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Direct, noninvasive measurement of brain glycogen metabolism in humans

Gülin Öz [a], Pierre-Gilles Henry [a], Elizabeth R. Seaquist [b], Rolf Gruetter [a,*]

[a] *Department of Radiology, Center for MR Research, University of Minnesota, 2021 6th St. S.E., Minneapolis, MN 55455, USA*
[b] *Department of Medicine, University of Minnesota, Minneapolis, MN, USA*

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Abstract

The concentration and metabolism of the primary carbohydrate store in the brain, glycogen, is unknown in the conscious human brain. This study reports the first direct detection and measurement of glycogen metabolism in the human brain, which was achieved using localized ^{13}C NMR spectroscopy. To enhance the NMR signal, the isotopic enrichment of the glucosyl moieties was increased by administration of 80 g of 99% enriched [1-^{13}C]glucose in four subjects. 3 h after the start of the label administration, the ^{13}C NMR signal of brain glycogen C1 was detected (0.36 ± 0.07 μmol/g, mean ± S.D., $n = 4$). Based on the rate of ^{13}C label incorporation into glycogen and the isotopic enrichment of plasma glucose, the flux through glycogen synthase was estimated at 0.17 ± 0.05 μmol/(g h). This study establishes that brain glycogen can be measured in humans and indicates that its metabolism is very slow in the conscious human. The noninvasive detection of human brain glycogen opens the prospect of understanding the role and function of this important energy reserve under various physiological and pathophysiological conditions.

Keywords: Brain glycogen; Astrocytes; Neurotransmission

1. Introduction

Glycogen is the main storage form of glucose in mammals and is found in high quantities in the liver and muscle and, to a lesser degree, in the heart and kidney. Albeit at much lower concentrations, the brain also contains glycogen, which is primarily located in astrocytes (Wiesinger et al., 1997). Although accepted as the principal energy reserve in the CNS by many (Dringen et al., 1993; Swanson, 1992; Watanabe and Passonneau, 1973), the role of glycogen in the brain is largely unknown.

Studies in astrocyte cultures have shown that the cellular glycogen content is regulated by the concentration of glucose in the culture medium. Thus, glycogen was synthesized when glucose was abundant (Cummins et al., 1983) and degraded under glucose deprivation (Dringen and Hamprecht, 1992). This regulation is thought to result in a neuroprotective role for astrocytic glycogen under glucose deprivation, which was observed both in mixed cultures (Swanson and Choi, 1993) and the rat optic nerve (Wender et al., 2000). Glycogen may also support axonal function under normal physiological conditions by responding to sudden increases in energy demand during neurotransmission (Swanson et al., 1992; Wender et al., 2000). Several lines of evidence suggest that this is accomplished by breaking down glycogen to lactate, which is then transferred to neurons as fuel (Dringen et al., 1993; Wender et al., 2000). Interestingly, glycogen has been observed to accumulate after hypoglycemia, ischemia (Folbergrova et al., 1996), brain injury (Guth and Watson, 1968; Shimizu and Hamuro, 1958) and cell swelling (Dombro et al., 2000). Several studies suggested the involvement of brain glycogen in sleep cycle regulation (Kong et al., 2002; Petit et al., 2002). These changes in glycogen content may be brought about by a number of factors (Wiesinger et al., 1997) including hormones (Dringen and Hamprecht, 1992) and neurotransmitters (Hamai et al., 1999; Sorg and Magistretti, 1992; Swanson et al., 1990).

Apart from these studies in cell cultures and animal models, our understanding of glycogen metabolism in the human brain has been hampered by the lack of a noninvasive method to detect glycogen in the conscious human. To date, the few isolated studies in humans have been limited to biopsies (Castejon et al., 2002; Lowry et al., 1983) and postmortem samples (Gertz et al., 1985). The biopsy samples were collected under conditions of surgery, therefore

* Corresponding author. Tel.: +1-612-625-6582; fax: +1-612-626-2004.
E-mail address: gruetter@cmrr.umn.edu (R. Gruetter).

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such investigations are not widely applicable, in addition to the fact that the results may be influenced by anesthesia. The postmortem studies, on the other hand, bear another challenge due to the well-known rapid postmortem breakdown of glycogen (Choi et al., 1999; Lowry et al., 1964; Nelson et al., 1984; Swanson et al., 1989). A recent study underlined the challenges associated with biochemical extraction of brain glycogen and suggested that previous studies may have underestimated its levels in resting rodent brain (Cruz and Dienel, 2002). These issues and the important role of glycogen in cerebral carbohydrate metabolism suggested by the cell culture and animal studies render a noninvasive method to detect glycogen in the conscious human brain essential.

Nuclear magnetic resonance spectroscopy (NMR) enables noninvasive studies of neurochemistry in vivo (Gruetter, 2002) and has been instrumental in furthering the understanding of liver and muscle glycogen metabolism (see, e.g. Casey et al., 2000; Kunnecke and Seelig, 1991; Shulman et al., 1990; Shulman and Rothman, 2001; Van Den Bergh et al., 2000 and references therein). ^{13}C NMR has been shown to provide accurate measurements of glycogen concentrations based on the C1 resonance at 100.5 ppm that is well resolved from the C1 resonances of α- and β-glucose (Gruetter et al., 1991, 1994a). In contrast to other tissues, however, the glycogen concentrations reported in the mammalian brain range from 2 to 5 μmol/g (Choi et al., 1999 and references therein), which precludes detection of natural abundance ^{13}C glycogen signals from individual subjects. Therefore, transfer of label from infused ^{13}C glucose to glycogen is necessary in order to increase the ^{13}C isotopic enrichment and hence the sensitivity of the experiment. Additionally, when measuring brain glycogen by NMR, any contribution to the signal from the 10-fold more concentrated glycogen in the superficial muscle tissue needs to be eliminated. One preliminary study reported evidence for a glycogen signal detected from the head of a subject following subtraction of a pre-infusion spectrum in conjunction with use of surface dephasing gradients (Chhina et al., 2001). However, considering the potential contribution of non-cerebral muscle glycogen to the NMR, use of three-dimensional localization methods is of paramount importance in establishing the cerebral origin of the signal. While many localization methods have been described for in vivo NMR spectroscopy, localization of a molecule as large as glycogen (10^7–10^9 Da) with short longitudinal (T_1) and transverse (T_2) relaxation times presents particular challenges that were recently overcome by use of a non-echo method for the detection of brain glycogen in the rat (Choi et al., 1999, 2000). However, the localized measurement of human brain glycogen additionally requires that specific absorption rate (SAR) limits set forth by the FDA be observed, while maintaining optimal sensitivity.

The purpose of the present study was to establish the detection of the fully localized ^{13}C NMR signal of glycogen in the human brain and to estimate the rate of label incorporation into glycogen from [1-^{13}C]glucose.

2. Experimental procedures

2.1. Subjects

Four healthy males (age 41 ± 5.2 years, BMI 26.4 ± 1.13 kg/m^2, mean ± S.E.M.) were studied after giving informed consent using procedures approved by the Institutional Review Board: Human Subjects Committee. On the morning of the study, subjects reported between 6 and 7 a.m. to the General Clinical Research Center in the fasting state. An intravenous catheter placed antegrade in a forearm was used for infusion and a catheter placed retrograde in a foot was used for blood sampling. Venous blood was arterialized by heating the lower extremity bearing the catheter with preheated pads and water-soaked towels. A total of 80 g of [1-^{13}C]glucose (Isotec Inc., Miamisburg, OH, prepared as 20% (w/v) D-glucose in water with 99% isotopic enrichment) was administered into the arm vein in the form of 10–20 g bolus injections approximately every hour in order to raise and maintain the isotopic ^{13}C enrichment of blood glucose above 50% for the first 4.5 h of the study. To achieve this, plasma glucose concentrations were maintained above 100 mg/dl, which were measured immediately on a nearby glucose autoanalyzer (Beckman, Fullerton, CA) in blood samples taken from the foot vein every 10 min. Additional blood samples were frozen for the later determination of plasma insulin concentrations by a chemiluminescent assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA), as well as the isotopic enrichment of the plasma glucose by gas-chromatography–mass spectroscopy (GC–MS) as described previously (Gruetter et al., 2001).

2.2. MR spectroscopy

All measurements were performed on a 4 T, 90 cm bore magnet (Oxford Magnet Technology, Oxford, UK) with an INOVA console (Varian, Palo Alto, CA). Subjects were positioned supine on the patient bed with the occipital lobe just above the surface coil, which was a quadrature 14 cm ^{1}H surface coil combined with a 9 cm diameter linearly polarized ^{13}C coil (Adriany and Gruetter, 1997). Subjects wore earplugs to minimize gradient noise and were positioned in the coil holder using cushions to minimize head movement. In three of the four subjects, two sessions of NMR measurements were performed per study (the first session ∼2 h, the second ∼1 h in length), between which the subject was taken out of the magnet for a break. The position of the voxel relative to the plane of the ^{13}C coil (*y*-dimension in Fig. 1) was kept constant between sessions. This resulted in a maximum displacement of the position relative to the head of 5% of the voxel dimensions.

Following the positioning of the subject in the magnet and prior to the ^{13}C NMR measurements, the following adjustments were performed in each session: after tuning the coil, axial magnetic resonance imaging was acquired using a multi-slice RARE sequence (repetition time (TR) = 4 s,

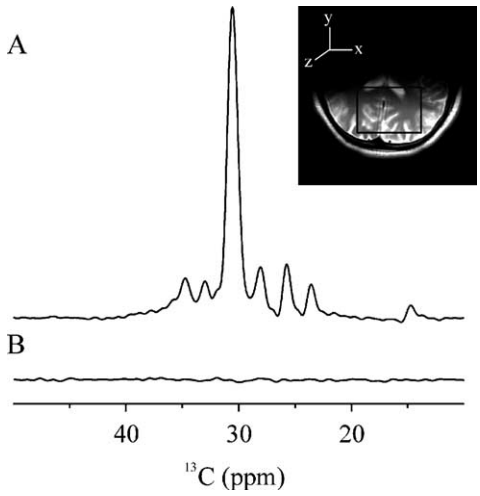

Fig. 1. Proton-decoupled ^{13}C NMR spectrum of the human head without (A) and with (B) localization (TR = 0.3 s, 32 scans). The voxel in the occipital lobe is shown on the transverse RARE image.

echo train length = 8, echo time (TE) = 60 ms, seven slices) to determine the voxel position for spectroscopy. All first- and second-order shim terms were adjusted using FAST(EST)MAP (Gruetter and Tkac, 2000), which resulted in water linewidths of 10–11 Hz in a 210 ml voxel.

The localization was achieved by outer volume suppression (OVS) in three dimensions combined with one-dimensional image-selected in vivo spectroscopy (ISIS) (Ordidge et al., 1986), similar to what was achieved in rat brain (Choi et al., 2000), following modification and optimization of the method for use on humans. Briefly, OVS was achieved with double-banded broadband hyperbolic secant pulses (6–8 ms) applied as nominal 90° pulses and the selection of the slice parallel to the ^{13}C coil was improved with one-dimensional ISIS. The time required for the localization was 35 ms. RF power requirements were calibrated in phantoms relative to the signal from a small sphere containing 99% enriched ^{13}C formic acid placed at the ^{13}C coil center. For this, double-chambered phantoms were employed and the signal from the outer chamber was suppressed. The power calibrations were checked each time after positioning the subject in the magnet and throughout the measurement by assessing the suppression of the extra-cerebral lipid signal. Excitation was achieved with a 2 ms adiabatic half-passage 90° pulse. Bi-level WALTZ-16 (Shaka et al., 1983) was used for nuclear Overhauser effect (NOE) generation during the relaxation delay (275 ms) and for decoupling during acquisition (25 ms). Data were acquired in blocks of 1024 scans (5 min), each of which was stored separately on disk prior to summation of five of these blocks for each data point. Processing of the spectra consisted of line broadening (30 Hz), zero-filling to 32k points, Fourier transformation and baseline correction.

Quantitation of the ^{13}C label in the C1 position of glycogen was done by the external referencing method as described previously (Choi et al., 1999; Gruetter et al., 1991). A phantom that contained 450 mM natural abundance oyster glycogen as measured using the amyloglucosidase assay (Gruetter et al., 1991), was prewarmed and spectra were acquired from the same voxel position using identical acquisition parameters as in vivo. The following equation that takes into account the 1.1% natural abundance of ^{13}C in the phantom was used to calculate the label concentration in brain glycogen:

$$[^{13}\text{C glycogen}] = \frac{I_{\text{Glyc}}(\text{brain}) \times I_{\text{FA}}(\text{phantom}) \times 450\,\text{mM} \times 0.011}{I_{\text{FA}}(\text{brain}) \times I_{\text{Glyc}}(\text{phantom})} \quad (1)$$

where I denotes integrated signal intensity, and the subscripts Glyc glycogen and FA formic acid. Determination of peak areas was accomplished using the built-in spectrometer software. Concentrations were converted to μmol/g based on a specific density of brain tissue of approximately 1 g/ml. The normalization of the glycogen integral to the formic acid signal was applied to correct for small differences in coil loading in vivo and in vitro (~2 dB).

3. Results

Following validation in double-chambered phantoms (not shown), the efficiency of the NMR method to localize signals in three dimensions was verified in the human brain. It has been shown that the strong lipid signal in natural abundance ^{13}C NMR spectra of the human head is from extra-cerebral tissue and normal human brain does not contain detectable triacyl-glycerol resonances (Gruetter et al., 1994b, 1996). Application of the localization method to the human head routinely suppressed the triacyl-glycerol resonances at 30.5 ppm more than 100-fold (n = 10 subjects without glucose infusion, Fig. 1), indicating minimization of extra-cerebral signals.

The low brain glycogen concentration, on the order of a few μmol/g, precluded natural abundance detection of brain glycogen in an individual subject, therefore, the glucosyl moieties in glycogen were enriched with ^{13}C label in four healthy human subjects by infusing [1-^{13}C]glucose. In all four subjects the C1 resonance of cerebral glycogen was unambiguously detected at 100.5 ppm in the localized ^{13}C NMR spectra, along with the C1 resonances of β- and α-glucose (Fig. 2).

Preliminary studies showed that the reliable detection of brain glycogen C1 signal was possible only after 2 h from the start of ^{13}C labeled glucose administration, which implied that metabolism of brain glycogen was slow. Therefore, NMR data acquisition was started 2.5 h after the start of glucose administration in three subjects and the change in the ^{13}C NMR signal of brain glycogen was followed over time. Plasma glucose (which ranged between 4.3 and 12.8 mM) and insulin concentrations (3–33 pmol/l) as well as the ^{13}C isotopic enrichment of plasma glucose were measured in

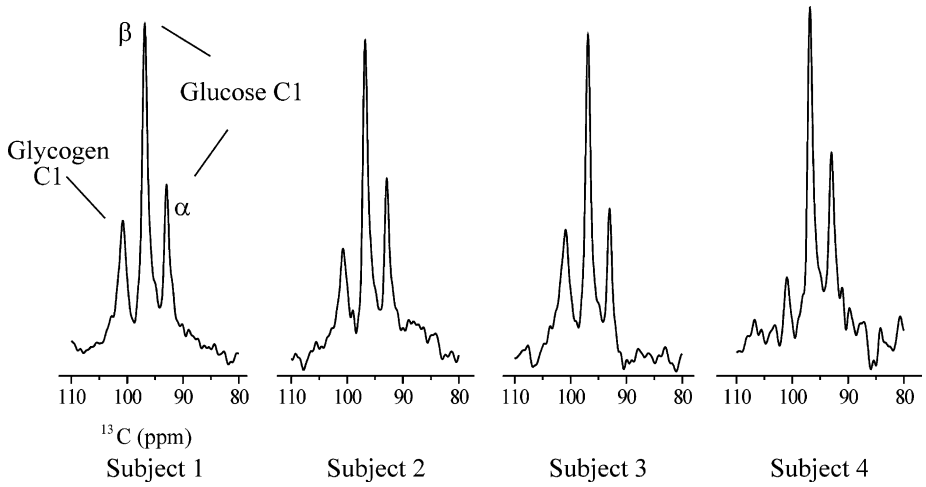

Fig. 2. Localized ^{13}C NMR spectra acquired from 210 ml volumes in four subjects. Spectra were averaged over 75 min in subjects 1, 2 and 3 and 65 min in subject 4 (TR = 0.3 s). Processing consisted of exponential multiplication corresponding to a line broadening of 30 Hz, zero-filling, fast Fourier transformation and phase correction. The spectra are shown without baseline correction. The spectrum of subject 4 was acquired earlier in the study and at lower isotopic enrichments compared to the other three.

all studies. The infusion protocol raised the ^{13}C isotopic enrichment of plasma glucose rapidly to 60–70%. After all of the ^{13}C label was administered, the isotopic enrichment slowly decreased to 35% in the last 2 h of the study. The glucose isotopic enrichment for one of the subjects is shown along with the NMR time course in Fig. 3. The intensities of the glucose resonances varied due to the changes in plasma and concomitant brain glucose concentrations, as well as due to changes in isotopic enrichment. The brain glycogen signal, on the other hand, showed a steady increase. In order to estimate the rate of label incorporation into glycogen C1, NMR spectra were averaged over 25 min and the amount of ^{13}C label was estimated at different time points based on the external reference method using Eq. (1) (Fig. 4). Linear regression of all data points between 2.5 and 4.5 h (measured in the first session from three subjects) resulted in a rate of label incorporation of 0.11 ± 0.04 μmol/(g h) ($R = 0.76$, $P = 0.01$) with a y-intercept not significantly different from 0. Performing a linear regression in the individual subjects through zero yielded rates of 0.11, 0.12 and 0.13 μmol/(g h) indicating very small inter-individual differences. Since the average isotopic enrichment of the precursor glucose over this period was 64%, the flux through the glycogen synthesis pathway was estimated at 0.17 ± 0.06 μmol/(g h).

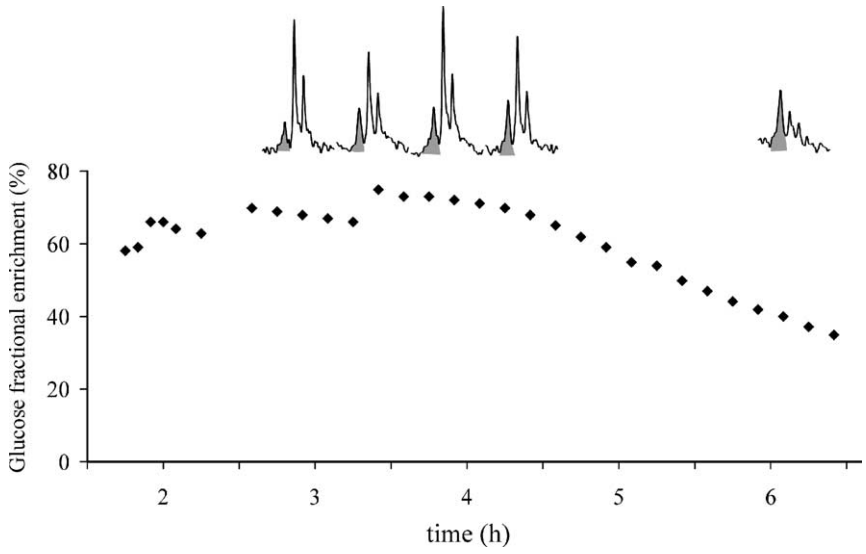

Fig. 3. The temporal changes in plasma glucose isotopic enrichment (y-axis) and brain glycogen signal in one study. Administration of labeled glucose was begun at time zero. NMR spectra averaged over 25 min are shown in the top trace at the time points they were acquired. The glycogen peak is highlighted by the shaded area in these spectra.

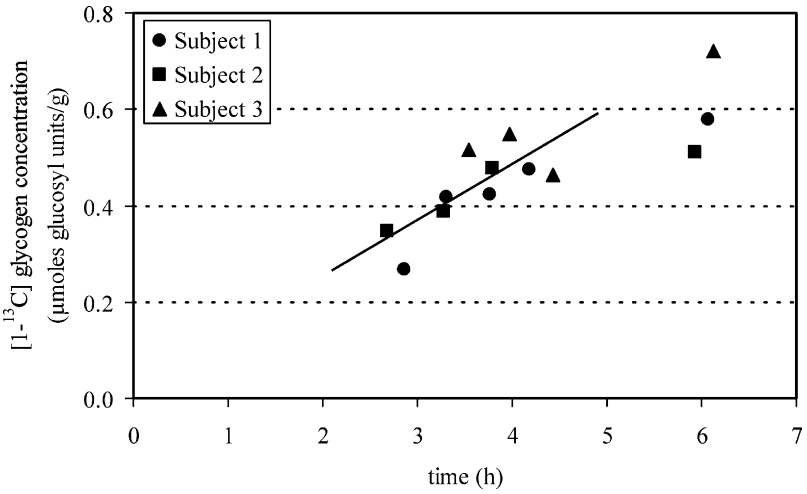

Fig. 4. Quantification of ^{13}C label (μmol/g) incorporation into glycogen C1 over time. Shown are the data from three different subjects, identified by individual symbols.

4. Discussion

In this paper the first noninvasive detection of brain glycogen metabolism in the conscious human is reported. The NMR signal of brain glycogen was measured after three-dimensional localization to minimize any potential contamination from the more concentrated muscle glycogen, which establishes the detection of cerebral glycogen in the conscious human brain. The accuracy of a method suitable for localization of the glycogen signal was previously tested in the rat brain (Choi et al., 1999, 2000). In the present study, similar NMR methods were used following extensive modification for human applications. The localization efficiency was thus validated in double-chambered phantoms mimicking the in vivo localization demands and in the human head. The 100-fold suppression of the lipid signal originating from outside the brain (Fig. 1) implied that contamination from a 10-fold more concentrated muscle glycogen would constitute a maximum of 10% of the glycogen signal. We therefore conclude that in the present study more than 90% of the glycogen signal was from the brain. An additional benefit of three-dimensional localization was that the signal was acquired from a well-defined volume. This ensured a reliable determination of ^{13}C label concentration by the external reference method, as judged from the highly reproducible rates of label incorporation (Fig. 4). The excellent reproducibility was in part due to a conservative time resolution of 25 min, however, the current sensitivity certainly would permit a temporal resolution of 10–15 min. Conversely, the sensitivity of the current experiment should allow reduction of the volume size from 210 to 140 ml, which is a spatial resolution that has been used in different, unrelated studies (Gruetter et al., 1994b; Shen et al., 1999).

The initial amount of ^{13}C labeled glycogen produced depends on the glycogen synthase flux and the isotopic enrichment of the metabolic precursor glucose-6-P. The isotopic enrichment of glucose-6-P in brain closely mimics that of plasma glucose, due to the lack of substantial activity of the gluconeogenic pathway. The isotopic enrichment of plasma glucose did not vary much during our NMR measurements (60–75% in the first session) resulting in a steady increase of the brain glycogen signal.

From the rate of label incorporation and the prevailing isotopic enrichment of plasma glucose, we estimated the glycogen synthase flux in the human brain at 0.17 μmol/(g h) in the period of 2.5–4.5 h after the start of glucose administration. This rate was approximately half that observed in rat brain after 2 h of glucose administration (Choi et al., 1999). It is interesting to note that metabolic rates in the conscious rat brain are typically two to three times faster than those in humans (Gruetter et al., 2001; Nakao et al., 2001). Independent of the precise value of the label turnover rate, the present study clearly demonstrates that metabolism of bulk brain glycogen is very slow. To what extent parts of the molecule might be subjected to accelerated turnover during, e.g. activation, however, remains to be determined.

We conclude that it is possible to study the metabolism of glycogen in the conscious human brain noninvasively using three-dimensional localization of the NMR signal. Furthermore, the synthesis and breakdown of brain glycogen can be followed at more specific locations and in smaller voxels. Whether the function of brain glycogen is to supply energy during neuronal activation (Swanson, 1992) or when energy supplies are limited, such as during anoxia and ischaemia (Castejon et al., 2002; Folbergrova et al., 1996; Swanson et al., 1989), studies to test these hypotheses in the human brain are urgently needed and the present study opens the prospect of achieving this goal. An important application of the localized spectroscopy of glycogen will be to examine the potential role of glycogen in hypoglycemia and in the mechanism that leads to hypoglycemia unawareness

following repeated episodes of insulin-induced hypoglycemia (Gruetter et al., 2000).

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