# The Linear Relationship between Cross-peak Volume and Concentration of Metabolites in 2D Localized MR Spectroscopy

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***Purpose:***

In the last decade, some effort was devoted to develop two-dimensional localized Magnetic Resonance Spectroscopy (2D L-MRS). MRS can evaluate the metabolites for *in vivo* clinical applications. However, there are many technical factors that could affect the quality of the spectrum, and lead to difficulty in interpretation. If the concentration of metabolites can be quantified, it may provide more reliable information (or to reveal the change of metabolites), which is directly related to the pathological conditions of certain diseases and disorders. The 2D localized MR Correlation Spectroscopy COSY (2D L-COSY) and 2D localized PRESS have capability of distinguishing overlapping metabolites because of their better spectral dispersion of J-cross-peaks [2,3] compared to traditional 1D MRS method. As such, they also provide a suitable method for quantification of metabolites. The linearity of the cross-peak volume integral with increasing metabolite concentration in 2D MRS was reported by Alonso et al. [1]. However, the measurements were done using phantoms by using ***non-localized*** phase-sensitive double-quantum filtered 2D COSY MRS method. The purpose of this study is to investigate the linear relationship between the cross-peak volume and metabolite concentration in two-dimensional ***localized*** MR Spectroscopy methods.

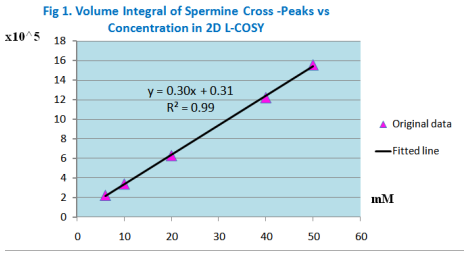
***Materials & Methods:***

All experiments were performed on a 4T whole-body scanner integrated with a SMIS console (Surrey Medical Imaging System, UK). A custom-made birdcage coil was used for both RF transmission and signal detection. The linearity of the cross-peak volume integral vs. metabolite concentration was assessed by studying solutions that contained five different concentrations (6, 10, 20, 40, 50 mM) of polyamine spermine, with pH=7.0. Another set of composite phantoms that contained polyamine spermine, choline, and creatine with 20, 40, 60 mM concentrations were also prepared. Two 2D localized MR spectroscopy sequences were tested in this study: 2D L-COSY and 2D L-JPRESS. The parameters for 2D L-COSY(90°-180°-90°) were: TR = 2 sec, $TE_{min}$= 28ms, Δ=8ms, Δ′= 6ms, and 64 $t_1$ increments, 1024 complex points. Voxel size 15x15x15 $mm^3$. The spectral window was 2.5 kHz in $F_2$ and 1.25 kHz along $F_1$ dimension. An 8-step phase cycling scheme was used on all three pulses. The parameters for 2D L-JPRESS(90°-180°-180°) were: TR = 2 sec, $TE_{min}$= 32ms, Δ=8ms, Δ′= 6ms, and 64 $t_1$ increments, 1024 complex points. The voxel size was 15x15x15 $mm^3$. The spectral window was 2.5 kHz in $F_2$ and 200 Hz along $F_1$ dimensions. A 16-step phase cycling scheme was used on all three pulses. The self customized MATLAB (Mathworks, Inc., USA) programs and matNMR (van Beek [4]) were utilized to analyze the spectra. The raw data was apodized with sine-bell windows along $t_1$ and $t_2$ and zero-filled to 128 x 1024 prior to 2D FFT.

For spermine, the cross-peak at 1.8ppm/3.1ppm, 2.1ppm/3.1ppm in 2D L-COSY spectrum and J-resolved cross-peaks around 1.8ppm, 2.1ppm and 3.1ppm in 2D L-JPRESS were integrated for linear regression analysis. For choline, 3.55/4.05ppm cross-peak in 2D L-COSY spectrum and J-resolved peak around 3.55pm in 2D L-JPRESS spectrum were used for linear regression analysis. Creatine diagonal peak at 3.1ppm was used as reference.

***Results:***

The linear relationships between the metabolite concentration and the integrated cross-peak volume measured by 2D Localized COSY are shown in Fig.1, and the results measured by 2D L-JPRESS are shown in Fig.2. The linear relationship is seen in both figures, indicating that both techniques could be applied for quantitative measurement of the metabolite concentration. We also investigated whether the linear relationship still holds using a composite phantom containing mixture of 3 metabolites. The linear relationship between integrated cross-peak volume and intensity of creatine diagonal peak is shown in Figure 3. The integrated choline cross-peak volume vs. concentration also showed a linear relationship (results not shown graphically, $r^2$ between 0.97~0.98).



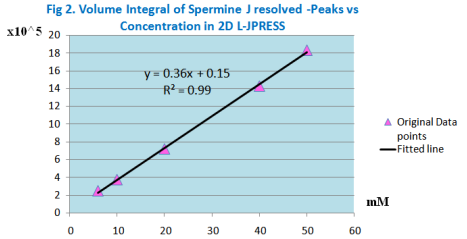




Fig.1 and 2 demonstrates the linear relationship between the concentration of spermine and the integrated cross-peak volume measured by 2D L-COSY and L-JPRESS.

***Discussion:***

The linear relationship between the integrated cross-peak volume and metabolite concentration in 2D Localized COSY and JPRESS was demonstrated. The linear correlation coefficient, $r^2$, was between 0.97~0.99, which shows very strong linearity. When diagonal-peak from creatine was used as a reference, the results indicate that diagonal peak of creatine can be used as an internal reference to quantitatively measure the change in the concentration of spermine and choline for *in vivo* applications, particularly for the prostate. An internal reference or external reference is usually used for quantification. The basic requirement for a reference peak, either internal or external is that its intensity needs to remain constant under different conditions. Creatine is an ideal metabolite to be used as internal reference signal in most of the biological systems since the creatine exists in most of the biological systems, and creatine peak usually remains constant in healthy and diseased (e.g. cancerous) tissue. It is commonly used for the MRS studies in the brain. However, since the creatine peak is very close to choline and spermine, the 1-D MRS technique may not be able to differentiate the peak to provide the reference for MRS of prostate. For two-dimensional MRS studies, the metabolite of interest must have one or more J coupling constants which was shown as scalar connectivity in COSY that can be detected by 2D L-COSY or J –resolved peak which can be detected by 2D L-JPRESS. Both 2D L-COSY and 2D L-JPRESS showed very good linear relationships. Our results also show that both spermine and choline correlation cross-peaks in 2D L-COSY spectrum showed better dispersion compared to spermine and choline J-resolved peaks in 2D L-JPRESS spectrum. In 2D L- JPRESS spectrum, choline J resolved peaks were close to background noise, while in 2D L-COSY spectrum, the cross-peaks of spermine and choline were well dispersed, which makes the quantitative analysis more reliable.

In summary, our results showed that the integrated cross-peak volumes in 2D Localized MR spectroscopy methods such as 2D L-COSY and 2D L-JPRESS spectra were proportional to the concentration of metabolites. The results are consistent with a previous study using non-localized 2D MRS [1]. The cross-peak volumes of metabolites in 2D Localized MRS can be used for quantitative analysis of metabolite ratio as in 1D MRS method for clinical applications.

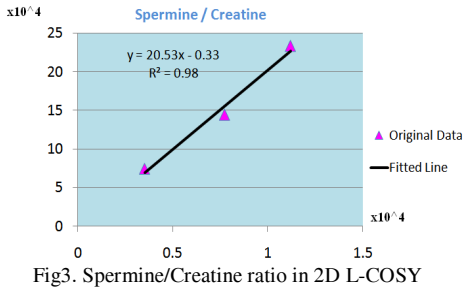



Fig3. Spermine/Creatine ratio in 2D L-COSY

***References:*** [1] Alonso et al. Magn. Reson. Med.(MRM) 1989:11:316-330 [2] Thomas et al. MRM 2001; 46:58-67. [3]. Yue et al. MRM 2002; 47:1059-1064. [4]. van Beek et al. J Magn Reson. 2007;187(1):19-26.