**SUPPLEMENTAL MATERIAL**

Acute Hyperglycemia Increases Brain Pregenual Anterior Cingulate Cortex Glutamate Concentrations in Type 1 Diabetes Mellitus

**S1** - MRS Data Analysis

The model basis set used for analysis was composed of 17 metabolite spectra that were simulated in-house using the Vespa (1) software graphical interface to the GAMMA magnetic resonance simulation library (2), including: alanine, aspartate, creatine (Cr), GABA, glucose (Glc), glutamine (Gln), glutamate (Glu), glycero-phospho-choline (GPC), phospho-choline (PC), phospho-creatine (PCr), myo-inositol (mI), scyllo-inositol, glutathione (GSH), lactate, N-acetylaspartate (NAA), N-acetyl aspartyl-glutamate, and taurine, as well as macromolecule and lipid spectra that were simulated by LCModel. To perform absolute quantitation of metabolites, we scaled the metabolite signal to the water signal from the same voxel using LCModel’s water scaling method and estimated the water molal concentration in each voxel by using the voxel partial volume fractions of grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) (3,4) obtained by segmentation of the T1-weighted structural image. There was a higher ratio of GM to white matter WM in the ACC (GM/WM = 4.11 ± 0.92) than in the OCC (GM/WM = 1.37 ± 0.26) voxels in this study. T1-weighted images were segmented using the FMRIB software library FSL v5.0.10 (https://fsl.fmrib.ox.ac.uk/fsl) (5) FAST segmentation tool. We report all metabolite concentrations in units of mmol/kg wet weight brain tissue.

**Table S1:** Reliability of MRS metabolite concentration measurements in pregenual anterior cingulate and occipital lobe cortices

Means ± SD [range] of full width at half max (FWHM), signal to noise ratios (SNR) and Cramér-Rao lower bounds (CRLB) for metabolites by group and condition. EU = basal euglycemia; HG = hyperglycemic clamp; Glu = glutamate; Glx = Glu + Gln (determined by LCModel fit); NAA = N-acetylaspartate; TCr = total creatine (Cr + PCr). A one-way ANOVA was used to determine p-values (p).

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| --- | --- | --- |
|  | **T1D** | **CONTROLS** |
|   | **EU** | **HG** |  | **EU** | **HG** |  |
| **(N = 13)** | **(N = 13)** | **p** | **(N = 11)** | **(N = 11)** | **p** |
|  | **Anterior Cingulate Cortex (ACC)** |
| **FWHM (Hz)** | 6.1 ± 1.1 [4.9 - 8.6] | 6.8 ± 2.0 [3.7 - 8.6] | 0.288 | 6.8 ± 1.5 [4.9 - 8.6] | 6.3 ± 1.4 [4.9 - 8.6] | 0.456 |
| **SNR** | 18 ± 3 [13 - 21] | 18 ± 3 [14 - 23] | 0.790 | 19 ± 5 [15 - 31] | 19 ± 3 [14 - 23] | 0.870 |
| **CRLB Glu (%)** | 6.9 ± 0.8 [6 - 8] | 6.9 ± 1.0 [5 - 9] | 0.970 | 7.2 ± 1.4 [5 - 10] | 6.8 ± 1.0 [6 - 9] | 0.489 |
| **CRLB Glx (%)** | 7.2 ± 0.9 [6 - 9] | 7.0 ± 0.8 [6 - 8] | 0.391 | 7.3 ± 1.0 [6 - 9] | 7.6 ± 1.0 [7 - 10] | 0.391 |
| **CRLB NAA (%)** | 4.3 ± 0.7 [3 - 5] | 4.3 ± 0.9 [3 - 6] | 0.057 | 4.6 ± 0.9 [3 - 6] | 4.4 ± 0.9 [3 - 6] | 0.917 |
| **CRLB TCr (%)** | 2.7 ± 0.5 [2 - 3] | 2.9 ± 0.5 [2 - 4] | 0.167 | 3.1 ± 0.7 [2 - 4] | 2.8 ± 0.4 [2 - 3] | 0.192 |
|  | **Occipital Lobe Cortex (OCC)** |
| **FWHM (Hz)** | 5.5 ± 0.6 [4.2 - 6.1] | 5.5 ± 0.7 [4.2 - 7.3] | 0.971 | 5.3 ± 0.9 [4.2 - 7.3] | 5.3 ± 0.9 [4.2 - 7.3] | 0.999 |
| **SNR** | 29 ± 3 [24 - 37] | 28 ± 4 [19 - 33] | 0.458 | 31 ± 5 [23 - 37] | 30 ± 3 [26 - 36] | 0.699 |
| **CRLB Glu (%)** | 6.0 ± 0.6 [5 - 7] | 6.2 ± 0.8 [5 - 8] | 0.190 | 6.0 ± 0.9 [5 - 8] | 6.1 ± 0.8 [5 - 7] | 0.729 |
| **CRLB Glx (%)** | 6.3 ± 0.5 [6 - 7] | 6.5 ± 0.7 [6 - 8] | 0.436 | 6.2 ± 0.7 [5 - 7] | 6.3 ± 0.5 [6 - 7] | 0.681 |
| **CRLB NAA (%)** | 3.1 ± 0.3 [3 - 4] | 3.0 ± 0.0 [3] | 0.337 | 3.1 ± 0.8 [2 - 5] | 3.1 ± 0.3 [3 - 4] | 0.999 |
| **CRLB TCr (%)** | 2.0 ± 0.0 [2 - 2] | 2.1 ± 0.3 [2 - 3] | 0.337 | 2.1 ± 0.3 [2 - 3] | 2.1 ± 0.3 [2 - 3] | 0.999 |

**S2 –** Exploratory analyses

**Table S2:** Concentrations of metabolites in mmol/kg wet weight of brain tissue (mean ± SD) and fractional amplitude of low frequency fluctuations (fALFF) in pregenual anterior cingulate cortex and occipital lobe cortex during euglycemia (EU) and hyperglycemia (HG). Includes values for the metabolites Glx and TCr and fALFF in slow-bands 4 and 5 for the exploratory part of the study. P-values are from the linear mixed effects model, with main effects of group, condition and condition x group interaction in the right side columns, and contrasts between HG and EU for each group in left side columns. P-values that meet the partial Bonferroni corrected threshold for significance (p = 0.039) are highlighted in bold. HG - EU = change from EU to HG in mmol/kg wet weight brain tissue or fALFF (mean); Glu = glutamate; NAA = N-acetylaspartate; fALFF-4 = fALFF in slow-band 4 (0.027 – 0.073 Hz); fALFF-5 = fALFF in slow-band 5 (0.001 – 0.027 Hz).

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| --- | --- | --- | --- |
|  | **T1DM** | **CONTROLS** |  **Linear Mixed Effects Model p** |
|  | **EU** | **HG** | **HG - EU** | **Contrast** | **EU** | **HG** | **HG - EU** | **Contrast** | **Group** | **Condition** | **Condition x Group** |
|  | **(n=13)** | **(n=13)** |  | **p** | **(n=11)** | **(n=11)** |  | **p** |
| **Metabolite** | **Anterior Cingulate Cortex (ACC)** |
| **Glu** | 12.3 ± 0.9 | 13.5 ± 2.0 | 1.2 | **0.014** | 12.8 ± 1.6 | 12.6 ± 1.1 | -0.2 | 0.578 | 0.706 | 0.194 | **0.035** |
| **Glx** | 15.0 ± 0.5 | 16.4 ± 2.6 | 1.4 | **0.016** | 15.2 ± 1.4 | 14.9 ± 1.4 | -0.3 | 0.663 | 0.189 | 0.183 | 0.049 |
| **NAA** | 8.9 ± 1.1 | 9.4 ± 1.1 | 0.5 | 0.043 | 8.9 ± 1.1 | 8.9 ± 1.2 | 0.0 | 0.971 | 0.575 | 0.154 | 0.170 |
| **TCr** | 7.1 ± 0.9 | 7.5 ± 0.7 | 0.4 | **0.008** | 7.3 ± 0.4 | 7.1 ± 0.6 | -0.2 | 0.277 | 0.644 | 0.291 | **0.009** |
| **Activity Index** |   |   |   |   |   |   |   |   |   |   |   |
| **fALFF-4** | 0.36 ± 0.04 | 0.36 ± 0.05 | 0.00 | 0.449 | 0.39 ± 0.03 | 0.34 ± 0.02 | -0.05 | **0.002** | 0.274 | **0.004** | 0.061 |
| **fALFF-5** | 0.18 ± 0.03 | 0.18 ± 0.02 | 0.00 | 0.953 | 0.19 ± 0.02 | 0.18 ± 0.03 | -0.01 | 0.520 | 0.603 | 0.656 | 0.601 |
| **Metabolite** | **Occipital Lobe Cortex (OCC)** |
| **Glu**  | 7.9 ± 0.6 | 7.8 ± 0.8 | -0.1 | 0.401 | 7.4 ± 0.7 | 7.7 ± 0.8 | 0.3 | 0.266 | 0.242 | 0.750 | 0.164 |
| **Glx** | 9.0 ± 0.5 | 8.9 ± 1.0 | -0.1 | 0.508 | 8.5 ± 0.6  | 8.8 ± 0.8 | 0.3 | 0.205 | 0.323 | 0.581 | 0.162 |
| **NAA** | 7.8 ± 0.4 | 7.7 ± 0.4 | -0.1 | 0.389 | 7.5 ± 0.3 | 7.5 ± 0.2 | 0.0 | 0.608 | **0.008** | 0.875 | 0.345 |
| **TCr** | 5.3 ± 0.3 | 5.4 ± 0.3 | 0.1 | 0.566 | 5.2 ± 0.2 | 5.3 ± 0.2 | 0.1 | 0.846 | 0.379 | 0.605 | 0.827 |
| **Activity Index** |   |   |   |   |   |   |   |   |  |   |   |
| **fALFF-4** | 0.41 ± 0.03 | 0.43 ± 0.04 | 0.02 | 0.196 | 0.44 ± 0.04 | 0.42 ± 0.04 | -0.02 | 0.088 | 0.656 | 0.667 | **0.033** |
| **fALFF-5** | 0.20 ± 0.04 | 0.18 ± 0.02 | -0.02 | 0.311 | 0.22 ± 0.03 | 0.20 ± 0.05 | -0.02 | 0.271 | 0.105 | 0.135 | 0.874 |

**S3** – Functional MRI processing and analysis

We analyzed rs-fMRI data using the FSL (fsl.fmri.ox.ac.uk) and DPARSF (rfmri.org) software packages to calculate the fractional amplitude of low frequency fluctuations in slow-bands 4 (fALFF4, from 0.027 to 0.073 Hz) and 5 (fALFF5, from 0.001 to 0.027 Hz) of the rs-fMRI time series BOLD signal fluctuations (6,7), which has been proposed to reflect the regional intensity of spontaneous neuronal activity of two distinct sets of oscillators (8) preferentially localized in grey matter (9). Using FSL, each fMRI time series underwent slice timing and motion correction, registration to the MNI-152 standard brain space with 2 mm isotropic resolution, Gaussian spatial smoothing by 8 mm FWHM, and high-pass filtering with a cutoff of 100 sec. The FSL motion correction factors were used to apply exclusion criteria for excessive head motion: time series mean displacement > 3 mm or mean rotation > 3º. No time series were excluded from the analysis by these criteria. Using DPARSF, each time series underwent further band pass filtering in the frequency ranges of 0.01 – 0.027 Hz and 0.027 – 0.08 Hz to obtain the time series in slow-band 5 and slow-band 4, respectively. The amplitude of low frequency fluctuations (ALFF) whole brain maps were calculated by taking the average square root of the power spectrum in each frequency band in each pixel. The fractional ALFF (fALFF) whole brain maps were calculated by taking the ratio of ALFF of each slow band to the average amplitude in all frequencies. The average fALFF in each MRS voxel (ACC and OCC) were extracted using the fslstats function of FSL and applying a mask of the voxel (obtained using in-house software) to the fALFF4 and fALFF5 maps.

**References**

1. Soher BJ, Semanchuck P, Young K, Todd D. Vespa – Simulation User Manual and Reference. 2012 Sep 21:1–68.

2. Smith S, Levante B, Meier B, Ernst R. Computer Simulations in Magnetic Resonance. An Object-Oriented Programming Approach. Journal of magnetic resonance Series A. 1994;106:75.

3. Ernst T, Kreis R, Ross BD. Absolute quantitation of water and metabolites in the human brain. I. Compartments and water. J Magn Reson B. 1993 Jul 24;102:1–8.

4. Gasparovic C, Song T, Devier D, Bockholt HJ, Caprihan A, Mullins PG, et al. Use of tissue water as a concentration reference for proton spectroscopic imaging. Magn Reson Med. 2006 Jun;55(6):1219–26.

5. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage. 2004;23 Suppl 1:S208–19.

6. Zang Y-F, He Y, Zhu C-Z, Cao Q-J, Sui M-Q, Liang M, et al. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. Brain Dev. 2007 Mar;29(2):83–91.

7. Zou Q-H, Zhu C-Z, Yang Y, Zuo X-N, Long X-Y, Cao Q-J, et al. An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: Fractional ALFF. J Neurosci Methods. 2008 Jul;172(1):137–41.

8. Buzsáki G, Draguhn A. Neuronal oscillations in cortical networks. Science. 2004 Jun 25;304(5679):1926–9.

9. Zuo X-N, Di Martino A, Kelly C, Shehzad ZE, Gee DG, Klein DF, et al. The oscillating brain: complex and reliable. NeuroImage. 2010 Jan 15;49(2):1432–45.