Multi-modality MR approach to assess metabolism and perfusion in the human brain during hypoglycemia

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Introduction: In type-1 diabetes, hypoglycemia unawareness is a serious complication of intensive insulin therapy. Hypoglycemia unawareness is thought to be the ultimate result of cerebral adaptation to recurrent hypoglycemic events. Despite possible roles for brain glycogen **[1]**, blood flow and glucose transport **[2]**, the underlying mechanism still needs to be elucidated. So far, little is known about the effect of hypoglycemia on cerebral glucose metabolism vis-à-vis glucose delivery and general energy demand. Magnetic Resonance (MR) provides several non-invasive methods to obtain complementary information on relevant processes, i.e. 13C MRS to follow uptake and metabolism of 13C-labeled glucose, 1H MRS to determine local glucose concentrations, 31P MRS to assess high-energy phosphate compounds and pH, and arterial spin labelling (ASL) to measure blood flow. The **aim** of this feasibility study was to realize and test these MR methods on a 3T MR system for the assessment of local changes in glucose uptake and (energy) metabolism in the human brain under hypoglycemic conditions.

Methods: Ten healthy volunteers (21.9 ± 1.6 yrs of age) underwent a hyperinsulinemic euglycemic-hypoglycemic glucose clamp **[3]** (with 13C-1-glucose in case of 13C MRS) inside the bore of a 3T Siemens Trio MR spectrometer. Plasma glucose was clamped at 5-6 mM (euglycemic phase) for about 60 min., allowed to fall to ~ 3 mM over 20 minutes, and maintained at that hypoglycemic level for about 60 min. Arterial blood was sampled every 5 min. to determine plasma glucose levels and, in case of 13C MRS, 13C-1-glucose isotopic enrichment (by hf ^{1}H NMR). Approval of the local ethics committee was obtained and all volunteers gave written informed consent. **13C-MRS experiments** were performed on five subjects. Stable enrichment with only little decay during hypoglycemia was obtained using a 30-ml bolus of 100% 13C-1-glucose 20% solution at the initiation of the clamp, followed by 30% 13C-1-glucose 20% solution for the remainder of the experiment. Initially, an LP 13C coil in combination with a CP 1H coil was used (n=2), which was later replaced by an improved CP volume coil for 1H with a CP surface coil insert for 13C **[4]** (n=3). The 13C-MRS experiment consisted of repetitive pulse-acquire experiments (200-μs rectangular excitation pulse, 75 scans, TR = 2 s, time res. of 2.5 min) with proton decoupling (WALTZ-16), and outer volume suppression bands to suppress lipid signals. Sets of 4 spectra acquired during infusion of 13C-1-glucose were added and reference spectra obtained before the 13C-labeled glucose infusion were subtracted. jMRUI **[5]** was used for signal integration. The other five subjects underwent a hyperinsulinemic euglycemic-hypoglycemic glucose clamp with unlabeled glucose for 1H MRS (n=5) and ASL experiments (n=4), generally using the standard CP head coil. Three of them also underwent 31P MRS, using initially a separate LP 31P coil in two and later a CP volume coil for 1H with a CP surface coil insert for 31P in one, making the coil change unnecessary. **1H-MRS experiments** consisted of measurements of volumes of 15-27 ml positioned in occipital gray matter with a PRESS localization sequence. Generally, 96 scans were acquired with TE =20ms and TR = 2 or 5 sec. 1H-MRS data were analyzed using the LCModel software **[6]**. For the **ASL measurements** a sequence based on QUIPPS-II **[7]** was applied. In the first subject no calibration was used, which turned out to be necessary for quantification. In the next three subjects the ASL measurement was combined with a proton density measurement to allow proper quantification of cerebral blood perfusion using home-written software. **31P-MRS measurements** consisted of pulse-acquire experiments using a 45º BIRP pulse for excitation and a WALTZ16 pulse train for proton decoupling and NOE enhancement.

Results: Under both glycemic conditions, 13C MRS resulted in spectra of good quality showing glucose signal increase as result of 13C-1-glucose uptake, glucose signal decline at the start of the hypoglycemic phase (not shown), and time-dependent conversion of glucose to glutamate, glutamine, and aspartate (Figs 1-2). Figure 2 shows that the time-dependent curves of glucose metabolite signals flatten during hypoglycemia. 1H MRS showed a decrease in brain glucose content, from concentrations of approx. 1 mM during euglycemia to values below detection limit during hypoglycemia. The ASL measurements resulted in quantitative perfusion maps showing clear differences between various brain regions with a higher blood perfusion rate in gray matter than in white matter (Fig. 3). Comparison of five measurements during euglycemic and hypoglycemic conditions performed in three subjects showed average overall brain perfusion rates of 47.5 ± 5.8 ml/min/100ml and 46.9 ± 4.6 ml/min/100ml, respectively. So, no detectable differences were observed in the overall blood perfusion rate in the brain of these healthy volunteers between both glycemic conditions. The 31P MRS experiments resulted in spectra of good quality with clearly resolved resonances of phosphoethanolamine, phosphocholine, intracellular inorganic phosphate, glycerophosphoethanolamine, glycerophosphocholine, phosphocreatine (PCr), and α,β,γ-ATP. Upon changing the glycemic condition to hypoglycemia, no change in the PCr/ATP ratio was observed, but the Pi resonance showed a very slight shift indicative for a pH increase in the order of ~0.01-0.02, which is in agreement with findings of Bischof et al. **[8].**

Discussion and conclusion: We showed that a hyperinsulinemic euglycemic-hypoglycemic glucose clamp can be safely and successfully applied in a 3T MR magnet. Human brain 13C MRS, 31P MRS, and ASL were all found to work properly under both glycemic conditions. The sensitivity of 1H MRS at 3T seems too low to detect brain glucose signals under hypoglycemic conditions. The ASL measurements indicated no change in blood perfusion during hypoglycemia, although an increase in blood flow was expected based on results obtained in rats **[9]**. However, the absence of an increase in blood flow may be explained by the rather mild hypoglycemic conditions (plasma glucose ~ 3 mM), as increased blood flow in these rats occurred at plasma glucose levels below 2 mM. All MRS methods indicated (subtle) changes between the two glycemic states. 13C MRS provided a wealth of data that can be used as input for modeling experiments **[10]** from which we expect to obtain new information on brain glucose metabolism under both euglycemic and hypoglycemic conditions. Successful implementation of the MR techniques mentioned allows future studies on diabetic patients with and without hypoglycemia unawareness to gain further insight in this syndrome.

References: 1. Gruetter R, J Neurosci Res 74 (2003), 179-183; **2.** Boyle PJ, et al., Proc Natl Acad Sci USA 91 (1994), 9352-9356; **3.** De Galan BE, et al., Diabetes 51 (2002), 790-796; **4.** Klomp DWJ, et al., ESMRMB 21 (2004), 276; **5.** http://www.mrui.uab.es/mrui/; **6.** Provencher SW, Magn Reson Med 30 (1993), 672-679; **7.** Luh WM, et al., Magn Reson Med 41 (1999), 1246-1254. **8.** Bischof MG, et al., Diabetologia 47 (2004), 648-651; **9.** Choi IY, et al., J Cereb Blood Flow Metab 21 (2001), 653-663; **10.** Gruetter R, et al., Am J Physiol Endocrinol Metab 281 (2001), E100-E112.

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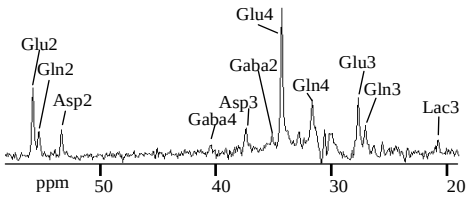

Fig. 1 ^{13}C MR spectrum of glucose metabolites in the human brain obtained during 30 min. under euglycemic conditions. Glu, glutamate; Gln, glutamine; Asp, aspartate; Lac, lactate.

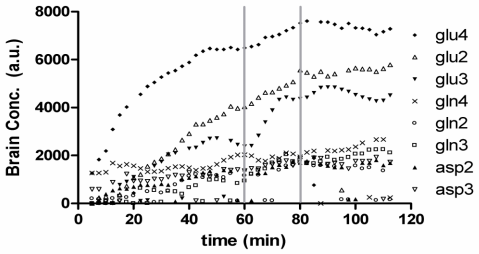

Fig. 2 Brain metabolite signals of 13C-1-glucose measured by 13C MRS during euglycemic (before 60 min) and hypoglycemic (after 80 min) conditions

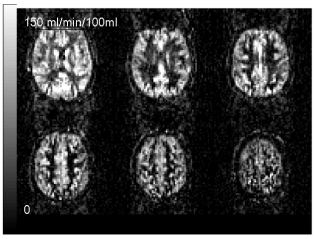

Fig. 3 Quantitative perfusion map of one subject under euglycemic conditions. The perfusion scale is shown at the left side and ranges from 0 to 150 ml/min/100ml.