The 1.28 ppm signal – A Translational Magnetic Resonance Spectroscopy Marker for Neurogenesis?

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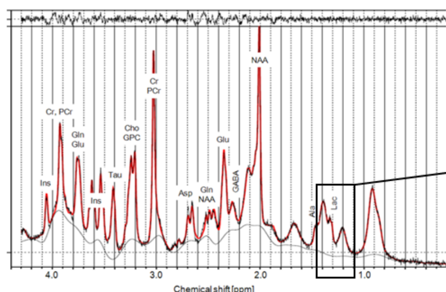
Introduction: Magnetic Resonance Spectroscopy (MRS) is a non-invasive, translational neuroimaging technique providing access to neurotransmitter and metabolite concentrations in the living human and animal brain. In a recent publication a small signal contribution at 1.28 ppm in the MRS spectrum of the living brain was proposed as a spectral fingerprint of neuronal progenitor cells, which hence might serve as a biomarker for neurogenesis (Manganas et al., Science 2007). This finding is still under ardent debate. Our study aimed at validating this putative biomarker using state-of-the-art MRS technology and data analysis in two well-established animal models of enhanced hippocampal neurogenesis, namely physical exercise in the running wheel (Praag et al., Nat Neurosci 1999) and early postnatal development.

Methods: *Animal Models:* Physical exercise (running wheel): 18 male Sprague Dawley rats were included, i.e. 6 controls and 12 'runners'. All animals underwent two MRS examinations, the 1st MRS at six weeks of age, the 2nd MRS 14 days later. Between the two MRI assessments the 'runner' group exercised voluntarily on a running wheel. Early postnatal brain development: 10 Sprague Dawley rat pups, i.e. 5 males and 5 females from two litters were included in the study. All animals underwent two MRS experiments, i.e. at age P4/P5 and at P17/20. Animals were sacrificed at the end of the experiment and their brains collected for double cortin (DCX) analysis, which serves as an independent measure of neurogenesis. *Animal Preparation for MRS:* Animals were anesthethized using 1.6% isoflurane in 25% oxygen and 75% air, and were intubated and artficially ventilated (exception: P4/P5 rats: free-breathing in face mask). Rectal temperature was monitored and maintained in physiological range. *MRS Hardware:* Data were collected on a Bruker BioSpec 9.4 T/30 cm MR scanner equipped with a 72 mm bird-cage resonator for excitation and a 4-loop phase-arrayed head coil for reception. In the P4/P5 aged rats advantage was taken of the enhanced SNR featured by a transmit/receive CryoProbe^{TM}. MRS Data Acquisition: MRS Spectra were acquired using PRESS single voxel spectroscopy. The highest available pulse bandwidth (3.2-4 kHz) and volume selection tailored to the metabolite range of interest were employed to minimize chemical shift displacements. Water suppression was accomplished using VAPOR, interleaved with outer volume suppression. In each animal three MRS spectra (TR 1.5 s, TE 10 ms, spectral width 4 kHz collected in 2048 complex points, 1024 averages, acquisition time 25 min) were obtained from an 11uL voxel placed in the left hippocampus, covering the neurogenic region of the dentate gyrus. *MRS Data Analysis:* Three spectra acquired for each animal in each session were averaged and metabolite quantification was carried out using LCModel (http://s-provencher.com).



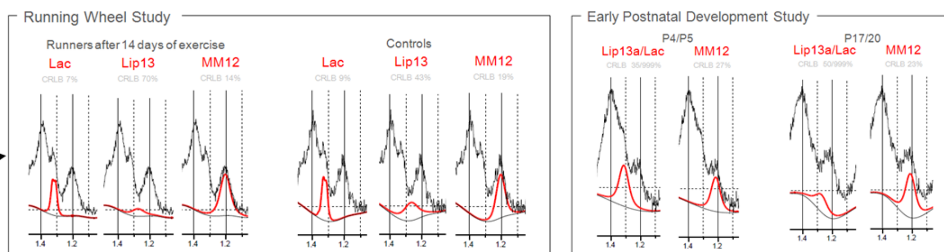


Fig. 1: Averaged group spectrum of the runner group after the running period. Black: measured spectrum and spline fitted baseline, red: LCModel fit, upper panel: fit residuals.

Fig. 2: Decomposition of the fitted signal from the boxed spectral area of interest of Fig. 1 into its metabolite components. Shown are the contributions of the **Lip13a+b** signal (1.28 ppm biomarker), macromolecules (**MM12**) and lactate (**Lac**), which may confound quantification of the small putative biomarker signal.

Results and Discussion: Quality of the MR spectra: Stable, reproducible spectra of high quality were obtained from all animals. Group averaged spectra showed improved SNR (factor of √(n=number of animals)) and detection reliability (CRLBs) compared to single subject data, which confirms high consistency and quality of the MR spectra [Fig. 1]. Neurochemical profile: Group averaged spectra were analyzed for differences in metabolite concentrations between groups. Runners and controls at baseline (1st MRS) and after the experimental running/control period (2nd MRS) did not reveal significant differences. In contrast, group spectra of the P17/P20 vs. P4/P5 group exhibited significantly larger concentrations of NAA, tCr and Glx and decreased levels of Tau, as known from literature (e.g. Tkac et al., Magn Reson Med 2003). 1.28 ppm biomarker: Concentrations of the putative biomarker were assessed through the contribution of the 'Lip13a+Lip13b' metabolite component of the modeled basis spectra. Comparison between runners and controls revealed a larger 1.28 ppm signal contribution in the control group, however with estimated standard deviations of SD >> 20% these signal estimates do not represent a reliable quantification [Fig. 2, left]. In the early postnatal development study the Lip13a+b signal could not be separated from the adjacent lactate component [Fig. 2, right]. In summary, in all spectra the biomarker could not be assessed reliably, regardless of whether neurogenesis was enhanced, either by exercise in the running wheel or at very early postnatal age. Corroborative evidence and data analysis: In contrast to Manganas et al. we analyzed our data with LCModel rather than with the originally proposed SVD algorithm, which as yet is not publicly available. Therefore, differences in the data analysis might explain that in our study the 1.28 ppm signal could not be quantified with appropriate statistical power. It is of interest that a similar approach to ours using an electroconvulsive shock (ECS) paradigm to boost neurogenesis and different anesthesia regimens has recently been presented (Smith et al., ISMRM2010). Their data revealed differences in several cerebral metabolites in the ECS group as compared to sham treated animals, but the 'Lip13a+Lip13b' as a measure of the 1.28ppm contribution could also not be estimated reliably using LCModel.

Using robust neurogenesis stimulation paradigms, state of the art MRS technology and optimized MRS methodology combined with LCModel spectral analysis, we found the putative 1.28 ppm biomarker too elusive as to justify its routine use as a robust biomarker.