024, and NS = 128). FID data were zero-filled to 16 K and processed using exponential line

broadening (LB = -2.0 Hz) and Gaussian line broadening (GB = 0.3) before Fourier transform. After Fourier transform zero and first order phase corrections were made.

RESULTS

The spectral result of [U-¹³C]₆glucose only infusion obtained from four subjects is shown in Fig. 1. The spectra were acquired during the time intervals after the beginning of [U-¹³C]₆glucose infusion given in Fig. 1. They showed that glutamate C5 singlet signal at 182.0 ppm, if any, was below noise level, indicating negligible spectral interference from hepatic gluconeogenesis. This result is compatible with our previous observation that found no detectable doublet signals when only [2-¹³C]glucose was infused (8, 9). The upfield lactate C1 doublet resonates at 182.4 ppm at 3 Tesla, close to the glutamate C5 singlet at 182.0 ppm. With optimized B₀ field homogeneity it is clear from Fig. 1 that lactate C1 does not cause significant spectral interference.

Figure 2 shows a time course of spectra of isotopomer turnover using the first infusion protocol ([U-¹³C]₆glucose was infused first and [2-¹³C]glucose was infused second). Each spectrum was acquired in 8.5 minutes. Glutamate C5 and C1 originating from [U-¹³C]₆glucose appeared as a doublet, while glutamate C5 and C1 originating from [2-¹³C]glucose appeared as a singlet at 182.0 ppm. Similar spectral patterns were also obtained for glutamine C5 and aspartate C4. Because the [U-¹³C]₆glucose infusion ended when data acquisition began, the doublet signals of detected metabolites decreased in the time course spectra. In contrast, the singlet signals of the metabolites increased due to the [2-¹³C]glucose infusion during data acquisition.

Figure 3 shows the sum of the spectra displayed in Figure 2, and provides more distinguishable isotopomer patterns. The doublet of glutamate C5 (centered at 182.0 ppm) originating from [U-¹³C]₆glucose was clearly identified with a ¹³C-¹³C homonuclear J coupling constant (J₄₅) of 50.5 Hz. Similarly, the doublet of glutamate C1 (centered at 175.4 ppm) was also distinctly observed with a J coupling constant (J₁₂) of 53.0 Hz. Because the chemical shifts of glutamine C5 (178.5 ppm) and aspartate C4 (178.3 ppm) are very close, their doublets partially overlap. From the summed in vivo ¹³C spectra obtained from the infusion of [U-¹³C]₆glucose only (Fig. 1), the J coupling constant was estimated to be 47.2 Hz for glutamine C5 (J₄₅), and 49.4 Hz for aspartate C4 (J₃₄). The measured coupling constants for these doublets are consistent with previous reports (7, 11). Doublets of glutamine C1 (centered at 174.9 ppm) and aspartate C1 (centered at 175.0 ppm) largely overlapped because their chemical shift differences are only 0.1 ppm apart. As a result, they appeared as one doublet with a broader linewidth. A small doublet centered at 183.3 ppm was assigned to lactate C1. The chemical shift assignment of the metabolites in our measurements is based on references (20, 21). Overall, the large homonuclear ¹³C-¹³C J coupling constants between an aliphatic carbon and a carboxylic or an amide carbon provided a relatively large separation between two peaks of a doublet, which allowed a clear separation of a singlet peak positioned between two doublet peaks.

The time course spectra and the summed spectrum obtained using the second protocol ([2-¹³C]glucose was infused first and [U-¹³C]₆glucose was infused second) are shown in Figures 4 and 5, respectively. Because the order of the infused substrates was reversed, the dynamic pattern of doublets and singlets were also reversed; thus, a decrease of the glutamate C5 singlet, and an increase of the glutamate C5 doublet were observed. Similar singlet and doublet resonances were observed in the summed spectrum of both infusion protocols (Figs. 3 and 5). The J coupling constants measured from Fig. 5 agree with those from Fig. 3. Note that in the bottom trace of Fig. 4 ([2-¹³C]glucose baseline) there are no detectable doublets above the noise level. In both protocols, the singlet resonances

originating from the infusion of $[2-^{13}\text{C}]$ glucose were in excellent agreement with those observed in our previous human brain studies that infused only $[2-^{13}\text{C}]$ glucose (9).

DISCUSSION

The present study distinctly detected the metabolism of two different ^{13}C labeled substrates in the carboxylic/amide spectral region with sequential administration of $[2-^{13}\text{C}]$ glucose and $[\text{U}-^{13}\text{C}_6]$ glucose. Singlet signals of brain metabolites originating from singly labeled $[2-^{13}\text{C}]$ glucose were clearly differentiated from doublet signals of the metabolites originating from doubly labeled $[\text{U}-^{13}\text{C}_6]$ glucose.

Several advantages are associated with this approach. First, the resonances of the carboxylic/amide carbons (174–183 ppm) of interest do not overlap with scalp lipid signals which resonate at the 172–173 ppm region. The metabolite signals are therefore without contamination from scalp lipids. Our previous study has shown that there are no other detectable resonances in this region (9). Second, carboxylic and amide carbon are located at ends of the carbon skeleton and can only form singlets or doublets. This significantly simplifies the spectral pattern of carboxylic and amide carbons. Previous studies demonstrated that when multiple aliphatic carbons were labeled with an infusion of either $[\text{U}-^{13}\text{C}_6]$ glucose (7) or $[1,2-^{13}\text{C}]$ acetate (22), the spectra of the aliphatic carbons of glutamate C4 (or glutamine C4) consist of a singlet and a doublet of doublets due to homonuclear couplings between C4 and C5, and between C4 and C3 with both substrates contributing to the glutamate C3–C4 signal. In contrast, for carboxylic or amide carbons, only one doublet can be formed which leads to a significant simplification. Splitting of the doublet peaks of carboxylic and amide carbons (~ 50 Hz) is relatively large compared with the linewidth of singlets or the individual peak of doublets at 3 T. As noted previously (9), broadband decoupling of the carboxylic/amide carbons can be achieved using relatively low RF power deposition. Therefore, acquiring the spectrum of carboxylic/amide carbons at 3 T presents several useful advantages.

One of the main goals of this study was to access potential interference due to labeling of lactate and hepatic gluconeogenesis. In studies performed by infusing $[1-^{13}\text{C}]$ glucose significant labeling of lactate C3 at 21.0 ppm has been consistently detected (3). As highlighted by Figures 2 through 5, lactate C1 centered at 183.3 ppm was observed. The glutamate C5 singlet (182.0 ppm) partially overlapped with an upfield peak of the lactate C1 doublet (at 182.4 ppm with lactate J_{12} of 55 Hz). Since the downfield resonance line of the lactate C1 doublet is fully resolved and equal in magnitude to its upfield line, spectral interference with the glutamate C5 singlet can be removed. Using the same principle, the interference of lactate C1 singlet signal with the downfield line of the glutamate C5 doublet can also be removed accordingly. As shown by Figs 2 and 4, the glutamine C5 singlet and doublet (178.5 ppm) partially overlap with the aspartate C4 singlet and doublet (178.3 ppm), respectively, at 3 Tesla. This has implications for studies with two substrates of different molecules, for example, $[\text{U}-^{13}\text{C}_6]$ glucose and $[1-^{13}\text{C}]$ acetate. Optimization of B_0 field homogeneity can only partially resolve the two metabolites. Complete spectral resolution requires spectral fitting or lineshape deconvolution (23). Fig. 1, the $[\text{U}-^{13}\text{C}_6]$ glucose baseline in Fig. 2, as well as the $[2-^{13}\text{C}]$ glucose baseline in Fig. 4 demonstrated that there is no significant interference from hepatic gluconeogenesis for the double glucose substrate experiment reported here. Whether it remains so or not needs to be investigated when a different substrate is infused. Based on our animal studies that used glucose and a different substrate (acetate, or β -hydroxybutyrate or lactate) (17) significant interference from hepatic gluconeogenesis is unlikely.

The results of Figs 2 and 4 together with our previous animal study therefore suggest that it is feasible to simultaneously detect the ^{13}C labeling of brain amino acids by two different labeling pathways, using two substrates of either the same molecule with different labeling patterns or different molecules (18). As a proof of principle for applications to human subjects the current study investigated the combined use of $[\text{U-}^{13}\text{C}_6]\text{glucose}$ and $[2\text{-}^{13}\text{C}]\text{glucose}$. The same strategy would not have been possible had $[\text{U-}^{13}\text{C}_6]\text{glucose}$ and $[1\text{-}^{13}\text{C}]\text{glucose}$ were used for the labeling of the aliphatic region because of the extremely complicated isotopomer patterns there as demonstrated previously (7) as well as the fact that both substrates will contribute some of the same isotopomer signals (e.g., the glutamate C3-C4 doublets). In contrast, because of the clean separation between the different labeling pathways carried on by the carboxylic/amide carbons both the wash-in and washout time course data were unambiguously detected in Figs 2 and 4 using the sequential infusion method proposed here.

CONCLUSIONS

This study successfully demonstrated the feasibility of sequentially infusing two different substrates and detecting ^{13}C labeling of different metabolites originating from different substrates in the spectral region of carboxylic/amide carbons in human subjects. Detection of ^{13}C spectra of carboxylic/amide carbons has several advantages, including low RF power deposition and that the spectra are not contaminated by lipid signals and that only a singlet and a doublet can be formed for one carboxylic or amide carbon, which simplifies the isotopomer pattern. The large ^{13}C - ^{13}C homonuclear coupling constants for carboxylic/amide carbons produced unambiguous separation between singlets and doublets for the same carbon at 3 T. Although we tested this approach using two different ^{13}C labeled glucose infusates, we expect that the method can be used to simultaneously monitor ^{13}C turnover from doubly labeled $[\text{U-}^{13}\text{C}_6]\text{glucose}$ and one singly labeled substrate, such as $[1\text{-}^{13}\text{C}]\text{acetate}$, $[1\text{-}^{13}\text{C}]\text{lactate}$, or $[1,3\text{-}^{13}\text{C}_2]\beta\text{-hydroxybutyrate}$.

Highlights

- Feasibility of ^{13}C isotopomer MRS of human brain is demonstrated.
- $[\text{U-}^{13}\text{C}_6]\text{glucose}$ and $[2\text{-}^{13}\text{C}]\text{glucose}$ generates doublets and singlets of brain amino acids, respectively.
- Different ^{13}C labeling pathways are simultaneously detected in the human brain at 3 Tesla.

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Figure 1.

In vivo baseline ^{13}C spectra acquired from four subjects after infusion of $[\text{U-}^{13}\text{C}_6]\text{glucose}$. Each spectrum was acquired over 8.5 minutes (NS=128) during the time interval indicated on each spectrum after the beginning of $[\text{U-}^{13}\text{C}_6]\text{glucose}$ infusion. Spectra a and c were acquired from two subjects who received infusion of $[\text{U-}^{13}\text{C}_6]\text{glucose}$ only, and spectra b and d were acquired from the other two subjects who received $[\text{U-}^{13}\text{C}_6]\text{glucose}$ infusion followed by $[\text{2-}^{13}\text{C}]\text{glucose}$ infusion. There is no noticeable glutamate C5 singlet observed at 182.0 ppm in any of these spectra. The upfield lactate C1 doublet resonates at 182.4 ppm at 3 Tesla, close to the glutamate C5 singlet at 182.0 ppm. With optimized B_0 field homogeneity it is clear from Fig. 1 that lactate C1 does not cause spectral interference.

Figure 2.

Time course spectra of isotopomer turnover with the first infusion protocol. Acquisition began at the end of [U- $^{13}\text{C}_6$]glucose infusion and at the beginning of [2- ^{13}C]glucose infusion. Each spectrum was acquired over 8.5 minutes (NS=128). Parameters for data processing: LB = -2.0 and GB = 0.3. Glu5: glutamate C5, Glu1: glutamate C1.

Figure 3.

Sum of the time course spectra in Figure 2. Isotopomer patterns were identified for the following ^{13}C labeled carbons: glutamate C5 and C1, glutamine C5 and C1, aspartate C4 and C1, and lactate C1 doublet. The upfield peak of an NAA C5 doublet was also observed. Glu5: glutamate C5, Glu1: glutamate C1, Gln5: glutamine C5, Gln1: glutamine C1, Asp4: aspartate C4, Asp1: aspartate C1. NAA5: N-acetyl-aspartate C5.

Figure 4.

Time course spectra of isotopomer turnover with the second infusion protocol. Acquisition began at the end of [2- ^{13}C]glucose infusion and the beginning of [U- $^{13}\text{C}_6$]glucose infusion. Each spectrum was acquired over 8.5 minutes (NS=128). Parameters for data processing: LB = -2.0 and GB = 0.3. Glu5: glutamate C5, Glu1: glutamate C1.

Figure 5.

Sum of the time course spectra in Figure 4. Glu5: glutamate C5, Glu1: glutamate C1, Gln5: glutamine C5, Gln1: glutamine C1, Asp4: aspartate C4, Asp1: aspartate C1. NAA5: Nacetyl-aspartate C5.