13C MRS shows altered cerebral glucose metabolism during acute mild hypoglycemia in humans

K. van de Ven[1], M. van der Graaf[1], B. de Galan[2], C. Tack[2], and A. Heerschap[1]

[1]Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [2]General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands

Introduction

Hypoglycemia is an unwanted side-effect of insulin treatment in people with diabetes that is potentially harmful for brain function. To prevent cerebral damage, hypoglycemia usually elicits typical warning symptoms and counterregulatory hormone responses, so that carbohydrates can be ingested to restore normal glucose levels [1,2]. It is unknown to which extent mild acute hypoglycemia affects glucose metabolism in the human brain. In vivo ^{13}C MRS is a unique tool to assess cerebral glucose metabolism, but has predominantly been used under hyperglycemic conditions in humans.

Aim: to investigate the effect of acute mild hypoglycemia on cerebral glucose metabolism in humans in vivo using ^{13}C- MRS.

Methods

Subjects: After an overnight fast, eight healthy volunteers (4 male/4 female, 23.2±2.5 yrs old) underwent two hyperinsulinemic (60 mU·m^{-2}·min^{-1}) glucose clamps. In random order, subjects were clamped at euglycemia on one day and at hypoglycemia on another day. Approval of the local ethics committee was obtained and all volunteers gave written informed consent.

Clamp conditions: Both experiments started with a continuous insulin infusion of 60 mU·m^{-2}·min^{-1}, followed 5 minutes thereafter by a bolus of 30 ml of 100% ^{13}C-1-glucose 20 % (w/w) solution infused over 10 minutes to increase plasma glucose ^{13}C enrichment. During the remainder of the experiments, plasma glucose levels were maintained for 2 hours at either ~5.0 mmol/l using 40% ^{13}C-1-glucose 20% (w/w) solution or at ~3.0 mmol/l using 50% ^{13}C-1-glucose 20% (w/w) solution. Arterial blood was sampled every 5 min to determine plasma glucose levels and ^{13}C-1-glucose isotopic enrichment (using high resolution ^{1}H-NMR).

^{13}C-MRS experiments: ^{13}C-MRS experiments were performed with an ISIS-DEPT sequence with ^{1}H decoupling (WALTZ-16), which combines ^{1}H-ISIS localization with ^{1}H-^{13}C polarization transfer. A 45° alpha pulse was used to simultaneously observe CH, CH_2 and CH_3 ^{13}C-MR signals. ^{13}C-MRS acquisition (72 scans, TR=2s, duration=2.5 min) of a voxel of ~125 ml in the occipital brain tissue was started 20 min before clamping to obtain 8 reference spectra, and continued throughout the entire clamp (± 2 h). All experiments were performed at 3T with an optimized volume coil for ^{1}H with a CP surface coil insert for ^{13}C [3].

Post-processing and quantification: The FIDs of 8 reference spectra were averaged and subtracted from all FIDs to remove baseline distortions due to residual lipid signals. To enhance SNR the FIDs were added in running averages of 15 min (6 spectra). These spectra were fitted in jMRUI with the AMARES algorithm. To quantify the spectra the natural abundance ^{13}C Myo-inositol signal was assumed to be equal to 6 mM [4]. In addition, the data were corrected with theoretical values for DEPT sequence (different intensities of CH, CH_2 and CH_3 signals) and a correction for the pulse profiles as measured in a phantom.

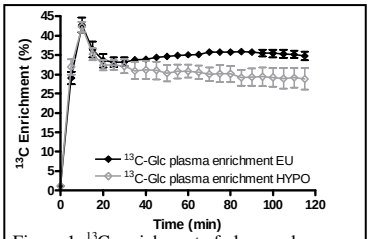

Figure 1: ^{13}C-enrichment of plasma glucose (mean± SEM).

Results

After an initial peak, plasma glucose levels were maintained at 5.18±0.06 mmol/l during euglycemia and at 2.95±0.21 mmol/l during hypoglycemia. ^{13}C-enrichment of plasma glucose also peaked initially, then stabilized at 35.11±0.85% during euglycemia and at 29.82±1.37% during hypoglycemia (fig. 1). Quality of the ^{13}C-MR spectra was sufficient to determine glutamate ($Glu_{2,3,4}$), glutamine ($Gln_{2,3,4}$), aspartate ($Asp_{3,4}$) and lactate (Lac_3) isotope signals (fig. 2). After correction for blood glucose isotope enrichment the increase of ^{13}C signals was determined (fig. 3). Under hypoglycemic conditions signals of Asp_3 and Glu_2 (fig. 3B/C) increased to higher levels than under euglycemic conditions, while signals of Asp_2 and Glu_3 (fig. 3A/D) were lower. Glu_4 reached lower signals during hypoglycemia compared to euglycemia. No differences were observed in lactate signals.

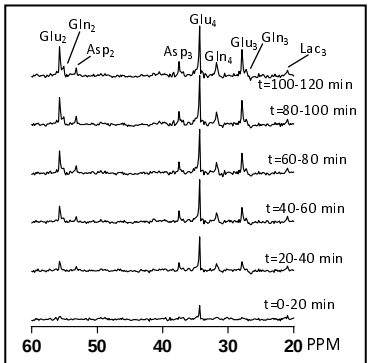

Figure 2: Representative spectra under hypoglycemic condition, averaged over 20 min

Discussion and conclusion

Successful ^{13}C-MRS measurements were performed under both euglycemic and hypoglycemic conditions in humans in vivo. Good spectral quality was obtained by an initial bolus of ^{13}C-1-glucose, which induces a high starting ^{13}C-enrichment of plasma glucose, followed by a stable ^{13}C-enrichment during the remainder of the study. We found differences in the formation of Asp_2, Asp_3, Glu_2 and Glu_3 as well as in Glu_4 between the two glycemic conditions. The opposing response of Asp_3 and Glu_2 versus Asp_2 and Glu_3 [5] suggests upregulated anaplerosis under hypoglycemic condition in order to replenish TCA-cycle intermediates.

We conclude that ^{13}C-MRS with ^{13}C-1-glucose infusion is feasible under hypoglycemic conditions and that hypoglycemia alters cerebral glucose metabolism.

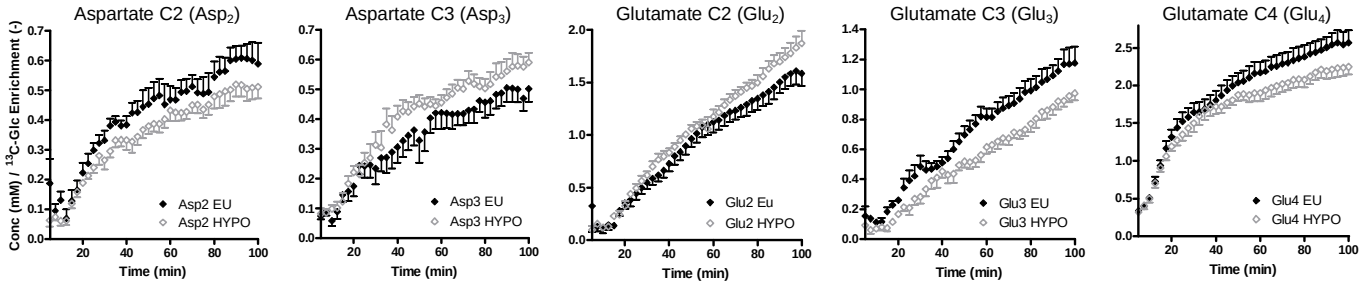

Figure 3: Concentration over ^{13}C plasma glucose enrichment (mean ±SEM) of A) Asp_2, B) Asp_3, C) Glu_2, D) Glu_3, E) Glu_4. Eu- (black) and hypoglycemia (gray)

References: 1. Cryer PE, et al., Diabetes Care. 2003; 26:1902-12; **2.** De Galan BE, et al., Neth J Med 2006; 64:269-79; **3.** Klomp DW, et al., MRM 2006, 55:271-8; **4.** Ross B, et al., NMR in Biomed 2003, 16:358-69; **5.** Gruetter R, et al., Am J Physiol Endocrinol Metab 2001; 281:100-12.

Acknowledgments: Dutch Diabetes Research Foundation (Grant 2004.00.012) and NIH (Grant R21 DK069881) for financial support.