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Optimized [1-^{13}C]glucose infusion protocol for ^{13}C magnetic resonance spectroscopy at 3 Tesla of human brain glucose metabolism under euglycemic and hypoglycemic conditions

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Abstract

The effect of insulin-induced hypoglycemia on cerebral glucose metabolism is largely unknown. ^{13}C MRS is a unique tool to study cerebral glucose metabolism, but the concurrent requirement for [1-^{13}C]glucose administration limits its use under hypoglycemic conditions. To facilitate 13C MRS data analysis we designed separate [1-^{13}C]glucose infusion protocols for hyperinsulinemic euglycemic and hypoglycemic clamps in such a way that plasma isotopic enrichment of glucose was stable and comparable under both glycemic conditions. ^{13}C MR spectra were acquired with optimized ^{13}C MRS measurement techniques to obtain high quality ^{13}C MR spectra with these protocols.

Keywords

Hypoglycemia; ^{13}C; Magnetic Resonance Spectroscopy; Brain glucose metabolism

Introduction

Since glucose is of vital importance to preserve brain function, the human body takes great effort in avoiding hypoglycemia to ensure constant glucose supply to the brain. In people with type 1 diabetes, however, loss of endogenous insulin production and consequent insulin injection therapy may lead to erratic glucose fluctuations and a considerable risk of hypoglycemic episodes. Moreover, failure of glucose counterregulatory hormone or symptom responses, normally elicited at blood glucose levels below ~3.8 mmol/L, may increase the risk of deeper glucose reductions accompanied by declines in cognitive function (Cryer, Davis et

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Disclosure/conflict of interest

The authors have no conflicts of interest to disclose relevant to this paper.

al., 2003). Although the brain is thought to play a central role in the detection of hypoglycemia and the initiation of counterregulatory responses, the effect of hypoglycemia on brain glucose metabolism is largely unknown.

^{13}C MR spectroscopy is a unique tool to study glucose metabolism in the brain non-invasively. Because of the low natural abundance of the ^{13}C isotope (1.1%) metabolic conversions can be visualized after administering ^{13}C-enriched compounds by infusion or oral intake and then monitoring the passage of the isotope through different metabolic pathways. Since more than two decades ^{13}C-labeled glucose has been successfully applied for in vivo studies of cerebral glucose uptake and metabolic conversions, starting first in animals (Rothman, Behar et al., 1985) and later also in humans (Beckmann, Turkalj et al., 1991). Since then, studies perfomed with ^{13}C MRS or ^{1}H-observe ^{13}C-edited MRS in combination with ^{13}C-labeled compounds have provided novel information on important neurochemical processes (e.g. see de Graaf, Mason et al., 2003, Gruetter, Adriany et al., 2003, Garcia-Espinosa, Rodrigues et al., 2004).

To ensure optimal detection ^{13}C in the brain, experiments using ^{13}C-glucose for isotopic enrichment have been commonly employed after administration of high doses of the label and under hyperglycemic conditions. As the blood-brain barrier usually limits the capacity for glucose uptake in the brain, these studies have greatly advanced our understanding of in vivo glucose metabolism by the intact human brain. However, direct MRS assessment of brain metabolism under hypoglycemic conditions is complicated. The effect of hypoglycemia on cerebral glucose metabolism has been studied by ^{13}C MR spectroscopy in brain slices (Bachelard, Badar-Goffer et al., 1993) and recently in animals (Jiang, Herzog et al., 2009). ^{13}C MRS of the human brain was applied during hypoglycemia to monitor glycogen utilization and formation (Oz, Kumar et al., 2009) and monocarboxylic acid transport (Mason, Petersen et al., 2006), but none of these studies used simultaneous infusion of ^{13}C labeled glucose to follow its metabolism.

In this study, we tested the feasibility to study human brain glucose metabolism directly by ^{13}C MRS at 3 Tesla under euglycemic and hypoglycemic conditions after 1) improving SNR for reliable measurements at lower plasma ^{13}C enrichment levels and 2) by designing a ^{13}C-enriched glucose infusion protocol that resulted in plasma glucose ^{13}C enrichment of sufficient magnitude to perform data analysis of ^{13}C signals from glucose derived metabolites. We specifically aimed for stable and broadly similar plasma glucose ^{13}C enrichment levels under both glycemic conditions, so as to facilitate ^{13}C MRS dynamic data analyses and making the results of both experiments directly comparable. Previously we reported on the technical adjustments to the MR coil design (Klomp, Renema et al., 2006) and the pulse sequence (Klomp, Kentgens et al., 2008). Here we present the infusion protocols,

Materials and methods

Subjects

Written informed consent was obtained from 7 healthy volunteers (female, mean age±sd, 21.8 ±2.0 years) to participate in one of the protocols of the study. The study was approved by the institutional review board of the Radboud University Nijmegen Medical Centre. All subjects came to the Magnetic Resonance facility at 8.15 in the morning in fasting condition. The brachial artery of the non-dominant arm was cannulated (Angiocath 20-gauge, Beckton Dickinson, Sandy, UT) under local anaesthesia (Xylocaine 2%) for frequent blood sampling. An intravenous catheter was inserted into an antecubital vein of the contralateral arm for infusion of insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) and [1-^{13}C]glucose 20% (w/w) (Campro, Veenendaal, The Netherlands) at the required enrichtment percentage. After an equilibration period of 30 minutes, subjects were positioned supine on the MR scanner bed

with the head inside the dedicated RF coil, and then placed headfirst inside the bore of the magnet.

Glucose infusion protocols

All volunteers were subjected to one of two different hyperinsulinemic glucose clamp protocols which included a eu- and a hypoglycemic period, as a modification to a previously described method (de Galan, Tack et al., 2002). All clamps were initiated 30 minutes after the subject was placed into the scanner and baseline MRS data had been acquired.

Protocol 1 (n=4)—This protocol consisted of a hyperinsulinemic euglycemic-hypoglycemic clamp in which plasma glucose was sequentially clamped at 5.0 mmol/L and 3.0 mmol/L for 45 minutes each. Insulin was infused at a rate of 60 $mU.m^{-2}{\cdot}min^{-1}$ during the euglycemic phase, but increased to 120 $mU.m^{-2}{\cdot}min^{-1}$ upon initiation of the hypoglycemic phase. A bolus of 6 g [1-^{13}C]glucose with a fractional enrichment of 100% was infused over the first 10 minutes of the clamp, followed by variable infusion of 30% enriched [1-^{13}C]glucose during euglycemia. During hypoglycemia 100% enriched [1-^{13}C]glucose was used in one volunteer to maximize SNR in this phase. Later experiments were performed with 30% enriched [1-^{13}C]glucose during hypoglycemia. The [1-^{13}C]glucose infusion rate was adjusted by arterial plasma glucose levels measured at 5-minute intervals to maintain plasma glucose at the target level, while part of the sampled blood was stored for later determination of plasma glucose isotopic enrichment.

Protocol 2 (n=3)—This protocol consisted of hyperinsulinemic euglycemic and hypoglycemic clamp studies performed in random order, separated by four weeks. During both clamps, the insulin infusion rate was set at 120 $mU.m^{-2}{\cdot}min^{-1}$ for 10 minutes and then at 60 $mU.m^{-2}{\cdot}min^{-1}$ for the remainder of the experiments. Except for the similar bolus of 100% labeled [1-^{13}C]glucose at the beginning of each clamp, [1-^{13}C]glucose with 40% fractional enrichment was used for the euglycemic clamp and [1-^{13}C]glucose with 50% fractional enrichment was used for the hypoglycemic clamp, aiming at similar plasma glucose ^{13}C enrichment levels.

MR Spectroscopy

All studies were performed on a 3T MR system (Magnetom Trio, Siemens, Erlangen, Germany). In protocol 1 a combination of a ^{1}H circularly polarized and a ^{13}C linearly polarized surface coil (Adriany and Gruetter, 1997) was used for the first volunteer. Acquisition was performed with a basic ^{13}C-FID sequence with outer-volume suppression (OVS). The other experiments were performed with a new coil design consisting of a ^{1}H volume coil and a circularly polarized ^{13}C surface coil (Klomp, Renema et al., 2006), while the acquisition sequence was optimized from a ^{13}C-FID with OVS to a sequence with ^{1}H to ^{13}C cross-polarization to increase SNR. In protocol 2 the optimized coil-setup was used together with a more advanced DEPT sequence for ^{1}H to ^{13}C polarization transfer combined with ^{1}H-ISIS localization (Klomp, Kentgens et al., 2008). Adiabatic ^{13}C pulses were used in all sequences to ensure homogeneous excitation using the ^{13}C coils, as well as WALTZ-16 proton decoupling (Shaka, Keeler et al., 1983) to simplify the spectra and enhance SNR. In all experiments one spectrum consisted of 72 repetitions of 2 seconds allowing a time resolution of 2.5 minutes. Eight reference spectra were obtained before the start of [1-^{13}C]glucose infusion, to correct dynamic spectra for natural abundance ^{13}C signal and residual lipid signals. In the unlocalized experiments all signal from the occipital brain tissue was acquired, while in the localized ISIS-DEPT experiments a voxel of ~125 ml was placed in this region.

Data analysis

^{13}C MRS data processing—The reference spectra were averaged and subtracted from the dynamic spectra to remove all natural abundance ^{13}C signals including those originating from lipids. To enhance SNR the corrected spectra were added in running averages of 15 min. The spectra were fitted in jMRUI (Naressi, Couturier et al., 2001) with the AMARES algorithm (Vanhamme, van den Boogaart A. et al., 1997). The SNR of the spectra was defined as the ratio of the highest signal intensity (Glutamate-C4) in a running average spectrum at t=100 min divided by the standard deviation (SD) of the noise in the region 100–120 ppm (SNR= Glutamate-C4/SDnoise).

Blood samples—Arterial blood was sampled for all measurements. Plasma glucose was measured in duplicate by the glucose oxidation method (Beckmann Glucose Analyzer II, Fullerton, CA). All other blood samples were immediately stored on ice for 30 minutes, followed by centrifugation for 15 minutes at 3000 rpm. The supernatant was stored at −80°C to obtain plasma glucose isotopic ^{13}C enrichment at a different day using high resolution ^{1}H-NMR at 500 MHz.

All data are expressed as means ± standard deviation (sd).

Results

In all experiments, plasma glucose levels peaked 10–15 minutes after initiation of the clamps at 7–8 mmol/L. In protocol 1 glucose levels stabilized first at 5.5±0.2 mmol/L, followed by a hypoglycemic phase in which glucose levels stabilized at 2.7±0.2 (figure 1a). In protocol 2 glucose values were stable at 5.2±0.1 and 2.9±0.1 mmol/L for the euglycemic and hypoglycemic phase, respectively (figure 1b).

Plasma glucose ^{13}C enrichment also peaked early during the clamps (figure 1c/d), and subsequently remained stable during the euglycemic phases of all protocols. During hypoglycemia, fractional enrichment increased steeply when 100% enriched [1-^{13}C]glucose was infused and decreased when the 30% enriched [1-^{13}C]glucose was continued, despite doubling of the insulin infusion rate (protocol 1, figure 1c). The use of different label percentages for the separate euglycemic and hypoglycemic clamp experiments (protocol 2, figure 1d) resulted in stable and comparable plasma isotopic enrichment of [1-^{13}C]glucose during euglycemia (36.0±0.6%) and hypoglycemia (34.6±1.4%).

During all experiments ^{13}C MR spectra were acquired continuously, clearly showing signals of glucose and several of its downstream metabolites. The increase in signals commonly observed in time-series of spectra obtained under hyperglycemic conditions were also observed under euglycemic and hypoglycemic conditions (figure 2a). The SNR of the spectra was calculated as 41.3±7.4 during euglycemia and 33.8±10.3 during hypoglycemia. The course of label incorporation into various glucose metabolic compounds was evaluated from these spectra. The data showed steep increases of label incorporation into glutamate-C4 during the first 30 minutes followed by a more gradual increase for the remainder of the experiments. The experiment in which 100% [1-^{13}C]glucose was used during the hypoglycemic phase of the clamp showed ongoing label incorporation of all metabolites during hypoglycemia, indicating continuing glucose uptake and metabolism (figure 2b).

Discussion

This study presents a [1-^{13}C]glucose infusion protocol that together with enhanced ^{13}C MRS sensitivity enables the use of ^{13}C MRS at 3T for investigating brain glucose metabolism under in vivo euglycemic and hypoglycemic conditions. To do so, we optimized MRS hardware and

sequences and tested various infusion protocols. Our final protocol, consisting of euglycemic and hypoglycemic clamps performed on separate days and with the use of different label percentages for [1-^{13}C]glucose, resulted in comparable and stable plasma ^{13}C enrichment of sufficient magnitude over time, while maintaining stable plasma glucose values. The spectra were of good quality for the assessment of ^{13}C-labeled derivates of glucose. [KCC1]

Several factors potentially limit the use of ^{13}C MRS under eu- and hypoglycemic conditions for dynamic studies of glucose metabolism in the brain. The low sensitivity of ^{13}C MRS requires infusion of large amounts of ^{13}C-enriched glucose, whereas activated glucose counterregulation increases endogenous glucose production during hypoglycemia, thus reducing the requirement of exogenous glucose and therefore of [1-^{13}C]glucose. It was not feasible to perform eu- and hypoglycemic clamps consecutively in one experiment for a number of reasons. First, the 'history' of ^{13}C label at the start of the hypoglycemic period complicates the already challenging data analysis. Secondly, such experiments would take considerably longer for plasma isotopic enrichment to stabilize during hypoglycemia, if at all possible considering subject discomfort. These issues were overcome by performing the clamps on separate occasions, by using a bolus of 100% enriched [1-^{13}C]glucose to rapidly obtain target plasma enrichment levels, and by using higher [1-^{13}C]glucose label percentages for hypoglycemic experiments. Glucose ^{13}C enrichment values thus obtained were stable and of equivalent magnitude, which makes current results directly comparable and may aid in future data analysis. However, this approach assumes little intra-individual variation in basal neurometabolic rate over time, which may not always be the case. Future studies will therefore require careful randomization.

Performing ^{13}C MRS of the brain under euglycemic conditions may prove valuable to study in vivo brain glucose metabolism in situations where the assumption of limited glucose transport over the blood-brain barrier is not valid, such as in brain tumors. The successful application of ^{13}C MRS of the brain under hypoglycemic conditions creates new opportunities to investigate the effect of hypoglycemia on cerebral glucose metabolism beyond the blood-brain barrier directly. The use of [1-^{13}C]glucose at 40–50% isotopic enrichment resulted in sufficient SNR for volumes of ~125 ml, at costs that are much lower than would have been the case with higher isotopic enrichment or with the use of [1,6-^{13}C]glucose (de Graaf, Mason et al., 2003). Potentially, however, the use of higher isotopic enrichment would enable measurements in smaller voxels, so that information of specific parts of the brain, for example in the glucose sensing region of the hypothalamus, can be obtained.

In conclusion, we developed a ^{13}C glucose infusion protocol that enabled high quality ^{13}C MRS under both euglycemic and hypoglycemic conditions. Future work will need to address the dynamics in the level of several metabolites by modeling.

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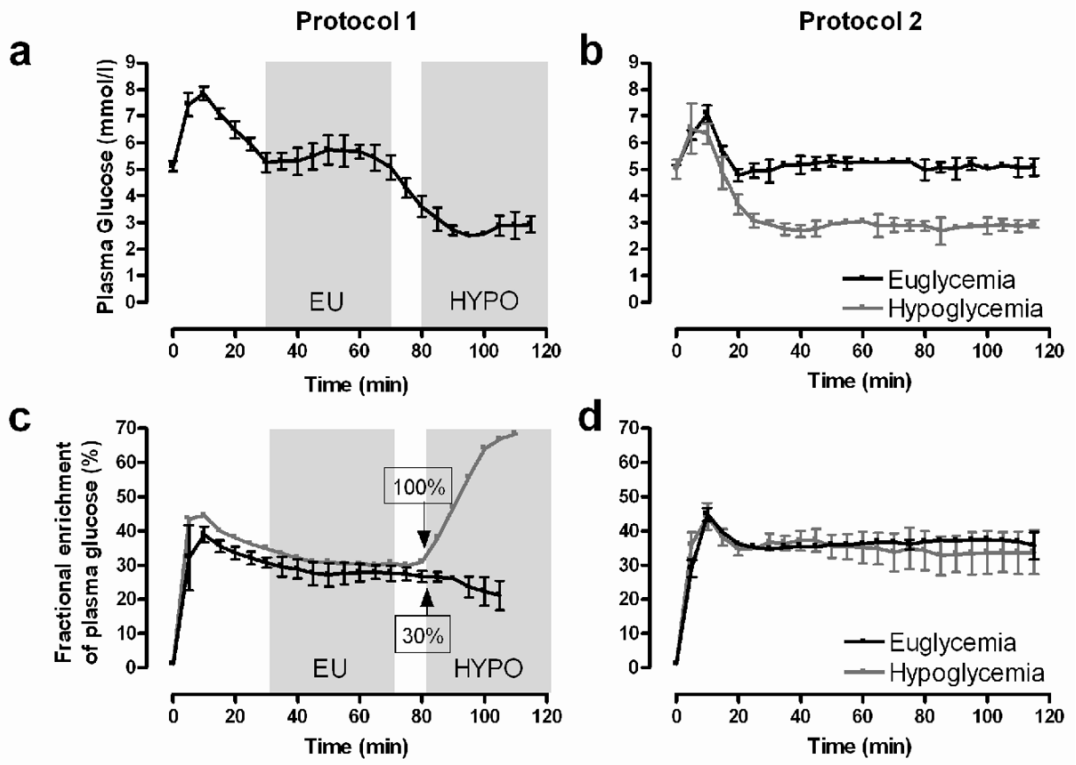

Figure 1.
Plasma glucose levels of protocol 1 (panel a) and protocol 2 (panel b), and corresponding fractional ^{13}C isotopic enrichment of plasma glucose (panels c and d). Data from 1 subject receiving 100% enriched [1-^{13}C]glucose during hypoglycemia (gray) is separated from the results of the other 3 subjects who received 30% enriched [1-^{13}C]glucose (black) (panel c). All data are expressed as mean ± sd.

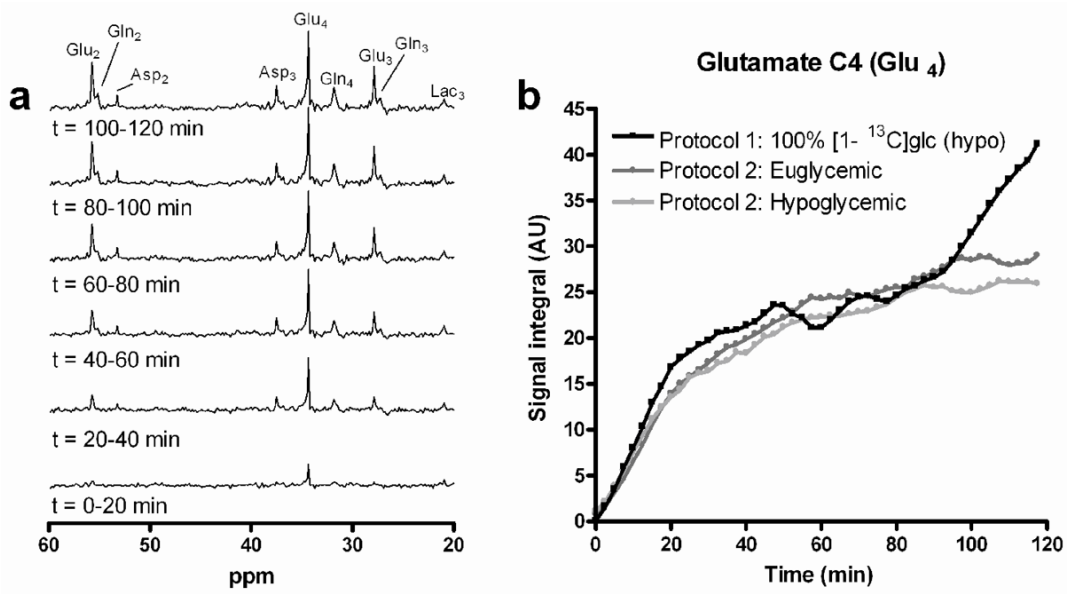

Figure 2.

a) Representative MR spectra of one subject under hypoglycemic condition (protocol 2), averaged over 20 minutes. b) Curves of Glutamate-C4 signal integral in protocol 2 as a function of time under hypoglycemia (light gray), under euglycemia (dark gray) and for protocol 1, where a euglycemic period with 30% enriched [1-^{13}C]glucose infusion was followed by a hypoglycemic period with infusion of 100% enriched [1-^{13}C]glucose (black).