High-energy phosphate transfer in human muscle: diffusion of phosphocreatine

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PHOSPHOCREATINE (PCr) serves as the main short-term energy reserve in muscle, heart, and brain. PCr generates ATP to fuel cellular processes, including ion transport and muscular contraction, via the creatine kinase (CK) reaction. The CK reaction is central to muscle energetics, buffering ATP levels during periods of intense activity via consumption of phosphocreatine (PCr). PCr is believed to serve as a spatial buffer of high-energy phosphate between sites of energy production in the mitochondria and sites of energy utilization in the myofibrils via diffusion. Knowledge of the diffusion coefficient of PCr ($D_{PCr}$) is thus critical for modeling and understanding energy transport in the myocyte, but $D_{PCr}$ has not been measured in humans. Using localized phosphorus magnetic resonance spectroscopy, we measured $D_{PCr}$ in the calf muscle of 11 adults as a function of direction and diffusion time. The result shows that the diffusion of PCr is anisotropic, with significantly higher diffusion along the muscle fibers, and that the diffusion of PCr is restricted to a ~28-$\mu$m pathlength assuming a cylindrical model, with an unbounded diffusion coefficient of ~0.69 × 10$^{-3}$ mm$^2$/s. This distance is comparable in size to the myofiber radius. On the basis of prior measures of the CK reaction kinetics in human muscle, the expected diffusion distance of PCr during its lifetime in the CK reaction is ~66 $\mu$m. This distance is much greater than the average distances between mitochondria and myofibrils. Thus these first measurements of PCr diffusion in human muscle in vivo support the view that PCr diffusion is not a factor limiting high-energy phosphate transport between the mitochondria and the myofibrils in healthy resting myocytes.

myocyte; energy metabolism; creatine kinase shuttle; human studies

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PHOSPHOCREATINE DIFFUSION IN THE HUMAN MUSCLE

METHODS

Diffusion MRS

The prevailing technique in MR encodes diffusion with a pair of magnetic field gradient pulses. This pulsed-field gradient method sensitizes the signal to the microscopic motion of the molecules, which translates into a phase dispersion, causing signal attenuation (41). This attenuation is modeled as a monoexponential decay

\[ S = S_0 \exp(-bD) \]  

where \( S \) and \( S_0 \) are the diffusion-weighted and reference signals, respectively, \( D \) is the apparent diffusion coefficient, and \( b \) is a diffusion weighting factor determined by the gradient waveform. For \( b \)-values, respectively, \( D \) is the apparent diffusion coefficient, and \( D_{app} = D_{app} \), is fitted to the model represented by Eqs. 3 and 4. In practice, the infinite series of Eq. 4 was truncated to the first 20 terms, and a brute-force search in the two-dimensional space spanned by \( D \) and \( R \) \((0.1 \leq D \leq 1.0 \times 10^{-3} \text{ mm}^2/\text{s} \) and \( 1 \leq R \leq 100 \mu \text{m} \)) was performed to determine the best-fit parameters.

Our study design for measuring \( D_{PCr} \) in humans assumes the possibility of anisotropic and/or restricted diffusion: complete characterization of diffusion would require the measurement of the complete diffusion tensor using different diffusion gradient orientations and \( t_{diff} \) (26). To minimize losses in signal-to-noise ratio (SNR) during \( t_{diff} \), diffusion MR experiments typically employ spin-echo or stimulated-echo pulse sequences. The stimulated-echo pulse sequence consists of three radio-frequency (RF) pulses. During the “mixing time” (TM) between the second and third pulses, the magnetization is in the longitudinal (z) direction and does not accumulate spin-spin relaxation (T2) losses (Fig. 2). Thus this sequence permits measurements at long \( t_{diff} \) by increasing TM with minimal penalty in SNR for the diffusing metabolite. T2 losses accumulate only during the echo period (TE), which is the sum of the periods between the first and second pulses and between the third pulse and signal acquisition (Fig. 2). Accordingly, TE is kept as short as possible. To enable \( D_{PCr} \) measurements with different \( t_{diff} \) up to \(-1 \) s for muscle PCr with \( T_2 \) of \(-300 \) ms and a longitudinal relaxation time (T1) of \(-6-7 \) s at 3 T (12), we performed all experiments with the stimulated-echo pulse sequence. Experiments were repeated with the diffusion gradients directed along the three principal Cartesian axes to document anisotropy and determine the diagonal elements of the diffusion tensor, \( D_{xx} \), \( D_{yy} \), and \( D_{zz} \).

The main challenge with in vivo human \( ^{31} \text{P} \) diffusion spectroscopy is low SNR because of the combined effect of the low sensitivity of \( ^{31} \text{P}-\text{MRS} \), the low concentration of PCr in muscle \((-25 \mu \text{mol/g wet wt vs. } \sim 86 \text{ mol/kg for water protons}) \), and \( T_2 \) relaxation effects, and the fact that diffusion weighting further attenuates the signal. Our first task was to develop a robust \( ^{31} \text{P} \) diffusion MRS protocol that could be performed in an examination time suitable for human studies. We used a reduced receiver bandwidth, signal averaging, and depth-resolved surface coil spectroscopy (DRESS) (6) to localize the \( ^{31} \text{P}-\text{MRS} \) signal of PCr to a relatively large, high-SNR, elongated slice compatible with the human calf.

![Diagram](http://ajpcell.physiology.org/)

Fig. 2. Stimulated-echo depth-resolved surface coil spectroscopy (DRESS) pulse sequence for localized \( ^{31} \text{P} \) diffusion spectroscopy. Diffusion gradients are shaded. RF, radio frequency; \( G_s \), slice-selection gradients; \( G_d \), diffusion gradients; TM, mixing time; TE, echo time, AQ, signal acquisition.

Fig. 1. Creatine kinase (CK) shuttle hypothesis. ATP is created in the mitochondria, and the reverse CK reaction transfers the phosphoryl group to creatine (Cr) to produce phosphocreatine (PCr). PCr then diffuses to the myofibrils, where it is converted back to ATP via the forward CK reaction to power muscle contraction. Cr diffuses back to the mitochondria to complete the cycle. \( D_{Cr} \) and \( D_{PCr} \), diffusion coefficients for Cr and PCr.
Optimizing the $D_{PCr}$ Measurements

The precision with which $D_{PCr}$ can be measured depends on the SNR of the acquired PCr signals and on the selected $b$ values and averaging scheme. For a given total scan time ($T$), we seek to optimize the diffusion MRS sequence repetition time ($TR$), the $b$ values, and the fraction of time spent acquiring each $b$ value.

To optimize TR for a partially saturated stimulated-echo experiment with three ($\pi/2$) RF pulses (3), we note, after averaging individual signals, that

$$\text{SNR} \sim \sqrt{\frac{T}{TR} e^{-TM/T_1} e^{-TE/T_2} \left[ 1 - e^{-TR - (TM + TE/2)} \right]} \quad (5)$$

If TM and TE are much shorter than TR, the value of TR that maximizes the SNR ($TR_{opt}$) is

$$TR_{opt} = 1.256T_1 \quad (6)$$

For muscle PCr with $T_1 = 6-7$ s, $TR_{opt} = 7.5-8.8$ s. If (TM + TE/2) $\ll$ TR, $TR_{opt}$ is given by the solution to the following fixed-point problem

$$TR = \log \left( \frac{2TR}{T_1} + 1 + \frac{TM + TE/2}{T_1} \right)T_1 \quad (7)$$

The solution progressively increases TR with increasing (TM + TE/2). However, for 50 $\leq$ TM $\leq$ 1.0 ms, optimizing TR using Eq. 7 results in 7.9 s $\leq$ TR $\leq$ 11.0 s, which does not improve SNR by >3% compared with a fixed TR of 8 s. Therefore, TR = 8 s was used for all 31P studies.

In choosing $b$ values, the optimum precision in $D$ is arguably achieved by an MR experiment consisting of only two $b$ values (13, 23). Devoting the total time for the experiment to averaging and improving the SNR of these two points is better than acquiring multiple $b$ values with lower SNR. In the two-point experiment, one $b$ value ($b_0$) is set to zero or very low (to dephase the transverse magnetization between stimulated-echo pulses) to maximize the SNR of the first signal measurement ($S_0$). Optimization then focuses on the high $b$ value ($b$) with corresponding signal $S$. $D$ obtained from the two measured signals is

$$D = \frac{1}{b} \left[ \log(S_0) - \log(S) \right] \quad (8)$$

For the case where $b$ is unconstrained by practical system limitations (11, 22, 23, 48), the optimum value of $b$ ($b_{opt}$) can be derived from the variance in $D$ ($\sigma_D^2$), given by an error propagation analysis of Eq. 8. For $S_0$ and $S$, each measured in a single acquisition

$$\frac{\sigma_D^2}{b^2} \left[ \left( \frac{\partial \log(S_0)}{\partial S_0} \right)^2 + \left( \frac{\partial \log(S)}{\partial S} \right)^2 \right] = \frac{1}{b^2} \left[ \left( \frac{\sigma_s}{S_0} \right)^2 + \left( \frac{\sigma_s}{S} \right)^2 \right] \quad (9)$$

where $\sigma_s$ is the standard deviation in the noise of $S_0$ or $S$. If $f$ is the fraction of the acquisition time spent acquiring the $b_0$ measurement and $N$ is the total number of excitations in the combined experiment (the $b_0$ experiment + the $b$ experiments), then

$$\sigma_D^2 = \frac{\sigma_s^2}{b^2N\sigma_0^2} \left[ f + \frac{1}{(1-f)e^{-2bf}} \right] \quad (10)$$

Denoting $\psi = S_0/\sigma_s$ as the SNR per TR of the $b_0$ experiment and dividing by $D$ yields the relative error in $D$ or the reciprocal of the “diffusion-to-noise ratio” (DNR)

$$\frac{\sigma_D}{D} = \frac{1}{\text{DNR}} = \frac{1}{\sqrt{N\psi bD}} \sqrt{1 + \frac{e^{2bf}}{(1-f)}} \quad (11)$$

Numerically minimizing this term gives the values of $b$ and $f$ that maximize the precision in $D$ with $b_{opt} = 1.278/D$ and $f_{opt} = 0.218$.

With these values, the smallest error in $D$ is

$$\frac{\sigma_D}{D_{opt}} = 3.59 \sqrt{N\psi} \quad (13)$$

with TR = 8 s and a total scan time $T = N\cdot TR = 5$ min, $N \approx 40$, and precision in the calculated $D$ is 14–23% for $\psi = 2.5–4$. Unfortunately, as in the present case, the $b_{opt}$ prescribed by Eq. 12 is often unachievable because of system limitations on G, TE, SNR, and eddy current artifacts. This constrains the choice to the highest practical $b$ value ($b_{max}$). Minimization of Eq. 11 with respect to $f$ with constant $b = b_{max}$ shows that the experiment is optimized when

$$f_{opt} = \frac{1}{1 + e^{b_{max}D}} \quad (14)$$

Figure 3 plots $\beta$ as a function of the product $bD$. The curve is relatively flat around the optimal value of $bD = 1.278$, with the error doubling when $b$ is reduced to 0.33/$D$.

For the present study of human skeletal muscle, an expected $D_{PCr}$ of $\sim 0.5 \times 10^{-3}$ mm$^2$/s based on animal studies (9, 32, 43) yields $b_{opt} = 2.600$ s/mm$^2$ from Eq. 12. However, in practice, we were constrained in the worst case to $b_{max} = 1,000$ s/mm$^2$ at the shortest $t_{wait}$ with TE = 80 ms and a maximum $G = 31$ mT/m. From Eq. 16 with $b_{opt} = 0.5$, this corresponds to a worst-case ~1.5-fold increase in scatter in $D$ compared with what would have resulted with the unconstrained optimum prescription of Eq. 12. Figure 3 also shows the values of $bD$ used in the present study for the expected $D_{PCr}$ of $0.5 \times 10^{-3}$ mm$^2$/s.
Table 1. Diffusion coefficient of water in inorganic phosphate agarose gel phantom

<table>
<thead>
<tr>
<th></th>
<th>$D_x \times 10^{-3}$ mm$^2$/s</th>
<th>$D_y \times 10^{-3}$ mm$^2$/s</th>
<th>$D_z \times 10^{-3}$ mm$^2$/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated-echo DRESS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 ms TM</td>
<td>2.06</td>
<td>2.04</td>
<td>2.11</td>
</tr>
<tr>
<td>100 ms TM</td>
<td>2.06</td>
<td>2.03</td>
<td>2.08</td>
</tr>
<tr>
<td>150 ms TM</td>
<td>2.04</td>
<td>2.02</td>
<td>2.06</td>
</tr>
<tr>
<td>200 ms TM</td>
<td>2.07</td>
<td>2.05</td>
<td>2.04</td>
</tr>
<tr>
<td>400 ms TM</td>
<td>2.08</td>
<td>2.05</td>
<td>2.03</td>
</tr>
<tr>
<td>1,000 ms TM</td>
<td>2.06</td>
<td>2.02</td>
<td>2.01</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.06 ± 0.01</td>
<td>2.03 ± 0.01</td>
<td>2.05 ± 0.04</td>
</tr>
<tr>
<td>DTI</td>
<td>2.02 ± 0.03</td>
<td>2.01 ± 0.03</td>
<td>2.02 ± 0.04</td>
</tr>
</tbody>
</table>

Values were obtained with stimulated-echo depth-resolved surface coil spectroscopy (DRESS) protocol and $^1$H-MRS surface coil and with proton diffusion tensor imaging (DTI) using a conventional spin-echo sequence and the scanner's conventional SENSE $^1$H phased-array coil. DTI values are means ± SD of values in a region of interest in the middle of the phantom. TM, mixing time; $D_x$, $D_y$, and $D_z$, diagonal elements of the diffusion tensor.

In practice, the number of signal averages was increased for measurements of $D_{PCr}$ at longer t$_{diff}$ or TM values to compensate for T$_1$ losses and to maintain a comparable SNR and precision in $D$ for all experiments, and experimental variations in $f$ from $f_{0}$ affected the precision of $D$ by <2%.

Experiments

All MR studies were done on a 3.0-T clinical MRI system (Achieva, Philips Healthcare, Best, The Netherlands) with a broadband MRS capability. All diffusion studies were preceded by scout $^1$H-MRI, followed by main-field (B$_0$) shimming to optimize the field homogeneity in the region of interest (37).

Phantom studies. The stimulated-echo DRESS diffusion pulse sequence (Fig. 2) was first validated with $^1$H-MRS by measuring $D$ for water in an agarose gel phantom doped with 100 mM Na$_2$PO$_4$ solution at room temperature using a $^1$H transmit/receive circular 8-cm-diameter water coil. Water has an isotropic unrestricted diffusion constant of $\sim$2.0 × 10$^{-3}$ mm$^2$/s at room temperature (30). $D$ was measured along the three Cartesian axes, with TR/TE = 2,000/62 ms, $b$ = 560 s/mm$^2$, $b_0$ = 10 s/mm$^2$, N/S = 2/0.5, and TM = 50, 100, 150, 200, 400, and 1,000 ms. Reference values for water diffusion were obtained by running a standard MRI spin-echo diffusion tensor imaging (DTI) protocol with a SENSE coil array (TR/TE = 2,000/62 ms, $b$ = 560 s/mm$^2$, and N/S = 2/0.5) and calculating the average values of $D_{xx}$, $D_{yy}$, and $D_{zz}$ in a central region of interest in the phantom.

To test whether anisotropic diffusion could be measured independent of the $B_0$ direction (z-axis) of the magnet and MR coils, a bundle of asparagus was scanned with the $^3$P-MRS stimulated-echo DRESS diffusion protocol. Water diffusion in asparagus has been shown to be anisotropic (8). The scan parameters were the same as for the $^1$H-MRS agarose gel experiment. After reorientation of the longitudinal axis of the fibers in the asparagus bundle parallel to each of the three principal Cartesian axes of the MRI scanner, the experiments were repeated using the scanner’s whole body MRI coil and surface coils.

With $^3$P-MRS using a 17-cm transmit/8-cm receive $^3$P surface coil pair (12), the stimulated-echo DRESS diffusion protocol was next applied to measure the diffusion of the 100 mM Pi ($D_{PCr}$) present in a cylindrical gel phantom. $D_{PCr}$ was measured as a function of t$_{diff}$ = TM + 32 ms, with TM = 50, 100, 150, 200, 400, 700, and 1,000 ms, and diffusion gradients along the three Cartesian physical axes (TR/TE = 8,000/80 ms, $b_0$ = 10 s/mm$^2$, $b$ = 1,000 s/mm$^2$, and N/S = 200/5.5 for TM ≤200 ms and 30/0.33 for TM > 200 ms).

Human studies. Eleven healthy volunteers [7 men and 4 women, mean 26 ± 4 (range 22–34) yr old] provided informed consent after receiving an explanation of the study and protocol and were enrolled in this study, which was approved by the Johns Hopkins Institutional Review Board on human investigation. Subjects were positioned supine, with the left calf muscle resting on the 17-cm transmit/8-cm receive $^3$P surface coil set, with the long axis aligned parallel with the z-axis of the scanner. The leg was immobilized, and subjects were instructed to remain still during the examination. After scout $^1$H-MRI and shimming, the stimulated-echo $^3$P DRESS protocol was applied to localize the PCr signal to a 20-mm slice parallel to the coil at a depth of 30–60 mm from the coil surface. The RF pulses were optimized at the selected slice by application of a series of long-TR DRESS experiments with three to five different flip angles and interpolation of the results to determine the flip angle producing the maximum signal. The protocol parameters for $D_{PCr}$ measurements were as follows: TR/TE = 8,000/80 ms; bandwidth = 500 Hz; TM = 50, 100, 150, 200, 400, 700, and 1,000 ms, except in three subjects, where the 700-ms TM scan was omitted because of scan-time constraints; G ≤31 mT/m for $b$ = 1,000, 1,600, and 2,000 s/mm$^2$ for TM = 50, 100, and ≥150 ms, respectively; $b_0$ = 10 s/mm$^2$; and N/S = 35/29, 40/25, 42/24, 47/26, 50/0, 26, 55/0, 27, and 55/0.27 for the seven TM values, respectively. The protocol was repeated with diffusion gradients directed along the three Cartesian axes to obtain $D_{xx}$, $D_{yy}$, and $D_{zz}$.

Data Analysis

MRS signals were quantified as peak areas using the circle-fit routine CFTT (14), and the apparent $D$ was calculated from Eq. 8 for each gradient direction. $D_{av}$ was calculated as follows: $D_{av} = (D_{xx} + D_{yy} + D_{zz})/3$. The average diffusion distance was calculated from the Einstein-Smoluchowski equation (10): $x_i = \sqrt{2D_{av}t_{diff} + D_{zz}t_{diff}/3}$. Pair-t testing was used to determine whether differences in $D$ measured in each direction from the same subject were significant at $P < 0.05$.

$D_{av}$ as a function of t$_{diff}$ was fitted to the infinite-length, finite-radius cylindrical model for the restricting compartment given by Eqs. 3 and 4 (9, 33, 43) to obtain estimates of the radius of the cylindrical compartment and $D_i$. This analysis was performed with MATLAB (MathWorks, Natick, MA).

RESULTS

Phantom Studies

The $D$ of water measured with stimulated-echo DRESS along the three directions at different t$_{diff}$ is listed in Table 1.

Table 2. Diffusion coefficient of water in a bundle of asparagus

<table>
<thead>
<tr>
<th>Fiber Direction</th>
<th>$D_x \times 10^{-3}$ mm$^2$/s</th>
<th>$D_y \times 10^{-3}$ mm$^2$/s</th>
<th>$D_z \times 10^{-3}$ mm$^2$/s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body coil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x</td>
<td>1.51</td>
<td>0.47</td>
<td>0.39</td>
</tr>
<tr>
<td>y</td>
<td>0.47</td>
<td>1.42</td>
<td>0.41</td>
</tr>
<tr>
<td>z</td>
<td>0.69</td>
<td>0.59</td>
<td>1.35</td>
</tr>
<tr>
<td><strong>Surface coil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x</td>
<td>1.50</td>
<td>0.38</td>
<td>0.36</td>
</tr>
<tr>
<td>y</td>
<td>0.42</td>
<td>1.52</td>
<td>0.42</td>
</tr>
<tr>
<td>z</td>
<td>0.62</td>
<td>0.72</td>
<td>1.56</td>
</tr>
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</table>

Values were obtained with the stimulated-echo DRESS protocol and $^1$H MRS surface coil and with the scanner’s body coil. Diffusion experiment was repeated 3 times with the bundle oriented with the asparagus fibers parallel to the 3 Cartesian axes. Average of the diffusion coefficient ($D_i$) along ($D_0$) and perpendicular to ($D_z$) fibers in the 3 sample positions are listed.
Table 3. Diffusion coefficient of Pi in the agarose gel phantom

<table>
<thead>
<tr>
<th>TM, ms</th>
<th>( D_{xx} \times 10^{-3} \text{ mm}^2 / \text{s} )</th>
<th>( D_{yy} \times 10^{-3} \text{ mm}^2 / \text{s} )</th>
<th>( D_{zz} \times 10^{-3} \text{ mm}^2 / \text{s} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.68</td>
<td>0.69</td>
<td>0.68</td>
</tr>
<tr>
<td>100</td>
<td>0.89</td>
<td>0.81</td>
<td>0.83</td>
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<tr>
<td>150</td>
<td>0.92</td>
<td>0.88</td>
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<tr>
<td>200</td>
<td>0.70</td>
<td>0.70</td>
<td>0.80</td>
</tr>
<tr>
<td>400</td>
<td>0.75</td>
<td>0.78</td>
<td>0.76</td>
</tr>
<tr>
<td>700</td>
<td>0.81</td>
<td>0.72</td>
<td>0.69</td>
</tr>
<tr>
<td>1,000</td>
<td>0.71</td>
<td>0.76</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Mean ± SD

0.78 ± 0.10 0.76 ± 0.07 0.76 ± 0.07

Values were obtained with the stimulated-echo DRESS protocol and 31P-MRS surface coil set.

The 3D is isotropic and agrees with reported values for water measured at room temperature (30) and with the measurements obtained with diffusion tensor imaging (Table 1). The 3D of water measured in asparagus is reported in Table 2 and shows anisotropic diffusion, with a higher 3D along the fiber than in perpendicular directions. Orientation of the bundle of asparagus along the three axes did not significantly affect these measurements, confirming that neither the localization sequence nor the scanner biased the results in the B0 or any other direction. Table 2 also shows that the same results are obtained with the stimulated-echo DRESS protocol whether the surface detection coil or the scanner’s body MRI coil is used. The 31P diffusion measurements of Pi in the gel phantom are listed in Table 3. These results show no anisotropy or time dependence of \( D_{PPi} \), indicating unrestricted isotropic diffusion, as expected.

**Human Studies**

Figure 4 shows a representative set of spectra obtained from one volunteer. Comparable SNR is obtained at all TM values. The reduction in SNR with diffusion encoding along the z-axis is due to higher diffusion along that direction. Table 4 lists the mean 3D and diffusion distances in all subjects. Anisotropic diffusion is evident, with higher diffusion along the z-direction, substantially parallel to the fibers (Table 4), than in the x- or y-directions, at almost all diffusion times (\( P < 0.02 \) for \( D_{zz} \) vs. \( D_{xx} \) and \( D_{zz} \) vs. \( D_{yy} \), except at TM = 700 ms, where \( P = 0.07 \)).

Figure 5 shows \( D_{av} \) from all subjects as a function of \( t_{diff} \). \( D_{av} \) decreases with \( t_{diff} \), indicating restricted diffusion. The fit of \( D_{av} \) to the cylindrical model for restricted diffusion results in \( D_f = 0.69 \times 10^{-3} \text{ mm}^2 / \text{s} \) and a radius of the restricting compartment of 28 μm. The fitted model is also plotted in Fig. 5.

**DISCUSSION**

**PCr Diffusion Anisotropy and Restriction**

The results reported here are, to the best of our knowledge, the first measurements of PCr diffusion in human muscle. They show anisotropy and restricted diffusion with \( D_{PCr} \) starting, on average, at \( \sim 0.6 \times 10^{-3} \text{ mm}^2 / \text{s} \) and decreasing to \( \sim 0.3 \times 10^{-3} \text{ mm}^2 / \text{s} \) as a function of \( t_{diff} \leq 1 \text{ s} \) (Fig. 5). This result is consistent with prior 31P-MRS findings in rat and rabbit muscle in vivo, which show \( D_{PCr} \) between 0.7 and 0.2 \times 10^{-3} \text{ mm}^2 / \text{s} over the same range of \( t_{diff} \) (9, 32, 43). All these results are a little higher than those for excised fish and frog muscle studied at 20–25°C with \( D_{PCr} \) between 0.4 and 0.2 \times 10^{-3} \text{ mm}^2 / \text{s} (19, 20, 24, 49, 50). While the latter measures could be confounded by PCr degradation in vitro, correction for temperature alone would raise these measures of \( D_{PCr} \) to the same range of 0.6 to 0.3 \times 10^{-3} \text{ mm}^2 / \text{s}, based on either direct experimental data (9) or an indirect empirical expression applied to the diffusion of PCr as a solute (See Eq. 5 in Ref. 47). The one preliminary value for \( D_{PCr} \) of 0.65 ± 0.04 \times 10^{-3} \text{ mm}^2 / \text{s} in human muscle reported in 2003 (21) was for a single, albeit unclear, diffusion time and a single gradient direction perpendicular to the fiber axis. This value is a little higher than the earlier results for transverse diffusion in mammalian tissue, as well as our own values of \( D_{xx} \) and \( D_{yy} \) in Table 4.

**Fig. 4.** Scout images [A: axial (top), sagittal (bottom left), and coronal (bottom right)] and a typical stimulated-echo DRESS data set (B) acquired from a 20-mm-thick slice in the left leg of a volunteer. Spectra show the PCr peak and are scaled identically. Spectra were zero-filled to 2,048 points, filtered with a 5-Hz exponential filter, zero-order phase-corrected, and displayed with the same scale in a 200-Hz window. \( S_0 \), reference signal; \( S_x \), \( S_y \), and \( S_z \), signals in x, y, and z planes.
Similar to the animal studies that investigated anisotropic diffusion (9, 24, 43), we also found that PCr diffusion is 24–87% higher along the muscle fiber than in the transverse direction. However, in human calf muscle, our observation of dependence of \( D_{PCr} \) on \( t_{diff} \) is consistent with a \( \sim 30\)-\( \mu \)m restricting compartment radius compared with an 8- to 11-\( \mu \)m radius reported in two of the prior animal studies that employed the same cylindrical diffusion model (9, 43). A 44-\( \mu \)m compartment size for restricted PCr diffusion measured parallel to the rat leg was reported in a third study (32). Our estimate of \( \sim 30\ )\mu m \) for the cylindrical restricting compartment size results from a slower decrease in \( D_{PCr} \) with increasing \( t_{diff} \) in humans (Fig. 5) than in the animal studies that reported an 8- to 11-\( \mu \)m diffusion distance to the restricting barrier. Physiologically, this would be interpretable as a longer diffusion distance in human muscle than in the two animal studies, perhaps reflecting species differences of scale and/or mitochondrial density (17, 42, 44).

Our estimated diffusion distance of \( \sim 30\ )\mu m \) is comparable to myofiber radii of 25–50 \( \mu \)m (1, 18, 27, 35), which would be consistent with the sarcolemma serving as the ultimate lateral diffusion barrier, since PCr is exclusively intracellular. However, the \( t_{diff} \) dependence of \( D_{zz} \) in Table 4 is evidence for some restricted diffusion, even along the fiber direction, which was also seen in the prior animal data (24, 32, 43). It has been suggested that PCr diffusion may be restricted by intracellular organelles in the sarcoplasm (9, 24), such as the mitochondria, the sarcoplasmic reticulum, and/or other microstructures (24, 39), rather than by the sarcolemma. If significant, this would be expected to result in diffusion distances that are significantly smaller than the fiber radius and those observed here.

At the short end of the distance scale, the measurement of restricted diffusion distances in the <10-\( \mu \)m range requires experiments with shorter \( t_{diff} \) than can be accommodated by \( b_{opt} \) or \( b_{max} \) for our studies. However, we note that diffusion for PCr as a solute in aqueous solution can be estimated from Eq. 5 in Ref. 47 and is \( 0.91 \times 10^{-3} \) mm\(^2\)/s at 37°C, with the assumption that water viscosity is 0.7 cP and density is 1.0 g/ml. This is close to measured values for diffusion of PCr in aqueous solution of \( 0.74 \times 10^{-3} \) mm\(^2\)/s (9) and \( 0.81 \times 10^{-3} \) mm\(^2\)/s (32). These values place an upper limit for the free isotropic diffusion of PCr in tissue, at least in the absence of any facilitating mechanisms (29). In particular, the fact that the measured values of \( D_{zz} \) are approximately equal to this upper limit at the shortest \( t_{diff} \) studied (Table 4) means that significant intracellular restrictions to diffusion in the direction parallel to the fibers at shorter \( t_{diff} \) cannot exist. However, the lower initial values for \( D_{xx} \) and \( D_{yy} \) than for free \( D_{PCr} \) in aqueous solution would suggest that additional restrictions to lateral diffusion might well be resolvable with shorter-\( t_{diff} \) experiments.

**Implications for CK Reaction**

The apparent, bulk-tissue, time-averaged pseudo-first-order reaction rate constant (\( k \)) for the CK reaction previously measured by noninvasive \( ^{31}P\)-MRS in healthy human calf muscle was \( \sim 0.27\ )s\(^{-1}\) (7, 36). This leads to an apparent half-life for PCr in the CK reaction of log(2)/\( k \approx 2.6\ )s. Extrapolation of the fit to the cylindrical diffusion model in Fig. 5 for long diffusion times yields \( D_{xx} \) for PCr of \( \sim 0.28 \times 10^{-3} \) mm\(^2\)/s at 2.6 s. Thus, in a time equal to the half-life of PCr in the CK reaction, PCr would diffuse an average distance of \( \sim 66\ )\mu m \). This distance is very long compared with a mitochondria-myofibril distance of up to \( \sim 2\ )\mu m \) based on mitochondrial separations (17, 44). Table 4 shows that most of that distance is, in fact, traveled in \( \sim 1\ )s. On the basis of an extrapolated \( D \) of \( 0.08 \times 10^{-3} \) mm\(^2\)/s at 2.6 s perpendicular to the long axis in the cylindrical model, the lateral component of the diffusion distance is \( \sim 29\ )\mu m \) (which approximates the radius of the model cylindrical restriction). This is still large compared with the mitochondria-myofibril distance and is on the order of the typical radius of human muscle fibers (25–50 \( \mu \)m).

Therefore, these first measurements of PCr diffusion in human muscle appear to support the view that, on the time scale of CK reaction kinetics, PCr diffusion is not a factor.
limiting the supply of PCr to CK reactions occurring virtually anywhere in the myocyte, including the transfer of high-energy phosphate between the mitochondria and myofibrils in accordance with the PCr shuttle hypothesis. Such observations are similar to those from studies of isolated bullfrog muscle in which \( D_{\text{PCr}} = 0.28 \times 10^{-7} \text{mm}^2/\text{s} \), diffusion distance of PCr = 57 \( \mu \text{m} \), and lifetime PCr = 5.8 s (based on \( k = 0.17 \text{ s}^{-1} \)) in CK (50). This previous study (50) it was concluded that the energy shuttle hypothesis is not obligatory for energy transport between mitochondria and myofibrils. Although it did not include direct measurements of \( D_{\text{PCr}} \), another \( ^{31} \text{P-MRS} \) study of high-energy phosphate diffusion in crab muscle concluded that the interaction between mitochondrial ATP production rates, ATP consumption rates, and diffusion distances is not particularly close to being limited by intracellular metabolite diffusion (25).

While our data support and extends this view to healthy human muscle at rest, it behooves us to also consider periods of peak energy demand for muscular contraction, wherein the PCr shuttle’s putative role as a temporal-spatial energy buffer may come into play. A high jump, for example, requires in a period of \( \sim 0.2 \text{ s} \) a peak power that is some 15 times larger than the maximal aerobic energy capacity, which must therefore be supplied by PCr and CK (2). Even in this case, the assumption that \( D_{\text{av}} = 0.5 \times 10^{-3} \text{mm}^2/\text{s} \) for PCr at the shorter \( t_{\text{diff}} \) (Table 4) yields a diffusion distance of \( \sim 26 \mu \text{m} \) in 0.2 s, which should not limit delivery of PCr over mitochondrial-myofibril distances of several micrometers in healthy muscle.

**Study Limitations**

In the present study, measurement of \( D_{\text{PCr}} \) and investigation of the effects of anisotropy and restricted diffusion in human muscle in vivo imposed a number of practical limitations. 1) Use of the stimulated-echo DRESS sequence, while providing a large voxel with high SNR, does result in poorly defined boundaries in the plane parallel to the surface detection coil. Nevertheless, the lower leg extended well beyond the detection coil without a significant change in muscle fiber orientation and yielded results consistent with prior animal studies (9, 32, 43). 2) We were unable to perform the diffusion sequence with the optimal diffusion parameter set (Eq. 12) because of system constraints on \( b \). This resulted in some loss of precision for the shorter \( t_{\text{diff}} \) measurements (Fig. 5). 3) Diffusion measurements are sensitive to motion and gradient eddy currents, which cause additional signal dephasing that is potentially reflected by artificially high \( D \) measurements. Our phantom studies (Tables 1–3) were designed to rule out eddy current effects, and subject motion was limited by immobilization of the leg. In the future, motion might also be corrected by phasing each of the \( b \) values prior to averaging (15); unfortunately, the SNR of individual acquisitions was too low to allow this in the present study. 4) The protocol with seven \( t_{\text{diff}} \) measurement times and three gradient orientations takes \( \sim 2 \text{ h} \) to complete. Future studies would benefit from focusing on a particular \( t_{\text{diff}} \) and/or gradient orientation combination. 5) It was not possible to measure the diffusion coefficient of ATP (\( D_{\text{ATP}} \)) with the current protocol because of ATP signal loss associated with limitations in \( b \), the long TE, and \( J \) coupling (Fig. 4).

Finally, it should be noted that PCr’s participation in the CK reaction could affect \( D_{\text{PCr}} \) measurements to the extent that a fraction of the PCr signal generated from \( \gamma^{-}\text{ATP} \) via the reverse reaction will have diffused for some portion of \( t_{\text{diff}} \) as ATP, and not as PCr. Because the MRS pulses are not spectrally selected, all species undergoing chemical exchange, both PCr and \( \gamma^{-}\text{ATP} \), are excited and “tagged” for diffusion. Given that PCr is in equilibrium, the PCr signal depleted by the forward reaction is regenerated from \( \gamma^{-}\text{ATP} \) via the reverse reaction. For a CK reaction rate \( k = 0.27 \text{ s}^{-1} (7, 36), \sim 0.4 \times 27\% = 11\% \) of the PCr will exchange with \( \gamma^{-}\text{ATP} \) during a typical experimental \( t_{\text{diff}} \) of, e.g., 0.4 s. If it is assumed that the time at which \( \gamma^{-}\text{ATP} \) is converted to PCr is distributed uniformly during \( t_{\text{diff}} \), then 11% of the \( D_{\text{PCr}} \) measurement will comprise \( D_{\text{av}} (D_{\text{PCr}} + D_{\gamma^{-}\text{ATP}})/2 \), resulting in a 5% contamination of the \( D_{\text{PCr}} \) measurement by \( D_{\gamma^{-}\text{ATP}} \) due to exchange. The contamination is proportionately less for shorter \( t_{\text{diff}} \) and, hence, is not significant over the range of \( t_{\text{diff}} \) that primarily determines the restricting compartment size or \( D_{\text{f}} \).

**Future Directions**

It would be important to extend measurements of PCr diffusion in muscle to metabolic disorders involving energy transfer and to cytoarchitectural disorders, although the present work suggests that \( D_{\text{PCr}} \) would have to change rather dramatically to affect the spatial transfer of high-energy phosphate between mitochondria and myofibrils. In the myocardium, which has a higher density of mitochondria than skeletal muscle and a higher energy demand, significant reductions in the forward CK rate of ATP supply have been found in the failing human heart (40, 46). Although measuring diffusion in the heart is difficult because of its motion (16), elucidating whether reduced CK energy transfer in heart failure is due to altered PCr diffusion or other biochemical defects may provide useful insights for targeting therapy.

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**DISCLOSURES**

M. Schar is an employee of Philips Healthcare and is a visiting scientist at Johns Hopkins University.

**REFERENCES**

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