pH heterogeneity in tibial anterior muscle during isometric activity studied by $^{31}$P-NMR spectroscopy

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Abstract

Heterogeneity of pH in human muscle tissue during exercise and recovery, as monitored by nuclear magnetic resonance spectroscopy ($^{31}$P-NMRS), has been assessed in several studies (1, 17–19, 24, 25, 29, 30). A shift of the frequency of the inorganic phosphate ($P_i$) signal, relative to that of phosphocreatine (PCr) in the $^{31}$P-NMR spectrum, corresponds to a change in the intracellular pH. The occurrence of a broadened, or even split, resonance of $P_i$ during exercise is often attributed to the type II fiber pool increases with decreasing pH levels. This phenomenon is discussed in the context of the size principle stating that the smaller (type I) motor units are recruited first.

Houtman, C. J., A. Heerschap, M. J. Zwarts, and D. F. Stegeman. pH heterogeneity in tibial anterior muscle during isometric activity studied by $^{31}$P-NMR spectroscopy. J Appl Physiol 91: 191–200, 2001.—The occurrence of pH heterogeneity in human tibial anterior muscle during sustained isometric exercise is demonstrated by applying $^{31}$P-nuclear magnetic resonance (NMR) spectroscopy in a study of seven healthy subjects. Exercise was performed at 30 and 60% of maximal voluntary contraction (MVC) until fatigue. The NMR spectra, as localized by a surface coil and improved by proton irradiation, were obtained at a high time resolution (16 s). They revealed the simultaneous presence of two pH pools during most experiments. Maximum difference in the two pH levels during exercise was 0.40 ± 0.07 (30% MVC, $n = 7$) and 0.41 ± 0.03 (60% MVC, $n = 3$). Complementary two-dimensional $^{31}$P spectroscopic imaging experiments in one subject supported the supposition that the distinct pH pools reflect the metabolic status of the main muscle fiber types. The relative size of the $P_i$ peak in the spectrum attributed to the type II fiber pool increases with decreasing pH levels. This phenomenon is discussed in the context of the size principle stating that the smaller (type I) motor units are recruited first.

Keywords: pH; human; size principle; muscle fatigue; sustained isometric exercise; $^{31}$P nuclear magnetic resonance spectroscopy

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only use coil localization to visualize as many as possible temporal details (temporal resolution of 16 s). In one of the seven subjects, the coil profile and the spatial distribution of the \( P_i \) peaks during exercise were investigated.

**METHODS**

**Subjects.** Seven healthy men and women participated in the study, aged 22–48 (Table 1). All subjects were regularly engaged in low to moderate aerobic exercise. They were informed of the purpose of the experiments and gave their written consent. The protocol was approved by the local ethics committee of the Faculty of Medical Sciences of the Nijmegen University.

**Exercise.** Subjects took a supine position on the investigation table with the left leg slightly bent and supported with vacuum pillows (Fig. 1A). For the two-dimensional phase-encoded \( ^{31}P \) spectroscopic imaging (2D \( ^{31}P \) SI) measurements, the exercising leg was straightened to enable its horizontal alignment with respect to the static magnetic (\( B_0 \)) field. The angle of the left lower leg and the left foot was always 90°, and the foot was fixed with straps in a pedal. Subjects were supplied with visual feedback of the force and were verbally encouraged during exercise. All subjects performed both exercise protocols, separated by at least 2 days.

The ergometer was home built, was specially designed for use in the magnet, and was applicable for both dorsal and plantar ankle flexion. The force signal was digitally stored at a sample rate of 100 Hz. To enable synchronization afterward, a trigger signal of the NMR system was registered on the force system. The NMR data points were assigned to the midpoint of the two \( ^{31}P \)-NMR excitation pulses, applied for each measurement.

**Table 1. Subject and force parameters**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age, yr</th>
<th>100% MVC Before 60% MVC</th>
<th>Duration of 60% MVC, s</th>
<th>100% MVC Before 30% MVC, s</th>
<th>Duration of 30% MVC, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>25</td>
<td>282</td>
<td>81</td>
<td>268</td>
<td>608</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>31</td>
<td>214</td>
<td>112</td>
<td>241</td>
<td>387</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>47</td>
<td>318</td>
<td>167</td>
<td>312</td>
<td>660</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>33</td>
<td>277</td>
<td>151</td>
<td>317</td>
<td>348</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>27</td>
<td>258</td>
<td>114</td>
<td>273</td>
<td>376</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>22</td>
<td>256</td>
<td>96</td>
<td>207</td>
<td>580</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>23</td>
<td>336</td>
<td>80</td>
<td>295</td>
<td>660</td>
</tr>
<tr>
<td>Means ± SD</td>
<td></td>
<td>30 ± 9</td>
<td>277 ± 41</td>
<td>114 ± 34</td>
<td>276 ± 38</td>
<td>539 ± 144</td>
</tr>
</tbody>
</table>

MVC, maximal voluntary contraction; M, male; F, female; SI, spectroscopic imaging data.

Compared with a NMR spectrum obtained at rest, the phosphocreatine (PCr) peak is decreased and the inorganic phosphate (\( P_i \)) peak is increased and doubled. pH values are derived from the frequency of the \( P_i \) peaks relative to that of the PCr peak. The area of a peak is a measure for the tissue level of the corresponding molecule. PME, phosphomonoesters; PDE, phosphodiesterase; \( pH_{\text{low}} \) and \( pH_{\text{high}} \), values derived from, respectively, left (low-field) and right (high-field) \( P_i \) peaks.

Fig. 1. A: experimental setup. The subject lies in a supine position on the table. The U-shaped coil is placed over the tibialis anterior (TA) of the left leg. The left foot is strapped in the ergometer. The left leg is slightly bent and supported by vacuum pillows. The angle between foot and lower leg is 90°. The subject gets visual feedback of the force and is encouraged verbally during exercise. B: example of a \( ^{31}P \) nuclear magnetic resonance (NMR) spectroscopy (\( ^{31}P \)-NMRS) spectrum (2 acquisitions, repetition time = 7 s) measured 68 s after the start of a 60% maximal voluntary contraction (MVC) exercise (subject 6, see also Fig. 2).
The exercise consisted of an isometric plantar ankle flexion until fatigue at 30 or 60% MVC. After offset correction, two MVC measurements were done, from which the maximum was used (Table 1). The subject was permitted to deviate ±5% at 30% MVC or ±5% at 60% MVC. If he or she could no longer meet this condition, despite encouragement, exercise was stopped. MVC measurements and exercise were separated by at least 30 min. $^{31}$P-NMR data were collected during rest (3 min), exercise (Table 1), and recovery (15 min).

**Nuclear magnetic resonance.** All experiments were performed on a 1.5-T whole body system (Magnetom SP, Siemens Medical Systems, Erlangen, Germany). The surface coil was home built and specially designed to detect signals from the TA. It consisted of two concentric loops: 25 cm × 8.5 cm (tuned to the $^1$H frequency) and 20 cm × 3.5 cm (tuned to the $^{31}$P frequency). It was carefully placed over the TA and fixed with tape. Before $^{31}$P-NMRs data collection, a series of five transversal $^1$H-NMR images (gradient-recalled echo: echo time = 6 ms, repetition time (TR) = 70 ms, thickness = 10 mm, distance factor = 0.4) were made to validate the correct placement of the surface coil. In case of the two $^{31}$P SI measurements, this series was extended to 15 transversal $^1$H-NMR images divided over the length of the coil to control the horizontal position of the lower leg and consequently that of the TA. After the last 2D $^{31}$P SI measurement, this series of $^1$H-NMR images was repeated to confirm an unchanged leg position.

The homogeneity of the $B_0$ field was adjusted by using the proton signal from water, resulting in a peak width at half-height of ≤0.5 ppm. To overcome inhomogeneity of the $B_1$ field, an amplitude-modulated adiabatic 90° pulse (sincos), with a length of 2.6 ms, was used for excitation. The spectral excitation of this pulse was constant over a frequency range of 1,000 Hz (~39 ppm), which is sufficient to map all resonances present in skeletal $^{31}$P-NMR spectra. For optimal SNR per unit time, we used a TR of 7 s, according to Ernst and Anderson (6) (TR = 1.25 T1, where T1 is spin-lattice relation time) and to Thomsen et al. (23) (muscle T1 (PCr, P, ATP) ≈ 5.5 s). Two acquisitions were averaged per measurement. Including data storage, the time resolution became 16 s. The free induction decay (FID) was low-pass filtered at 5 kHz and sampled during 512 ms at a sample frequency of 4 kHz. During data collection, high-level (10 W) WALTZ4 proton irradiation was applied to decouple the $^{31}$P-$^1$H spin coupling (15). During the remaining time, low-level (0.6 W) WALTZ4 proton irradiation was applied to amplify the $^{31}$P-NMR signal by the nuclear Overhauser effect (2).

For localization of the $^{31}$P-NMR signal, 2D $^{31}$P SI without slice selection and with a matrix size of 8 × 8 was applied in the transverse direction. Each volume of interest had a nominal resolution of 1.5 × 1.5 cm$^2$. In the third dimension, the view of the surface coil dictated the localization (~20 cm). Acquisition time was 7.57 min (~8 × 8 × 7 s), taking one acquisition for every phase-encoding step. Data analysis. The data-analyzing method VARPRO (27) was used as fitting procedure. The first three data points of each FID were not included in the Fourier transformation to avoid the broad baseline component arising from the tibia bone. Starting values for the peak position and line width of PCr and P, were given. The peaks were assumed to have a Lorentzian line shape. If the P, resonance split into two resonances, equal line widths were not assumed because the origin of this phenomenon is part of the study. To avoid improper use of prior knowledge, a broadened P, peak was analyzed in two ways: as one peak and as two independent peaks. In the evaluation of the results, the lower bound of the theoretical statistical errors, the Cramer-Rao (CR) lower bound, was used. The result with the lowest CR lower bound in the calculated parameters (peak area, line width, and frequency) was accepted as the best fit. Sometimes, both results came up with CR lower bounds of more than 60% of the parameter values. Then the P, peak was excluded from the fit procedure.

Intracellular pH was calculated from the chemical shift of P, based on the equation $p$H = 6.75 + log(δ − 3.26/log(5.75 − δ), where δ equals the chemical shift of the P, peak (in ppm), relative to PCr. The curves of the P, were fitted by piecewise linear regression. Continuity was assumed, except for the transition of one pH to two pHs and vice versa. The distribution of data points over the successive regression lines was optimized by minimizing the sum of the residuals of the lines. This method is described by Vieth (28) for the combination of two regression lines. We extended this method to an arbitrary number of line pieces.

The 2D $^{31}$P SIs were analyzed with LUISE, a data-analyzing program supplied by Siemens. No zero filling, filtering, or offset correction was used in the k space (the spacial frequency space). The shift of the matrix grid was equal for both 2D $^{31}$P SIs (rest and exercise). The first 2D $^{31}$P SI was depicted on $^1$H-NMR images measured before exercise, and the last 2D $^{31}$P SI was depicted on $^1$H-NMR images measured after exercise.

Preprocessing of the spectra shown in this paper (Figs. 1B, 2B, 3B, and 4B) was as follows. Before Fourier transformation, the first three data points of the FID were skipped, an asymmetric Gauss window (center 25 ms, width 100 ms) was applied, and zero filling was performed to 4,096 data points.

**RESULTS**

**Exercise.** MVC ranged between 207 and 336 N. The duration of the exercises varied between 80 and 167 s for 60% MVC and between 348 and 660 s for 30% MVC (Table 1). Although toe extension muscles did not contribute to the delivered force, subjects tended to extend their toes when it became hard to maintain the desired force. This means that the extensor digitorum longus (EDL) and the extensor hallucis longus were also activated and could potentially contribute to the $^{31}$P-NMR signal. However, the extensor hallucis longus is situated distal to the TA and therefore did not contribute to the $^{31}$P-NMR signal. The influence of the EDL was studied with the 2D $^{31}$P SI results (see Two-dimensional phase-encoded $^{31}$P spectroscopic imaging below).

$^{31}$P-NMR spectroscopy with only surface coil localization, 60% MVC. An example of a $^{31}$P-NMR spectrum of the TA obtained during a 60% MVC exercise is shown in Fig. 1B. A doubling of the P, peak is clearly visible. Such a doubling of the P, NMR signal was evident in three subjects. The other four subjects showed a clear broadening of the line width of the P, peak during exercise and early recovery. In three of these subjects, a second P, peak was visible in the first spectrum after the end of exercise.

The data of three subjects in which doubled P, peaks were analyzed are summarized in Table 2. The values were derived from the fitted line pieces through the data points as described. The pH levels derived from the left (low field) and right (high field) P, peaks are designated as $p$H$_{high}$ and $p$H$_{low}$, respectively. The
slopes of pH<sub>high</sub> and pH<sub>low</sub>, the maximum difference between pH<sub>high</sub> and pH<sub>low</sub> during exercise, and the pH levels at the end of exercise are given. For pH<sub>high</sub> and pH<sub>low</sub>, the mean ± SD slopes are (-4.2 ± 4.4) x 10<sup>-3</sup> and (-8.4 ± 5.0) x 10<sup>-3</sup> pH units/s, respectively, and the mean of the maximum difference in pH levels during exercise is 0.41 ± 0.03 pH units. The mean ± SD values at the end of exercise are, respectively, 6.69 ± 0.26 and 6.28 ± 0.24. No slopes are given for subject 5 because two Pi peaks could only be analyzed just before the end of exercise and during recovery.

Figure 2A shows the results for the pH analysis

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**Table 2. 60% MVC: pH slopes, maximum differences, and end-of-exercise values**

<table>
<thead>
<tr>
<th></th>
<th>Slope of pH&lt;sub&gt;high&lt;/sub&gt;, x10&lt;sup&gt;-3&lt;/sup&gt; g&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Slope of pH&lt;sub&gt;low&lt;/sub&gt;, x10&lt;sup&gt;-3&lt;/sup&gt; g&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Max (pH&lt;sub&gt;high&lt;/sub&gt; minus pH&lt;sub&gt;low&lt;/sub&gt;)</th>
<th>pH&lt;sub&gt;high&lt;/sub&gt; at End of Exercise</th>
<th>pH&lt;sub&gt;low&lt;/sub&gt; at End of Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject 3</strong></td>
<td>-1.1</td>
<td>-4.8</td>
<td>0.43</td>
<td>6.98</td>
<td>6.55</td>
</tr>
<tr>
<td><strong>Subject 5</strong></td>
<td>-7.3</td>
<td>-11.9</td>
<td>0.42</td>
<td>6.50</td>
<td>6.12</td>
</tr>
<tr>
<td><strong>Subject 6</strong></td>
<td>-4.2 ± 4.4</td>
<td>-8.4 ± 5.0</td>
<td>0.41 ± 0.03</td>
<td>6.69 ± 0.26</td>
<td>6.28 ± 0.24</td>
</tr>
<tr>
<td><strong>Means ± SD</strong></td>
<td>-4.2 ± 4.4</td>
<td>-8.4 ± 5.0</td>
<td>0.41 ± 0.03</td>
<td>6.69 ± 0.26</td>
<td>6.28 ± 0.24</td>
</tr>
</tbody>
</table>

pH<sub>high</sub> and pH<sub>low</sub> are derived from, respectively, the left (low field) and right (high field) Pi peaks. Slopes, maximum difference, and end-of-exercise values are derived from fitted line pieces through the data points (see text).

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Fig. 2. Results of pH analysis and 31P-NMR spectra of subject 6, performing a 60% MVC exercise. A: pH. Start (at 0 s) and end of exercise (at 96 s) are marked with vertical lines. One (×) or two (○) Pi peaks were analyzed. Lines through data points are fitted as described in the text. Horizontal bar, just over the time scale, indicates the time interval corresponding to the spectral selection shown in B for the region with Pi peaks. B: Pi peak region of sequential spectra. Numbers below the spectra represent time (in s). Note the gradual appearance of 2 Pi peaks during exercise leading to 2 different pH levels (A and B), the decrease of the 2 pH levels and simultaneous increase of the relative size of the right Pi peak (A and B), and the relative quick recovery (= disappearance) of the left Pi peak (B).
Table 3. 30% MVC: pH slopes, pH differences, and end-of-exercise values

<table>
<thead>
<tr>
<th>Subject</th>
<th>Slope of pH&lt;sub&gt;high&lt;/sub&gt;, ( \times 10^{-3} ) s&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Slope of pH&lt;sub&gt;low&lt;/sub&gt;, ( \times 10^{-3} ) s&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Max (pH&lt;sub&gt;high&lt;/sub&gt; minus pH&lt;sub&gt;low&lt;/sub&gt;)</th>
<th>pH&lt;sub&gt;high&lt;/sub&gt; at End of Exercise (If Two)</th>
<th>pH&lt;sub&gt;low&lt;/sub&gt; at End of Exercise (If Two)</th>
<th>pH at End of Exercise (If One)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td>-0.7</td>
<td>-2.6</td>
<td>0.42</td>
<td>6.24</td>
<td>6.25</td>
<td>6.04</td>
</tr>
<tr>
<td>Subject 2</td>
<td>-1.8</td>
<td>-1.8</td>
<td>0.34</td>
<td>7.01</td>
<td>6.61</td>
<td>6.45</td>
</tr>
<tr>
<td>Subject 3</td>
<td>-0.1</td>
<td>0.2</td>
<td>0.48</td>
<td>6.90 ± 0.16</td>
<td>6.54 ± 0.11</td>
<td>6.35 ± 0.10</td>
</tr>
<tr>
<td>Subject 4</td>
<td>-0.8</td>
<td>-2.9</td>
<td>0.30</td>
<td>6.46</td>
<td>6.38</td>
<td>6.34</td>
</tr>
<tr>
<td>Subject 5</td>
<td>-0.6</td>
<td>-0.5</td>
<td>0.36</td>
<td>6.79</td>
<td>6.46</td>
<td>6.41</td>
</tr>
<tr>
<td>Subject 6</td>
<td>0.0</td>
<td>-0.6</td>
<td>0.46</td>
<td>6.41</td>
<td>6.41</td>
<td>6.41</td>
</tr>
<tr>
<td>Subject 7</td>
<td>-0.9</td>
<td>1.6</td>
<td>0.46</td>
<td>6.41</td>
<td>6.41</td>
<td>6.41</td>
</tr>
<tr>
<td>Means ± SD</td>
<td>-0.7 ± 0.6</td>
<td>-1.4 ± 1.1</td>
<td>0.40 ± 0.07</td>
<td>6.90 ± 0.16</td>
<td>6.54 ± 0.11</td>
<td>6.35 ± 0.10</td>
</tr>
</tbody>
</table>

pH<sub>high</sub> and pH<sub>low</sub> are derived from, respectively, the left (low field) and right (high field) Pi peak. Slopes, maximum difference, and end-of-exercise values are derived from fitted line pieces through the data points (see text).

before, during, and after an exercise at 60% MVC performed by subject 6. Figure 2B shows stack plots of spectra for the Pi peak region for the same period. During exercise, two Pi peaks gradually appear. With lowering of the pH levels, the relative size of the right (high-field) Pi peak increases (Fig. 2, A and B). This Pi peak corresponds to pH<sub>low</sub>. The subsequent recovery of the left (low-field) Pi peak is remarkably quicker than the recovery of the right Pi peak (Fig. 2B).

31P-NMR spectroscopy with only surface coil localization, 30% MVC. All seven subjects showed doubled Pi peaks during the 30% MVC exercise. The results of the pH analysis for all subjects are summarized in Table 3. The values were derived from fitted line pieces through the data points as described. The slopes of pH<sub>high</sub> and pH<sub>low</sub>, the maximum difference in pH<sub>high</sub> and pH<sub>low</sub> during exercise, and the pH levels at the end of exercise are given for each subject. If pH<sub>high</sub> or pH<sub>low</sub> consisted of two line pieces, the slope from the first line piece is given. The mean ± SD slopes for pH<sub>high</sub> and pH<sub>low</sub> are, respectively, (−0.7 ± 0.6) and (−1.4 ± 1.1) \( \times 10^{-3} \) pH units/s, and the mean of the maximum difference in pH levels is 0.40 ± 0.07 pH units. The mean pH values (± SD) at the end of exercise are 6.90 ± 0.16 and 6.54 ± 0.11 for pH<sub>high</sub> and pH<sub>low</sub>, respectively, and 6.34 ± 0.09 if only one Pi peak is left at the end of exercise.

Figure 3A shows the results for the pH analyses during rest, exercise, and recovery at 30% MVC as analyzed from the data of subject 6. Figure 3B shows the Pi peak region of the spectra, corresponding to the most remarkable changes in peak area distribution over both Pi peaks and to the course of the peak after the end of exercise. pH heterogeneity appears during a large part of the exercise but is absent in the first and last spectra during exercise (Fig. 3A). The relative size of the right Pi peak (corresponding to pH<sub>low</sub>) increases as the highest pH level decreases (Fig. 3, A and B, between 415 and 510 s). pH<sub>high</sub> declines faster than pH<sub>low</sub> so that the two Pi peaks merge into one large peak, corresponding to one low pH level (Fig. 3, A and B, starting after 510 s).

Figure 4A shows the results of pH analyses of rest, exercise, and recovery at 30% MVC, performed by subject 3. Figure 4B shows the Pi region of the spectra during the final 8 min of exercise. For clarity, every second available spectrum is skipped in this presentation. Again two Pi peaks appear. In this subject, these two peaks last until the end of exercise and, in addition, the peaks and the calculated pH values stabilize during a long period compared with the other subjects. This behavior was the reason to select this subject for the spectral imaging session presented below, which has a much lower time resolution (8 min).

Two-dimensional phase-encoded 31P spectroscopic imaging. The 2D 31P SI measurement required an acquisition time of 8 min. Too much shifting of the Pi peaks during these 8 min would cause a spread and flattening of the Pi peaks. Therefore, the 2D 31P SI measurements were performed on subject 3, who showed two stable pH levels during 30% MVC in the measurement with only surface coil localization (Fig. 4A). Two 2D 31P SI measurements were acquired in one session. The first was measured during rest, immediately preceding the exercise. The signal intensities reflect the sensitivity profile of the 31P coil (Fig. 5A). Four of the 64 voxels contained almost all signal intensity. These voxels cover the TA (3 1⁄2 voxel) and part of the EDL (1⁄2 voxel). Because the signal intensity is higher in the most ventral voxels, more than 3⁄8 of the total signal intensity originates from the TA. The signal intensity of PCr is highest in the medial ventral (left upper) voxel.

The second 2D 31P SI (Fig. 5B) was measured during the last 8 min of an exercise at 30% MVC. In this 2D 31P SI, the highest intensity of PCr is now observed for the lateral ventral (right upper) voxel. The medial ventral (left upper) voxel, in which PCr is most declined, shows a doubled Pi peak. Five voxels clearly reveal increased Pi peaks. Fig. 5C shows enlargements of the corresponding spectra obtained during rest and during exercise. Obviously, a pH gradient from lateral (pH level of 7.0 in the lateral voxels) to medial (lowest pH levels of 6.3 and 6.7 in the most medial voxel) is present. A summation of all five spectra (not shown) during exercise results in two Pi peaks at pH 7.0 and 6.5, which is similar to the pH levels measured with only surface coil localization in the same subject (Fig. 4A). As expected, there is a good reproducibility within one subject (16).
DISCUSSION

*PH heterogeneity within the TA*. This study reveals pH heterogeneity in the TA during sustained isometric exercise at both sides of the anaerobic threshold (30 vs. 60% MVC). This pH heterogeneity is not caused by the contributions of different muscles in the leg because the only other muscle within the field of view of the coil (the EDL), which was sometimes activated by the subjects, contributes to less than 1/8 of the total $^{31}$P-NMR signal.

The combination of the two 2D $^{31}$P Sls, measured during rest and during exercise at 30% MVC, respectively, shows that during exercise PCr is more decreased at the medial side than at the lateral side of the TA. In this medial part of the muscle, a doubling of the $P_i$ peak occurs during the last 8 min of exercise. Whether the resulting two $P_i$ peaks were present simultaneously during the 8 min acquisition time or one after the other cannot be concluded from these data. The $^{31}$P-NMRS data, acquired with only surface coil localization, however, show that the two pH levels occur simultaneously during the last 6½ min of the exercise, in this subject. Therefore, the doubled $P_i$ peak must be ascribed to two pH compartments in the medial part of the TA. The size of these compartments has to be smaller than the nominal voxel size of $1.5 \times 1.5 \times 20 \text{ cm}^3$. As argued by Vandenborne et al. (25), differences between intra- and extracellular pH, or intrace-
lular pH differences, are unlikely to be the origin of the distinct P\textsubscript{i} peaks. Remaining sources are difference in activation or a partial limitation of the oxygen supply, both within a voxel size of 1.5 \times 1.5 \times 20 \text{ cm}^3. We have no anatomic indications for two distinct areas of blood supply within this voxel. Neither do we have anatomic indications for two distinct areas of muscle activation otherwise than on the level of muscle fiber types. Therefore, the following discussion proceeds from the most probable interpretation linking the left (pH\textsubscript{high}) peak to the slow-twitch (type I) motor units in the muscle and the right (pH\textsubscript{low}) one to the fast-twitch (type II) motor units.

**pH gradient within the TA.** The fact that the pH shows a declining gradient from lateral to medial within the TA is possibly caused by differences in blood supply (20). The compliance of the tibia bone is smaller than the compliance of the membrane surrounding the anterior compartment. During a sustained isometric exercise, this could possibly lead to a gradient in intramuscular pressure. If it is assumed that the mean arterial blood pressure and the local metabolic vasodilatation are homogeneously distributed over the whole TA, then blood flow is lowest closest to the tibia bone.

**Comparing 60% and 30% MVC.** Directly after the start of exercise, a small transient increase of pH is visible in all experiments with only coil localization (also in Fig. 2A, 3A and 4A). This reflects the proton consumption of PCr, which initially is the main energy source in these experiments (4).

In both exercise levels, the decrease of the pH\textsubscript{low} is roughly twice as fast as that of pH\textsubscript{high} (Tables 2 and 3). These pH slopes can be used as a measure of glycolytic activity (13). The factor of two corresponds well with the difference of phosphofructokinase activity in fiber types I and II, also a measure of glycolytic activity (7).

Recovery of both P\textsubscript{i} peaks could only be followed in two cases because the P\textsubscript{i} peaks tend to disappear...
Fig. 5. A: 31P 2-dimensional phase-encoded spectroscopic imaging (2D 31P SI) of the left lower leg of subject 3 during rest, preceding a 30% MVC exercise. In each voxel, that part of the spectrum containing Pi and PCR is shown. TA is outlined with a broken line. Note that 4 of the 64 voxels contain most of the 31P-NMR signal (outlined with a solid line). These 4 voxels cover mainly the TA and partly the extensor digitorum longus. The highest PCR peak occurs in the medial ventral (left upper) side of these 4 voxels. Large white area on left of the 1H NMR image is the inner side of the tibia bone. Small white spot is the inner side of the fibula bone. Long white area on right is an artifact of the 1H coil (high-flux area). B: 2D 31P SI of the same left lower leg as in A measured during the last 8 min of the fatiguing 30% MVC exercise after measuring A. TA is outlined with a broken line. In each voxel, the same part of the spectrum as in A is shown; however, the y-axis is rescaled. Note that the Pi peak is increased in 5 voxels (outlined with a solid line). The PCR peak is highest in the lateral ventral (right upper) voxel, and the Pi peak is broadened in the medial (left) voxels covering the TA. C: enlargements of 5 voxels covering the TA. Dashed lines originate from the 2D 31P SI measured during rest; solid lines are from the 2D 31P SI measured during 30% MVC exercise. Scaling of the x- and y-axes is the same for all. Numbers at the Pi peaks are the corresponding pH levels. Note the doubled Pi peaks in the medial (left) voxels. Furthermore, note the pH gradient from lateral (right) side to medial (left) side of the TA.
quickly in the noise after exercise. In these two cases (both after 60% MVC), the P_i peak ascribed to the type I fibers disappears much faster than the one ascribed to the type II fibers. This can be explained by the pH dependency of the recovery of P_i (10) and the higher oxidative capacity of the type I fibers (21) and is in agreement with the observations of others (1, 18, 19, 24, 29, 30).

The pH heterogeneity is more difficult to measure during 60% MVC than during 30% MVC exercise, although the pH differences at the end of exercise are similar (Tables 2 and 3). An obvious explanation is that fewer data points are available for a 60% MVC exercise. Moreover, a faster shifting of both P_i peaks limits proper analysis.

**Distribution of peak areas of P_i.** A number of issues are relevant in relating the distribution of peak areas to the recruitment of motor units.

1) Johnson et al. (12) and Polgar et al. (20) investigated fiber type distribution and sizes in young, healthy, male subjects. In the superficial region of the TA, they found a mean percentage of type I fibers of 73% and a mean cross section of type I and type II fibers of $2.4 \times 10^{-9}$ m$^2$ and $3.4 \times 10^{-9}$ m$^2$, respectively. Therefore, it is assumed that 64% of the TA cross section consists of type I fibers.

2) With increasing central neural drive, motor units are recruited according to the size principle of Henneman (8), whereby smaller type I motor units become active before larger type II motor units.

3) Vandeborre et al. (26) showed that, in contrast to results of experiments on animals, human muscle with predominantly type I fibers had the same PCr content as muscle with predominantly type II fibers. Thus, it is assumed that type I fibers and type II fibers have the same PCr content in rest.

4) PCr consumption might be higher in type II fibers because PCr also acts as a proton buffer (31).

5) If local ischemia arises, a part of the P_i is trapped by mitochondria and becomes invisible for $^{31}$P-NMRS (11). Already recruited motor units will especially “lose” some of their P_i signal. From the size principle, it can be predicted that the peak area of type I fibers is most affected.

In the theoretical case that all motor units will be equally recruited during the whole isometric exercise, around or somewhat less than 64% of the PCr consumption is attributed to type I motor units (see above, issues 1, 3, and 4). If two P_i peaks can be discerned, one expects a somewhat larger left peak. If ischemia occurs, a decrease of P_i in all active fibers is expected, leading to a proportional decrease of both P_i peaks (issue 5). The uptake of P_i in the mitochondria is possibly larger in type I fibers because of a larger mitochondrial density in that fiber type. In that case, a larger decrease of the left P_i peak is expected.

During sustained isometric exercise at 30 or 60% MVC, the prediction is more complicated because only a part of the motor units is recruited at the start of exercise. Fatigue and/or a deterioration of blood supply forces the system to recruit more motor units. Even at sustained isometric exercise at 30% MVC, deterioration of blood supply may occur, caused by an increase of intramuscular pressure (3). Two P_i peaks appear as soon as most type I units and a part of the type II units are recruited (issue 2) and intracellular protons accumulate. A relatively larger left P_i peak and a smaller right P_i peak are expected. The difference between the peak areas depends on the number of type II motor units that is necessary at that time and on the level of ischemia (issue 5). The more type II units involved, the larger the right P_i peak; the more ischemia, the smaller the left P_i peak.

Our results (Figs. 2, 3, and 5C) nicely illustrate that an increase of the relative size of the right P_i peak coincides with a notable decrease of pH$_{\text{high}}$ and pH$_{\text{low}}$. This is particularly clear during the last part of the exercise at 30% MVC, where (in most of our subjects) a striking increase of the right P_i peak occurs in coincidence with an accelerated decline of pH$_{\text{high}}$. All together, this points to a deterioration of blood supply combined with anaerobic glycolysis. This fits well with the mechanisms sketched above: the lower the blood flow, the less efficient type I fiber contributions and thus the more type II motor units have to be recruited, according to the size principle, to maintain the expected force.

Yoshida and Watari (29) showed the development of P_i peaks in one subject during a progressive dynamic exercise at an intermediate frequency (0.8 Hz) of the biceps femoris. Their results (Fig. 5) show that a substantial increase of the low pH peak area coincides with a shift of both P_i peaks toward the PCr peak (after 3–3½ min of exercise). Although their exercise is non-isometric, and thus the size principle is disrupted (e.g., Ref. 9), the development of P_i peak areas and positions suggests an orderly recruitment of motor units.

In conclusion, this study reveals pH heterogeneity in the TA during sustained isometric exercise below and above anaerobic threshold. The fact that the spatial pH distribution shows a declining gradient from lateral to medial within the TA (one subject) is attributed to intramuscular differences in blood supply. The pH dependency of relative sizes of both P_i peaks, in the temporally and spatially characterized $^{31}$P-NMRS data, can be explained by the size principle of motor unit type-related orderly recruitment of motor units.

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**REFERENCES**


