**Deterioration of neuronal and glial intermediary metabolism, neurochemical profiles and brain morphology in insulin-resistant Goto-Kakizaki rats: a multimodal magnetic resonance study *in vivo***

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**TARGET AUDIENCE –** Scientists and clinicians interested in neurological disorders associated with brain insulin resistance.

**PURPOSE –** Impaired insulin signalling in diabetes deteriorates brain structure and function leading to cognitive deficits. While the neurodegeneration process has been largely studied in diabetes, early metabolic modifications associated to such events remain to be elucidated. Aiming at identifying early biomarkers of diabetic encephalopathy, we performed a multimodal magnetic resonance imaging (MRI) and spectroscopy (MRS) study in insulin-resistant Goto-Kakizaki (GK) and Wistar rats at 2, 4 and 6 months of age, under 1.5% isoflurane anaesthesia, to evaluate brain morphology and neurochemical profiles. $^{13}$C MRS was then employed to detect isotopomers of glutamate, glutamine and aspartate during [1,6-$^{13}$C]glucose infusion under α-chloralose anaesthesia, which allowed quantifying metabolic pathways of brain energy metabolism.

**METHODS –** Before each MR session, spontaneous alternation behaviour was evaluated in a Y-maze and exploratory and locomotor behaviours were tested in an open field arena, as previously detailed [1]. MRS experiments were performed on a 14.1 T/26 cm horizontal bore magnet with homebuilt surface coils. Longitudinal MRS were performed under isoflurane at 2, 4 and 6 months of age with SPECIAL with TR=4 s and TE=2.8 ms [2] in the hippocampus and cortex. $T_2$-weighted MRI was performed with a fast-spin-echo sequence with TR=4 s and TE=40 ms. At 6 months of age, $^{13}$C MRS during infusion of [1,6-$^{13}$C]glucose under α-chloralose anaesthesia was performed as detailed previously [3] with semi-adiabatic distortionless enhancement by polarization transfer (DEPT) combined with 3D-ISIS for $^{1}$H localization [4]. LCModel was used for analysis of both $^{1}$H [2] and $^{13}$C spectra [5]. The scaling of $^{13}$C fractional enrichment (FE) curves was based on MRS of brain extracts [3]. A two-compartment model was used to analyse the time courses of aliphatic carbons of glutamate, glutamine and aspartate, and variance of parameters was determined by Monte-Carlo analyses [3].

**RESULTS –** At all ages, GK rats displayed smaller hippocampus (-17±2% to -21±1%, P<0.001), cortex (-8±2% to -12±1%, P<0.001) and whole brain (-7±1% to -11±1%, P<0.001), but larger ventricular volume (+52±10% to +92±6%, P<0.001), compared to controls. Hippocampal and cortical neurochemical profiles were affected by insulin resistance: out of 20 metabolites, GK rats displayed reduced glutamine (-14±3% to -25±2%, P<0.001) and choline (-11±5% to -19±4%, P<0.001) in both regions; higher taurine (+7±3% to +16±2%, P<0.001) and ascorbate (+35±6% to +51±5%, P<0.001), and reduced alanine (-18±3% to -29±4%, P<0.01) in the hippocampus; lower cortical aspartate (-15±6% to -31±6%, P<0.01). Spatial memory performance evaluated as the spontaneous alternation in a Y-maze was lower in GK than Wistar rats (-14±5% to -21±3%, P<0.01), and interestingly correlated to hippocampal concentrations of ascorbate (r=-0.47, P<0.001), glutamine (r=0.37, P=0.003) and taurine (r=-0.36, P=0.004). Mathematical modelling of $^{13}$C time courses *in vivo* revealed that insulin resistance caused mitochondrial oxidation rate to be reduced in neurons (-16±2%, P<0.001) but increased in astrocytes (+25±3%, P<0.001). Additionally, both glutamatergic neurotransmission (-19±2%, P<0.001) and glutamine synthesis (-16±2%, P<0.001) were reduced in the brain of GK rats compared to controls.

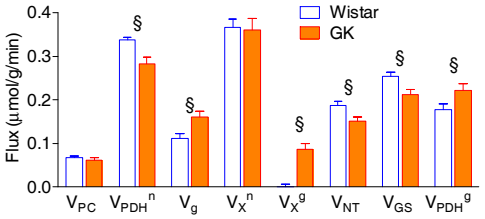


**Figure 1**. Metabolic fluxes of brain energy metabolism are modified by insulin resistance in GK rats, relative to Wistar rats. Data are mean±SD. §P<0.001 in a preliminary analysis with independent t-tests (n=7 each group).

**DISCUSSION/CONCLUSION –** The brain of insulin-resistant GK rats had impaired mitochondrial metabolism and abnormal metabolic interactions between neurons and astrocytes, compared to control Wistar rats. This led to neurochemical alterations that were associated with the degree of brain dysfunction, namely impaired memory performance. Thus, non-invasive detection of neurochemical profiles by MRS may be useful in tracking progression of diabetic encephalopathy.

**REFFERENCES** – [1] Duarte *et al*. (2012) PLoS ONE 7(4), e21899. [2] Mlynárik *et al*. (2006) Magn Reson Med 56(5):965. [3] Duarte *et al*. (2011) Front Neuroenergetics 3:3. [4] Henry *et al*. (2003) MRM 50:684. [5] Henry *et al*. (2003) NMR Biomed 16:400

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