# The influence of the external magnetic field strength on correlations between metabolites

**R. Taylor[1,2], P. Williamson[1,3], and J. Théberge[1,2]**
[1]Medical Biophysics, University of Western Ontario, London, ON, Canada, [2]Lawson Health Research Institute, London, ON, Canada, [3]Psychiatry, University of Western Ontario, London, ON, Canada

**Introduction:** An *in-vivo* $^1$H-MRS spectrum consists of many metabolite spectral signatures that often have significant degrees of overlap. This can make it difficult to reliably quantify these metabolites separately. Recent advances in technology have allowed the development of ultra-high field 7T magnets. It is known that as magnetic field strength ($B_0$) increases, so does the spectral dispersion in a linear manner. Additionally, strongly coupled metabolites at low field strengths are not as strongly coupled as $B_0$ increases. For example, the main excitatory neurotransmitter, glutamate (Glu), and its precursor, glutamine (Glu), which are of interest in many neuropsychiatric disorders, are severely overlapping at 1.5T. When $B_0$ is increased to 7T it becomes possible to visually discern the resonances from the protons on the C4 carbon of both metabolites. However, even at 7T, there is still strong overlap in the resonances corresponding to the C3 and C2 protons. Glu and Gln are not the only metabolites with strongly overlapping spectral signatures. In fact, there are many other metabolites that overlap with Glu and Gln, including GABA, glutathione (GSH), NAAG, and Myo-inosital (Myo). No study, to this author's knowledge, has characterized how the correlations between metabolites in *in vivo* spectra change with field strength. The purpose of this study is to examine how independently we can quantify metabolites in a $^1$H-MRS spectrum and how separate quantification is affected by increasing $B_0$ and SNR. It is generally expected that the magnitude of correlations between metabolite estimates will decrease as $B_0$ increases, particularly in metabolites that experience strong coupling at low $B_0$.

**Methods:** Simulations were produced for 22 metabolites (Glu, Gln, NAA, NAAG, Cr, PCr, Myo, Cho, PCho, GPCho, GABA, Ser, Gly, Scy, Peth, α-glc, β-glc, Asp, Ala, GSH, Lac, Tau) using software based on the GAVA simulation environment[1] and modified in-house (JT). They were made to represent true *in vivo* spectra for four common $B_0$, 1.5T, 3T, 4T, 7T (Fig. 1), acquired with an ultra-short echo STEAM sequence (TE=6ms TM=32ms) with the number of transients (nt)=64 and nt=256. J-coupling constants and chemical shifts were found in the literature[2],[3]. An empirically determined macromolecule (MM) signature was added to each template as well. Global constraints were set for phase ($0^{th}$ and $1^{st}$ order), chemical shift, and LW, but all metabolites had independent amplitudes. An additional chemical shift constraint was placed on the MM signals, leading to a total of 27 fitting parameters. SNR was determined relative to the height of a reference peak equivalent to the NAA $CH_3$ singlet at 2.01ppm often used to measure SNR. This peak was placed at 0 ppm sufficiently offset from the metabolites so as to not interfere with the quantitation. The literature suggests at nt=1 an SNR of 20-25 is possible at 7T[4]. The average of these values (22.5) was used to calculate the SNR for the 7T templates from the common SNR-nt relationship, $SNR_2/SNR_1=(nt_2/nt_1)^{0.5}$. The SNR for the remaining $B_0$ templates were determined following the assumption of linear SNR change with $B_0$. This resulted in SNRs of 360 and 180 (7T), 206 and 103 (4T), 154 and 77 (3T), 77 and 38.5 (1.5T) for nt=256 and nt=64, respectively. Lorentzian linewidths (LW) were set using the observation that LW increase at 1.35 Hz/T[5] resulting in 9.45Hz, 5.4Hz, 4.05Hz, and 2.025Hz for 7T, 4T, 3T, and 1.5T, respectively. To ensure reliable results, 200 noisy realizations were produced and fit using our fitting software previously described in the literature[6] for each $B_0$ template. This resulted in a total of 1600 simulations. Pearson's correlation were calculated and Sidak's adjustment for multiple correlations corrected the alpha value accordingly ($p<0.00004$ from $p<0.01$).

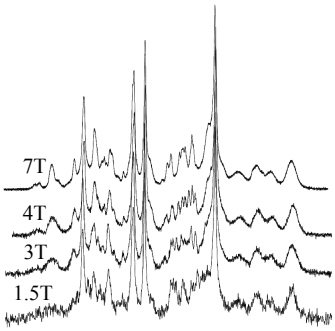


*Figure 1. Example spectra for each $B_0$ with SNR= 360 (7T), 206 (4T), 154 (3T), and 77 (1.5T)*

| $B_0$ / SNR | +'ve | -'ve | total |
|---|---|---|---|
| 1.5T / 38.5 | 9 | 22 | 31 |
| 1.5T / 77 | 8 | 21 | 29 |
| 3T / 77 | 4 | 13 | 17 |
| 3T / 154 | 5 | 11 | 16 |
| 4T / 103 | 3 | 8 | 11 |
| 4T / 206 | 3 | 9 | 12 |
| 7T / 180 | 5 | 4 | 9 |
| 7T / 360 | 3 | 6 | 9 |

*Table 1. A summary of all the significant correlations ($p<0.01$) found at each $B_0$ and SNR.*

**Results and Discussion:** Metabolite correlations tables were produced for each $B_0$ and SNR template (253 possible correlations per template). Table 1 shows the total number of significant positive and negative correlations observed in each case. Most correlations found were negative. It is clear that metabolite correlation generally decreases as $B_0$ increases. For example, Glu and Gln are significantly correlated at 1.5T, 3T, and 4T, but not at 7T. Other main significant negative correlations and how they change with $B_0$ are shown in Fig. 2. The presence of strong negative correlations implies that for any metabolite area estimation that is overestimated or underestimated there will be an opposite estimation error in the other metabolite. These negative correlations make sense because any error in area estimation will leave more or less area to be accounted for by other metabolites in the spectrum. Positive correlations, particularly at lower field strengths, can result from a chain effect. For example, if metabolite A partially overlaps metabolite B and is under fit, then metabolite B may be over fit. If metabolite B partially overlaps metabolite C, then metabolite C may be under fit, giving it a positive correlation with metabolite A. Not every significant correlation improved as $B_0$ increased. α-glc and β-glc stayed relatively constant, as did the strong positive correlations observed from Cr and PCr, and from Cho and PCho. NAA is the only metabolite that is not significantly correlated with any other metabolite at any field strength, likely due to the simplicity of fitting the strong $CH_3$ singlet. Increasing the SNR in the same $B_0$ did not have an overwhelming effect on the number of significant correlations as they were generally about equal. Achieving high SNR is important because precise quantification will minimize the effect of significant correlations. Comparison of $B_0$ at equal SNR (SNR=77 at 1.5T and 3T) showed improvements in the coefficients of variation (CV). Although this effect was variable throughout the metabolites, the average improvement was a factor of 1.76 when going from 1.5T to 3T. Otherwise, inversely proportional relationships exist between SNR and CV when compared at the same $B_0$ (not shown). Therefore, improvements in CV can be expected when going up in field strength from both the increased SNR and other intrinsic factors that improve the metabolite spectra as $B_0$ increases. Having a full set of metabolites *in vivo* increases complexity to the correlations between metabolites because multiple interactions can occur, effectively reducing the apparent correlation between two metabolites. Therefore, the correlations in this study should be interpreted as a lower limit. Additionally, exact seeding of metabolite fitting parameters was used to concentrate on the effect of noise alone. This study will be extended in the future to explore both the effect of inexact seeding on metabolite correlations and the interaction of multiple metabolites (3 or more rather than pair-wise).

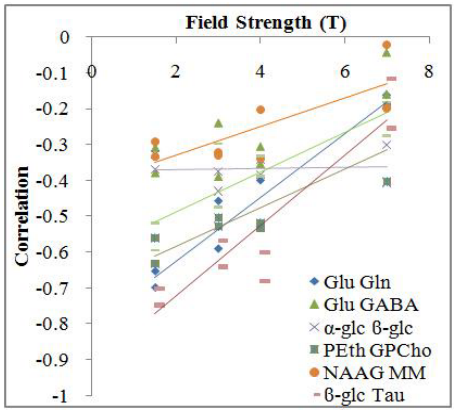


*Figure 2. Some of the main negative correlations found between metabolites as a function of $B_0$ are displayed with linear lines of best fit. Interacting metabolites are shown together in the legend.*

**Conclusion:** This study demonstrates quantitatively for the first time the general decrease in the magnitude of correlation as $B_0$ increases. This effect is most notably observed in coupled metabolites such as Glu and Gln. However, some significant correlations, such as α-glc and β-glc, do not weaken as $B_0$ increases. This study further demonstrates the potential benefits of going to high field in MRS.

**References:** [1] Soher BJ, et al. *J Magn Reson*. 185: 291-299 (2007). [2] Govindaraju V, et al. *NMR Biomed.* 13: 129-153 (2000). [3] Krawcsyk and Gradowska. *Journal of Pharmaceutical and Biomedical Analysis* 31:455-463 (2003). [4] Mangia et al. *J Cereb Blood Flow Metab* 27: 1055-1063 (2007). [5] Deelschand et al. *Journal of Magnetic Resonance* 206: 74–80 (2010). [6] Bartha et al. *NMR Biomed* 12:205-216(1999).