Assessment of Relative and Absolute Quantification Methods in Phosphorus Magnetic Resonance Spectroscopy

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Introduction: Phosphorus (^{31}P) MRS measurements are typically reported as relative concentrations and less often as absolute concentrations[1]. Absolute concentration methods include using internal or external references and phantom replacement methods[2]. Relative concentration methods include ratios to PCr, the sum of the three ATP peaks, or the total phosphorus signal[3,4]. This study compares three different methods of referencing phosphorus data and applies each referencing method to a subset of our control and patient schizophrenia data[5] to test whether different referencing methods can lead to different significant findings.

Methods: Informed, written consent according to the guidelines of the Health Sciences Research Ethics board at the University of Western Ontario, was obtained from all participants. Data was previously collected for our ^{31}P MRS studies of schizophrenia[5]. Scans were acquired on a 4.0 T whole body research scanner (Varian/ Siemens/UnityINOVA). A ^{1}H quadrature head-coil was used for shimming and to acquire sagittal, coronal (2D-FLASH) and transverse (3D MPRAGE) images for ^{31}P voxel location and to determine the grey matter, white matter and CSF ratios within these voxels. The ^{1}H coil was replaced with a ^{31}P quadrature head-coil, while the subject remained in the scanner. Localized ^{31}P spectra were acquired from 15cc effective voxels using an optimized 3D chemical shift imaging sequence with a spherically bound, random point omission, weighted k space. (TR = 500ms; pre-acquisition delay time = 1.905 ms; tip angle = 32°; matrix size (x, y, z) = 14X14X14 (zero-filled to 16X16X16); FOV (x, y, z) = 280 mm; data readout time = 400 ms)[6]. After left shifting 5 time domain points to remove the broad membrane baseline component, unfiltered spectra were fit in the time-domain using a non-linear, iterative fitting program based on the Marquardt-Levenberg algorithm using prior spectral knowledge[6]. T_2 weighting was removed from the fitted data by the fitting algorithm which automatically extrapolated metabolite amplitude values back to t=0. T_1 values for each metabolite were used to correct partial saturation due to the short TR.

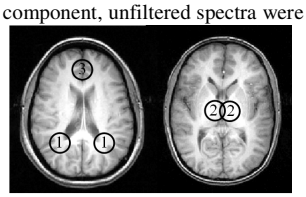

Figure 1. Voxel placement. 1) left & right parietal occipital; 2) left & right thalamus; 3) anterior cingulate

Metabolite concentrations were obtained with three different referencing methods:1) absolute concentrations using an external reference standard of 270 mM methylene diphosphonic acid which was separately corrected for T_1 saturation, RF-coil B_1 profile, reciprocity and reference–tube point spread function geomtery[6]; 2) relative concentrations by normalizing the data to total PCr plus ATP; 3) relative concentrations by normalizing data to total spectral signal. The three methods were analyzed by calculating and evaluating coefficients of variance and correlations from the left and right parietal occipital and left and right thalamus (Figure 1.). Finally the three methods were applied to the anterior cingulate (AC) and left thalamus (LTH) of patients and volunteers from our first episode studies[5,7] to determine the impact on statistical significant differences between groups. SPSS version 16.0 for Windows was used for all statistical calculations.

		Peth	PCh	GPeth	GPCh	PCr	γ-ATP	α-ATP	β-ATP
LTH	External	32.02	52.81	41.00	31.05	34.67	23.53	33.28	25.88
	PCr + ATP	23.77	38.30	34.88	24.26	19.27	10.86	22.92	24.92
	Total ^{31}P	18.76	37.47	32.67	18.43	25.88	15.56	23.22	28.68
RTH	External	31.23	55.07	44.78	41.20	43.69	30.00	28.63	35.21
	PCr + ATP	47.13	53.97	42.17	34.39	29.07	12.42	21.46	32.50
	Total ^{31}P	41.43	53.56	37.73	34.79	31.27	15.96	16.31	28.30
LPO	External	27.83	43.79	39.52	25.99	29.90	32.25	35.76	33.42
	PCr + ATP	29.23	46.64	36.37	29.72	11.31	10.52	12.82	15.12
	Total ^{31}P	25.33	46.93	38.08	27.67	17.30	16.98	21.81	16.60
RPO	External	38.99	43.12	30.66	27.71	31.73	41.34	42.17	52.11
	PCr + ATP	22.40	46.10	38.82	40.10	18.80	18.37	14.70	24.37
	Total ^{31}P	25.30	39.77	34.39	39.75	25.39	17.72	20.49	25.22

Table 1. Coefficients of variation(%). LTH: left thalamus, RTH: right thalamus, LPO: left parieto-occipital, RPO: right parieto-occipital.

Results: The relative methods of referencing returned the best coefficients of variation. Referencing to total phosphorus had the smallest coefficients of variation for the phosphomonoesters, Peth and PCh, and the phosphodiesters, GPeth and GPCh (Table 1.). Referencing to ATP + PCr had the smallest coefficients of variation for high energy metabolism PCr, α-ATP, β-ATP and γ-ATP.

Correlations between the two relative methods were high with r values ranging from 0.843 -0.989 ($p < 0.000001 – 0.000042$) for the phosphomonesters and phosphodiesters. Correlations between the absolute and PCr + ATP methods ($r = 0.642 - 0.895$, $p = 0.000003 - 0.055$) were higher than correlations between the absolute and total phosphorus methods ($r = 0.409 – 0.870$, $p = 0.000027 – 0.116$).

Results for the application of the three referencing methods to the first episode study data are summarized in Table 2. In the anterior cingulate, the observed increase in GPCh in patients as compared to controls was significant for all three referencing methods. In the left thalamus, PCh was decreased in patients as compared to controls, but was only significant for the total phosphorus referencing method.

	Region	AC	LTH
	Metabolite	GPCh	PCh
External Reference (mM)	Patient	0.9866 (0.350)	0.2244 (0.079)
	Control	0.5615 (0.255)	0.2961 (0.156)
	p-value	0.00026	0.108
	% difference	75.70	31.98
PCr + ATP	Patient	0.1523 (0.053)	0.0721 (0.036)
	Control	0.0853 (0.039)	0.0928 (0.034)
	p-value	0.00019	0.055
	% difference	43.99	28.71
Total Phosphorus	Patient	0.0727 (0.021)	0.0378 (0.015)
	Control	0.0432 (0.020)	0.0485 (0.018)
	p-value	0.00018	0.040
	% difference	40.54	28.49

Table 2. Comparison of different referencing methods Absolute: 1) external reference; Relative: 2) PCr + ATP 3) total phosphorus. Metabolite values for patients, controls, p -value and % difference.

Discussion: The high coefficients of variation from the absolute method may result from the numerous corrections made to both the data and external reference as error may be introduced with each correction. The correlations between the two relative methods were high as both depend on the large values of PCr and ATP relative to the smaller contribution from Peth, PCh, GPeth and GPCh. Correlations between the relative and absolute methods were not as high and may be explained by either subject to subject variation in total phosphorus and PCr + ATP and/or errors introduced by corrections when using the external reference method. When comparing the significance in the patients versus controls study for each region determined by the three different referencing methods, the external reference was the most conservative, followed by referencing to PCr +ATP and the least conservative, referencing to total phosphorus. When determining which reference method to use in a clinical study, the possibility of false significant results, when referencing to total phosphorus or the possibility of significant differences being overlooked, when using the external referencing method must be considered, particularly when metabolite differences are near the $p < 0.05$ level of significance.

There are obvious benefits and drawbacks to using each method. Metabolite concentrations can be obtained from the absolute method and therefore can be compared between studies, but there is the possibility of evaporation or contamination of the external reference over time. There is also the assumption that there are no geometric changes in the coil's RF field over time or from subject loading. The relative methods are easily applied and several systematic errors are removed. However, this method makes comparisons between studies difficult. In addition, these methods rely on the assumptions that there are no changes in *in vivo* T_1 or T_2 reference metabolites, and that concentrations of total phosphorus or PCr + ATP remain constant which may not be the case in certain pathologies or over a wide subject age range. Also, changes in PCr would likely be missed because of it's ratio to a relative reference dominated by PCr, however this is not an issue for this study as only changes in phosphomonoesters and phosphodiesters were being measured

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[1] Tofts PS et al, *NMR in Biomed* 1(1):1-10 (1998)
[2] Klunk, WE et al, Neurobiology of Aging, , 15(1) 133-140 (1994)
[3] Volz HP'Biol Psychiatry. Mar 15;41(6):644-8 (1997).
[4] Jayakumar PN et al, Biol Psychiatry. Aug 15;54(4):491-4 (2003)
[5] Jensen JE et al., *Br J Psychiatry,* 184:409-15 (2004)
[6] Jensen JE, et al, *NMR Biomed,*15:338-347(2002)
[7] Jensen JE, et al. *Br J. Psychiatry*; 180:39-44 (2002)