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

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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 13C-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 13C-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·m2·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·m2·min−1, followed 5 minutes thereafter by a bolus of 30 ml of 100% 13C-1-glucose 20% (w/w) solution infused over 10 minutes to increase plasma glucose 13C enrichment. During the remainder of the experiments, plasma glucose levels were maintained for 2 hours at either ~5.0 mmol/l using 40% 13C-1-glucose 20% (w/w) solution or at ~3.0 mmol/l using 50% 13C-1-glucose 20% (w/w) solution. Arterial blood was sampled every 5 minutes to determine plasma glucose levels and 13C-1-glucose isotopic enrichment (using high resolution 1H-NMR).

13C-MRS experiments: 13C-MRS experiments were performed with an ISIS-DEPT sequence with H decoupling (WALTZ-16), which combines H-ISIS localization with 1H-13C polarization transfer. A 45° alpha pulse was used to simultaneously observe CH, CH2, and CH3 13C-MR signals. 13C-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 H with a CP surface coil insert for 13C [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 13C 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, CH2 and CH3 signals) and a correction for the pulse profiles as measured in a phantom.

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. 13C-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 13C-MRS spectra was sufficient to determine glutamate (Glu1,2), glutamine (Gln1,2), aspartate (Asp1,2) and lactate (Lac)1,2 isoencephalin signals (fig. 2). After correction for blood glucose isotope enrichment the increase of 13C signals was determined (fig. 3). Under hypoglycemic conditions signals of Asp and Glu reached Glu1,2 signals (fig. 3A/D) were lower. Glu reached lower signals during hypoglycemia compared to euglycemia. No differences were observed in lactate signals.

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

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


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