# Quantification of Rat brain metabolites by ProFit: Preliminary evaluation of high fat diet induced obesity

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**Introduction**: The ProFit algorithm has been implemented for quantification of human brain metabolites [1]. Rats and mice may serve as subjects for modeling various pathological conditions, which can be studied by localized MRS. Increased spectral dispersion in localized J PRESS experiments [2] may improve the metabolite quantification. Regulation of energy metabolism is controlled by the brain, in which key central neuronal circuits process a variety of information reflecting nutritional state. Impairment of neuronal signaling can adversely affect autonomic output, resulting in increased feeding, weight gain, and altered hepatic glucose metabolism. In this study we have implemented ProFit based quantification of cerebral metabolites from Rat brain using max-echo sampled J PRESS approach [3] and have evaluated rats on high fat and placebo diets to determine the changes in metabolite concentrations in the thalamus region.

**Methods**: Localized max echo sampled J PRESS was implemented on a 7T ClinScan MRI/MRS scanner equipped with a 72mm volume resonator for RF transmit in combination with 20mm surface receive coil. Prior knowledge 2D basis spectra generated using the GAMMA [4] included 19 metabolites: creatine (Cr), N-acetylaspartate (NAA), glycerylphosphocholine (GPC), phosphorylcholine (PCh), alanine (Ala), aspartate (Asp), γ-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), glycine (Gly), glutathione (GSH), lactate (Lac), myo-inositol (mI), N-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), taurine (Tau), scyllo-inositol (Scy) and ascorbate (Asc). The method was validated in a phantom containing the same metabolites except Scy. The ProFit fitting of the J PRESS spectra was performed within MATLAB environment using a linear combination of the 2D basis spectra. For in vivo experiments, 7 male Fischer (strain: F344) rats, 3 fed with placebo diet (CE-2) and 4 with high fat diet (D12079B), were scanned when they were 13 weeks old using the following parameters: TR/minTE=4.0s/13ms, voxel size of 4x4x4 $mm^3$ (64µl), 16 averages, 50 $t_1$ increments with 1024 points with an acquisition time of ~ 53 minutes. The body weight and blood chemistry was monitored once in every week. None of the rats were diabetic according to the insulin resistance tests.

**Results**: The comparison of the metabolite ratio with respect to Cr shows a significant increase ($p<0.02$) for NAA, Ala, Gln, Glu and Lac for the high fat group as shown in Table 1. The accuracy of the fitting was characterized using Cramer-Rao lower bounds (CRLB) [5]. Cr, Ala, Asp, Gly, Glc, GSH, Lac, mI and Asc shows a lower CRLB compared to 1D approach even at 16.1 T [6]. The GABA quantification was better in rats on high fat diet and had larger CRLB for the rats on placebo diet possibly due to very low concentrations. Table 2 shows the diet composition for both the placebo (CE-2) and high fat (D12079B).

**Table 1: Metabolite ratios of placebo and high fat diet fed rats**

| Metabolite | Placebo Diet (n=3) | | | High Fat Diet (n=4) | | | |
|---|---|---|---|---|---|---|---|
| | /Cr | STD | CRLB % | /Cr | STD | CRLB % | p value |
| Cr | 1 | 0 | 0.95 | 1 | 0 | 0.973 | |
| ***NAA*** | 0.947 | 0.030 | 1.04 | 1.11 | 0.058 | 0.966 | ***0.007*** |
| GPC | 0.222 | 0.086 | 6.91 | 0.24 | 0.105 | 7.673 | 0.817 |
| ***Ala*** | 0.166 | 0.009 | 7.16 | 0.292 | 0.025 | 4.395 | ***<0.001*** |
| Asp | 0.765 | 0.103 | 4.43 | 0.892 | 0.219 | 4.135 | 0.403 |
| GABA | 0.060 | 0.022 | 31.47 | 0.093 | 0.013 | 19.775 | 0.051 |
| Glc | 0.634 | 0.060 | 3.87 | 0.746 | 0.073 | 3.433 | 0.083 |
| ***Gln*** | 0.490 | 0.025 | 5.02 | 0.659 | 0.054 | 3.965 | ***0.004*** |
| ***Glu*** | 0.980 | 0.060 | 2.34 | 1.23 | 0.114 | 2 | ***0.019*** |
| Gly | 0.227 | 0.017 | 5.09 | 0.272 | 0.034 | 4.478 | 0.094 |
| GSH | 0.344 | 0.039 | 3.05 | 0.395 | 0.012 | 2.82 | 0.053 |
| ***Lac*** | 0.210 | 0.140 | 7.55 | 0.53 | 0.081 | 2.56 | ***0.012*** |
| mI | 0.817 | 0.030 | 1.8 | 0.96 | 0.1 | 1.625 | 0.066 |
| NAAG | 0.076 | 0.016 | 13.3 | 0.066 | 0.022 | 17.675 | 0.537 |
| Tau | 0.219 | 0.075 | 10.53 | 0.255 | 0.067 | 9.053 | 0.534 |
| Asc | 0.544 | 0.129 | 3.65 | 0.548 | 0.178 | 3.993 | 0.977 |

**Conclusion**: This work exhibits the first quantification of rat brain metabolites using ProFit algorithm. The significant increase in metabolite ratio for Ala, Gln, Glu and Lac with respect to Cr is in agreement with increased glucose metabolism as shown by $^{13}C$ spectroscopy of rat brain after intravenous injection of $^{13}C$ Glc [7]. Our preliminary results clearly demonstrates that ProFit can be utilized to monitor changes in the metabolite concentrations during the process of high fat diet induced obesity and identify pre-diabetic markers.

**Table 2: Diet composition**

| | Placebo (CE-2) | High Fat (D12079B) |
|---|---|---|
| **Protein (%)** | 25.4 | 20 |
| **Fat (%)** | 4.4 | 21 |
| **Carbohydrate (%)** | 50.3 | 50 |
| **Water, Fiber and Others (%)** | 19.9 | 9 |

**References**

[1] Schulte, RF and Boesiger, P. NMR Biomed 2006; 19: 225-263
[2] Ernst RR, Bodenhausen G and Wokaun A. Principles of NMR Spectroscopy in one and two dimensions. Oxford (1987)
[3] Schulte, RF and Boesiger, P. NMR Biomed 2006; 19: 264-270
[4] Smith SA, Levante TO, et al. J. Magn. Reson. 1994; 106: 75-105
[5] Cavassila S, Deval S, Huegen C, et al.. NMR Biomed. 2001; 14: 278-283
[6] Sung-Tak Hong et al MRMedicine 000:000–000 (2010)
[7] Qu H et al, Dev Neurosci. 2000 Sep-Dec;22(5-6):429-36.