Measuring energy diffusion: phosphocreatine in human skeletal muscle

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Introduction The creatine kinase (CK) reaction plays a central role in energy transfer in the myocyte, shuttling high-energy phosphate (HEP) created in the mitochondria, to sites of utilization in the myofibrils [1,2]. At the mitochondria, the reverse reaction produces phosphocreatine (PCr) from adenosine triphosphate (ATP) and creatine (Cr). PCr diffuses to the myofibrils to regenerate ATP from adenosine diphosphate to fuel muscular contraction. The PCr energy transfer shuttle has been widely modeled. Knowledge of the diffusion coefficient of PCr, D_{PCr}, is key, but ill-defined in humans. Localized ^{31}P MRS is uniquely able to access and quantify PCr and forward CK flux noninvasively [3]. The aim of this study is to develop and optimize a ^{31}P MRS protocol for measuring D_{PCr}, and to characterize PCr energy transport in human muscle in relation to CK flux. We report D_{PCr} in 8 healthy subjects as a function of diffusion time (t_{diff}), and that PCr diffusion in muscle is anisotropic.

Methods A 3-pulse, stimulated echo sequence was chosen to permit D_{PCr} measurements with long t_{diff}'s (>>T_2) (t_{diff} =TM+32ms, TM is the mixing time). Diffusion encoding was provided via gradient lobes placed after the first and third RF pulses. Spectra were localized to a plane parallel to a 17-cm/8cm transmit/receive ^{31}P coil set using depth-resolved surface coil spectroscopy (DRESS) [4], with the first pulse slice-selective (TR/TE= 8000/80ms; bandwidth =500Hz). D_{PCr} was measured at t_{diff}'s corresponding to TM of 50,100,150,200,400,700, and 1000 ms, by acquiring one spectrum without diffusion gradients, and three spectra with diffusion gradients applied on the x, y, and z axes. The *b*-value was chosen to optimize precision of the D_{PCr} measurement ($1000 \le b \le 2000$ s/mm^2, $b = [\gamma.G.\delta]^2.t_{diff}$, with γ is the gyromagnetic ratio, G is the diffusion gradient strength, and δ is the diffusion gradient duration). Averaging was adjusted to maintain adequate and comparable signal-to-noise ratio for each acquisition. Peak areas were fitted using CFIT [5]. D_{PCr} was obtained in the three directions (D_{xx}, D_{yy}, D_{zz}) and the mean diffusivity D_{av} was calculated as $D_{av}=(D_{xx}+D_{yy}+D_{zz})/3$. The calf muscles of eight healthy subjects who gave informed consent were scanned on a Philips Achieva 3.0T system.

Table 1. *The diffusion coefficient of PCr in the calf muscle of eight healthy volunteers*

TM (ms)	D(x10^{-3} mm^2/s)			
	D_{xx}	D_{yy}	D_{zz}	D_{av}
50	0.44±.13	0.45±.10	0.81±.28	0.56±.12
100	0.41±.10	0.41±.10	0.58±.13	0.46±.08
150	0.43±.05	0.41±.06	0.60±.18	0.47±.07
200	0.46±.07	0.44±.07	0.57±.10	0.48±.04
400	0.39±.08	0.37±.09	0.51±.05	0.42±.06
700	0.38±.11	0.32±.11	0.40±.04	0.38±.06
1000	0.31±.12	0.30±.10	0.43±.08	0.35±.06

Results Fig. 1 shows typical spectra from one volunteer at TM=200ms. Table 1 lists average D_{xx}, D_{yy}, D_{zz}, and D_{av} for PCr as a function of TM for all subjects. D_{PCr} is significantly higher along the fiber direction (~z-axis) than transverse directions ($P \le 0.02$ for D_{zz} vs D_{yy} and D_{zz} vs D_{xx} at TM≤150ms). The slow decrease in D_{PCr} with TM suggests that diffusion is ultimately restricted.

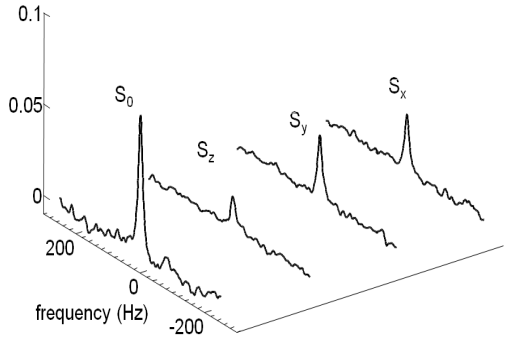

Fig. 1*. PCr spectra from the calf muscle using stimulated echo DRESS at TM=200ms, without (S_0) and with diffusion weighting along the three physical axes (S_x,S_y,S_z).*

Discussion We believe these are the first studies showing anisotropic and restricted PCr diffusion in human muscle. The values are comparable to data from animal limbs [6,7]. Prior ^{31}P saturation transfer MRS in healthy human muscle shows that ~27% of the PCr turns over through CK per sec [3]. This would yield a half-life for PCr of ~2.6s at rest. Our results show that during this half-life, PCr will have diffused ~65μm (extrapolated D_{av} ≈ 0.27 x10^{-3} mm^2/s). Based on rat muscle [8], the expected separation between mitochondria and myofibril is up to 2μm. If this is similar in humans, PCr diffusion would certainly be fast enough to transfer HEP between mitochondria and myofibrils. This situation may be altered in diseases that affect energy transfer.

References [1] Wallimann T. Curr Biol 1994; 4, 42. [2] Meyer RA et al. Am J Physiol Cell Physiol 1984; 246, 365. [3] Bottomley PA et al, Magn Reson Med 2002; 47, 850. [4] Bottomley PA et al, J Magn Reson 1984; 59, 338. [5] Gabr RE et al. J magn Reson 2006; 152. [6] de Graaf RA et al. Biophys J, 2000; 78, 1657. [7] Van Gelderen P et al. J Magn Reson B 1994; 103, 255. [8] Vendelin M et al. Am J Physiol Cell Physiol 2005; 288, C757.

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