In vivo L-COSY Identifies Neurochemical Changes in Professional Athletes with Repetitive Head Injuries

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Introduction: Approximately 3.8 million sports-related concussions occur in the USA each year. The long term effects of repetitive head injury such as those suffered by professional athletes has recently become of increasing concern given evidence of a neurodegenerative disorder called chronic traumatic encephalopathy marked by the accumulation of phosphorylated tau proteins in post-mortem studies(1). Our goal is to develop an *in vivo* non-invasive test that can be used to diagnose disease in lifetime of the athletes with repetitive head injury. Magnetic resonance spectroscopy is an ideal tool for measuring changes in brain biochemistry however conventional MRS has the disadvantage of overlapping resonances that can obscure specific metabolites. Jeener introduced 2D COrrelated SpectroscopY (2D COSY) in 1971(2) by proposing a simple two-pulse sequence (90x-t1-90x-Acq) which, after 2D Fourier transformation, produced a map of crosspeaks that represent the scalar couplings between the two protons it connects on the diagonal. Resonances that were composite in the 1D spectrum are not separated in a second dimension. This method when applied in vitro to cells an biopsies provided important information on the development of human diseases(3). The localized (L) COSY method has now been extended to examine the human brain *in vivo* by Thomas(4) and Ramadan(5). Our goal was to apply the L- COSY methods in professional athletes with a history of repetitive head injury to identify neurochemical changes that may be associated with chronic traumatic encephalopathy.

Methods: Five retired professional male athletes (mean age 43.6 $\pm$ 10.8 years) with 11-25 years (mean 8.2 $\pm$ 4.4 years) of exposure to head injury in contact sports (included collegiate and professional football, wrestling, and boxing), were scanned. Each athlete had experienced multiple concussions (including several with loss of consciousness) as well as repetitive subconcussive head trauma during their career. All five former athletes have reported clinical symptoms associated with either prolonged post-concussive syndrome or possible CTE including self-reported headaches, memory loss, confusion, impaired judgment, impulse control problems, aggression, and depression. Five healthy men (age and weight matched to athletes) (mean age 45.2 $\pm$ 12.6 years) ($p > 0.05$), with no history of head trauma, were recruited as controls**.** L- COSY was acquired on a 3T clinical MR scanner (TIM Trio, Siemens, Germany) using a 12 channel head coil. A 3x3x3 cm^3 voxel was localized in the posterior cingulate gyrus with: RF carrier frequency at 2.0 ppm, TR 1.7 s, weak water suppression using WET, spectral width=2000 Hz, increments size of 0.8 ms in 96 t1 increments giving an indirect spectral width of 1250 Hz, 8 averages per increment, and 512 data points were acquired in 256 ms. Scan time was 22 minutes. Raw COSY data was transferred to Matlab. Commercial 2D spectral processing software (Felix-2007, Accelrys, San Diego, CA, USA) was used for spectral reconstruction and analysis. Zero-padding to double the original data size, followed by apodization with skewed sine-squared window functions, in both dimensions was applied prior to magnitude two-dimensional Fourier transform (2DFT). The 2D spectra were chemical shift referenced and scaled to the prominent singlet diagonal peak of Cr (F2 = F1 = 3.02 ppm).

Results and Discussion: Representative L-COSY from an athlete with repetitive head injury and age-matched control is shown in Figure 1. A multitude of metabolites, not visible to conventional 1D MRS, such as γ-aminobutytic acid (GABA), amino acids such as threonine (Thr), histidine (His), aspartate(Asp), and macromolecules. Table 1 compares the results of the 5 controls with each of the individual athletes. L-COSY showed significant increases ($p<0.05$) in choline (Cho; 13%) and the combined resonances of glutamate and glutamine (Glx; 11%) in athletes with repetitive head injury when compared with controls. In addition, L-COSY showed increases in excitatory neurotransmitters of glutamine and Asp ($p<0.10$) of 12% and 14% respectively in the athletes. The athletes also showed a 8% decrease in GABA and 14% decrease in histidine ($p<0.10$) both of which are inhibitory neurotransmitters. Threonine is a structural amino acid and expected to increase due to diffuse axonal injury as reflected in the 15% increase in Thr associated with repetitive head injury. Finally, a 17% increase in lipid and macromolecules is also observed which is likely to be associated with the phospholipid cascade initiated by phospholipase in head injury(6).

Figure 1

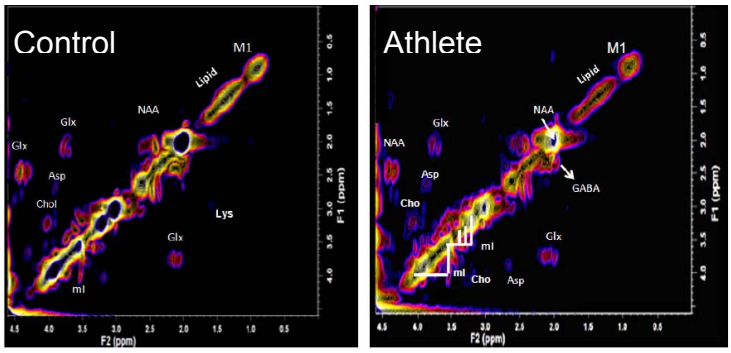

Table 1

Metabolite	*All Athletes*	1	2	3	4	5	*Control*
Choline**	**0.760 ± 0.041***	0.765	0.802	0.759	0.693	0.784	0.676 ± 0.019
Glx**	**0.105 ± 0.009***	0.105	0.114	0.093	0.113	0.097	0.094 ± 0.007
NAA	**1.31± 0.13**	1.41	1.47	1.18	1.29	1.19	1.24 ± 0.07
Glutamate*	**0.026 ± 0.004**	0.026	0.028	0.025	0.030	0.019	0.024 ± 0.003
Aspartate*	**0.048 ± 0.005**	0.041	0.055	0.045	0.050	0.047	0.043 ± 0.005
Threonine	**0.027 ± 0.005**	0.026	0.035	0.027	0.022	0.025	0.024 ± 0.003
GABA	0.026 ± 0.007	0.027	0.038	0.020	0.027	0.021	**0.029 ± 0.011**
Histidine*	0.024 ± 0.003	0.027	0.020	0.026	0.022	0.023	**0.027 ± 0.004**
Lipid	**0.176 ± 0.068**	0.184	0.113	0.121	0.221	0.242	0.151 ± 0.020
MM	**0.026 ± 0.005**	0.027	0.029	0.019	0.024	0.031	0.023 ± 0.005

** p<0.05; * p<0.10

Conclusion: This preliminary study demonstrates that L-COSY can measure a multitude of neurochemical changes in the MR visible metabolites in the human brain in professional athletes with a history of head injury. These results provide the basis for a larger study where L-COSY can contribute to a better understanding of the pathophysiology of repetitive head injury but more importantly may also provide a non-invasive, objective test for the early detection of chronic traumatic encephalopathy.

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