# Betainterferon treatment: Absolute Quantification of White Matter Metabolites in Patients with Multiple Sclerosis

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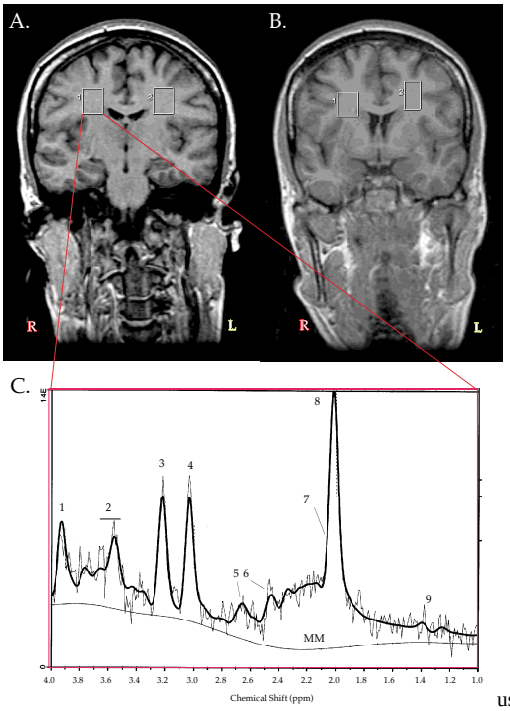



*Fig. 1 Schematic placement of the four PRESS-voxels (typically 4.9 mL) in white matter (A and B), and an example spectrum (C). Assignments: 1. Cr (methylene); 2. myoIns; 3. Cho (trimethyl); 4. Cr (methyl); 5. & 6. NAA; 7. NAAG (methyl); 8. NAA (methyl); 9. Lac. MM is the protein baseline.*

## *INTRODUCTION*

*Multiple Sclerosis (MS)* is a neurological disease of the central nervous system, where lesions appear in the white matter. This can be visualized using MRI. There are also reasons to believe that the normal appearing white matter (NAWM) (which appears normal using routine clinical MRI), is afflicted by certain impairment, which however will not give rise to differences in image contrast. MS is an inflammatory demyelinating disease for which several treatments that focus on retarding the progress of the inflammatory processes has been devised. We have investigated the dynamics of metabolite concentrations in white matter before and after treatment (exceeding three years for some subjects) of patients using beta-interferons. We wished to investigate how the absolute concentrations of different metabolites changed over extended treatment. And also if it was possible to reach a stabilization of the concentrations of metabolites that are known to be affected by the natural course of the disease (esp. NAA, Cho, *myo*Ins). We even hypothesized that NAA might increase as an effect of treatment. Limited data are available from similar studies, such as [Narayanan, 2001; Schubert, 2002], although typically not performed in an absolute quantitative manner, and for an extended length of the treatment.

## *MATERIALS AND METHODS*

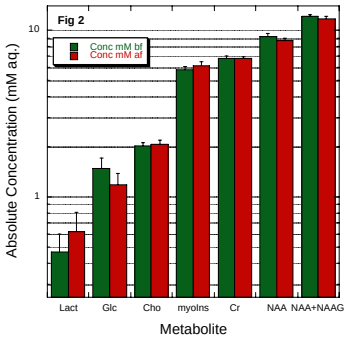
*Subjects* We included a series of patients who were scheduled for immunomodulatory treatment. The intention was to examine these patients prior to, after three months, and also after an extended period of treatment with betainterferons. All subjects a diagnosis of Clinical Definite MS according to the Poser criteria, but two were MR negative, still matching all clinical and CSF demandments for MS diagnosis. We also included a few untreated control-subjects (these patients declined the treatment for various reasons), the results of which are not reported here. Sixteen patients were examined and the average period of treatment was 2.0 ± SE 0.2 years (average age was 43 ± SE 2 years).

*MR* The MRI as well as the $^1$H-MRS examinations were performed on a GE Signa Horizon Echospeed Plus 1.5 T MR scanner (General Electric, Milwaukee, Wisconsin, USA) using a standard quadrature head coil. Axial scans for evaluating the presence of lesions, were obtained using a dual echo Fast Spin Echo (FSE). Providing both proton density (TR = 2300 ms, TE = 14 ms), and T2 weighted images (TR = 2300 ms, TE = 98 ms). Coronal T1 weighted scans for placement of the four spectral voxels were acquired using a 3D Fast Spoiled Gradient Recalled acquisition in steady state sequence (FSPGR), TR = 14.1 ms, TE = 5.4 ms, FOV = 35 cm with 4 mm slice thickness, using a flip angle of 20°. PRESS (Point Resolved Single Voxel Spectroscopy) was used to obtain $^1$H-MRS spectra at 63.87 MHz, using TR = 6.0 s, TE = 35 ms, with voxel dimension ca. 17x17x17 mm$^3$ (= 4.91 mL), and 64 transients. The PRESS version used was Probe-p (Proton brain exam-PRESS provided by the manufacturer), in which 167° pulses are used for the inversion pulses in order to achieve optimal bandwidth, slice profile, as well as power usage. Water suppression was obtained using CHESS (Chemical Shift selective imaging using Stimulated echoes). Four voxels were used for the MRS examination (see Fig. 1).

*Absolute quantification* The quantification of metabolite concentrations was performed using modified procedures [Gustavsson, 2007] based on Helms work [Helms, 2000], the main modification was that PRESS localization technique is used instead of STEAM providing a two-fold gain in signal to noise ratio. The most important part of the method was that the local sensitivity of the RF-coil used (for both transmission and reception of RF signal) was measured in each voxel. The reciprocity principle states a proportional relation between the sensitivity during transmission and reception in the NMR experiment and based on the local sensitivity measurement, a correction factor used for scaling of the received signal is obtained. LCModel was used for analysing the spectra, including to correct for a common protein baseline (see Fig. 1), and a two-sided paired t-test was used for testing the concentration differences for statistical significance.

Table 1. Absolute concentrations (mM aq.)

| | BEFORE | SD | AFTER | SD |
|---|---|---|---|---|
| Cho | 2.02 | 0.38 | 2.07 | 0.44 |
| Cr | 6.77 | 0.86 | 6.75 | 0.81 |
| *myo*Ins | 5.78 | 1.12 | 6.12 | 1.31 |
| NAA | 9.19 | 1.51 | 8.67 | 1.29 |
| NAA+NAAG | 12.05 | 1.45 | 11.75 | 1.49 |
| Glc | 1.48 | 0.97 | 1.18 | 0.82 |
| Lact | 0.47 | 0.53 | 0.62 | 0.76 |




## RESULTS

The absolute aqueous concentrations of metabolites in NAWM before and after treatment are shown in Table 1 and Fig 2. Note that these concentrations reflect the conditions in the aqueous phase, after the above-mentioned corrections. The absolute concentration of NAA was significantly (p = 0.02) lower (mean difference -0.52 mM aq.) in NAWM of MS patients after about two years treatment. In addition, patients showed higher concentration of *m*Ins (p = 0.03; mean difference +0.340 mM aq.). The average glucose concentration was also significantly lower after extended treatment.

## DISCUSSION

The lowered NAA and increased *myo*Ins concentrations is in line with the natural course of the disease, which is described by several investigators, mostly in untreated materials. From our results presented here it is not possible to confirm that the natural course of the disease slowed down as a consequence of the treatment, as it is not possble to compare the data with a matched set of control subjects (placebo). The only comparsion that is ethically possible, is to compare the patients with other patients that are untreated for a variety of individual reasons. This constitutes an investigational problem since such patients have particular reasons for not being treated, independent of if they are affected by a very mild disease, or by a severe disease; they are thus not suitable as a matched control material. Nevertheless for ethical reasons, it is clear that patients cannot be kept untreated because of the beneficial effect of the medication. We therefore plan to compare our data with results in other studies on treated patients, although these have only been treated for a brief period compared to the treatment period that is reported here. We will also perform a individual comparison by comparing the disability degree and metabolite development. In particular, we find the significantly lowered glucose concentration interesting, especially considering that the lactate concentration increased (although the latter was not statistically significant). This may be an effect of the natural course of the disease. Glucose has been a recent focus in MS research as a possible marker of inflammation, for example in the cerebrospinal fluid.

## LITERATURE

**Gustavsson, M., et al** (2007) Am J Neuroradiol, **28**:1306-12.; **Helms G.** (2000) NMR Biomed,13: 398-406.; **Sarchielli P, et al.** (1998) J Neurol Neurosurg Psychiatry, 64: 204-212.; **Schubert F, et al.** (2002) Magma 14: 213-222.