Cerebral glucose uptake in humans at hypoglycemic plasma levels follows reversible Michaelis-Menten kinetics

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Introduction
Cerebral glucose levels in humans can be measured non-invasively by 13C MRS to determine the kinetics of glucose transport into the brain. This transport can be described by a reversible Michaelis-Menten (MM) model [1], which is characterized by a half-maximal transport constant (Km) and maximal transport rate (Vmax) or maximal transport rate relative to CMRglc (Tmax/CMRglc). Several studies investigated the validity of the MM kinetic model and have determined these kinetic parameters for the brain of healthy subjects [1,2], for white (WM) and gray matter (GM) separately [3,4] and for the effect of insulin [4]. However, all these studies focused on brain glucose content as a function of plasma glucose levels under euglycemic (plasma glucose ~5 mM) and hyperglycemic (plasma glucose up to 30 mM) conditions. The effects of hypoglycemia on cerebral glucose levels have been studied by proton MRS [5,6] in humans, but have not been quantified and kinetic parameters were not presented. In rats it was demonstrated that the MM kinetic properties hold for hypoglycemic plasma levels of < 5 mM glucose and that values of the kinetic parameters are comparable to what has been reported for humans [7]. Another study demonstrated that chronic hypoglycemia increases brain glucose and consistently increases Tmax/CMRglc [8]. The aim of this study was to measure human cerebral glucose content during hypoglycemia using 13C MRS. In order to calculate values of reversible MM kinetic parameters for glucose transport and to compare these with previously reported data on glucose transport into the brain.

Methods
Eight healthy volunteers (4 male/4 female, 23.2±2.5 yrs old) were subjected to two hyperinsulinemic (60 mU·m-2·min-1) glucose clamp experiments after an overnight fast [9]. They were clamped at euglycemia (~5mM) on one day and at hypoglycemia (~3mM) on another day, 4 weeks apart. The clamps were designed to provide stable and comparable plasma levels of [1-13C]glucose. As such, a bolus of 6 g of 100% [1-13C]glucose 20 % (w/w) solution was infused over 10 min followed by infusion of [1-13C]glucose for the euglycemic and hypoglycemic clamp, respectively, at a variable rate to maintain target plasma glucose levels. Arterial blood was sampled every 5 min to determine plasma glucose concentration and [1-13C]glucose isotopic enrichment by high resolution 1H NMR. For in vivo measurements a DEPT sequence was used in combination with ISIS localization and 1H decoupling. 13C-MRS acquisition (72 scans, TR=2s, duration=2.5 min) of a voxel of ~125 ml in the occipital brain 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 1H with a CP surface coil insert for 13C [10].

Post-processing and quantification: MR spectra acquired during steady-state (~5.0 and ~3.0 mM, t=0-100 min) were averaged and then phased in the region 70 – 100 ppm (including glucose and myo-inositol signals) with JMRUI. The peaks of α- and β-glucose in the summed spectra were fitted with the AMARES algorithm. To quantify metabolite levels the natural abundance 13C myo-inositol signals were assumed to represent 1.1% of 6 mM [11]. In addition, glucose signals were corrected for the pulse profile as measured in a phantom, the 13C enrichment of plasma glucose and for 5% contamination by blood vessels.

Results
In 13C MR spectra of the brain there was an obvious difference in glucose signal intensity between the euglycemic and hypoglycemic state with respect to the natural abundance myo-inositol signals (Fig 1). Steady-state brain glucose levels were comparable to previously reported values in rats [7]. An example of the spectra together with the best fit of the data and a 95% confidence interval are presented in figure 2A, together with the best fit of the data and a 95% confidence interval. By linear regression we derived from a reversible MM kinetic model values for the parameters: Tmax/CMRglc = 2.93 and Ks = 5.27 mM. In figures 2B and 2C, we plotted our data points together with the linear relations between plasma and brain glucose levels reported previously in humans at euglycemic and hyperglycemic conditions [1,4] and in rats under hypoglycemic conditions [7].

Discussion and conclusion
Since glucose is the primary fuel of the brain, it is important to characterize brain glucose values as a function of plasma glucose values especially at hypoglycemia. Previously, brain glucose levels and values for MM kinetic parameters were estimated for euglycemic and hyperglycemic plasma levels, with rather large standard deviations that made these values less reliable for hypoglycemic conditions [1,4]. In our study we demonstrated that under moderate hypoglycemic conditions signals for glucose in the brain were still detectable in all subjects. Furthermore, the estimated brain glucose levels are in line with extrapolation of brain glucose values at higher plasma glucose concentrations and within the error of MM parameters published for this data [11]: Tmax/CMRglc = 2.3±0.2, Ks = 0.6±0.2 mM. [4]: WM: Tmax/CMRglc = 2.15±0.25, Ks = 1.96±2.45 mM, WM: Tmax/CMRglc = 2.24±0.23, Ks = 0.98±2.13 mM). Our data agrees even better with a rat study performed during hypoglycemia [7]: Tmax/CMRglc = 2.7±0.13, Ks = 3.3±1.0 mM, which also estimated brain glucose levels to approach zero at plasma glucose levels of ~2 mM. Whether the linear reversible MM kinetic relationship still holds at even lower plasma glucose values in humans is cannot be derived from our data. We conclude that we successfully measured brain glucose values at moderate hypoglycemia (~3 mM) in humans, and kinetic parameters confirm a reversible MM model for glucose transport at hypoglycemia.


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