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Published in:
Journal of Applied Physiology

Publication date:
1994

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Quistorff, B., Mizuno, M., & Secher, N. H. (1994). ^{31}P -NMR spectroscopy, rsEMG, and histochemical fiber types of human wrist flexor muscles. *Journal of Applied Physiology*, 76(2), 513-538.

³¹P-NMR spectroscopy, rsEMG, and histochemical fiber types of human wrist flexor muscles

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³¹P-NMR spectroscopy, rsEMG, and histochemical fiber types of human wrist flexor muscles. *J. Appl. Physiol.* 76(2): 531–538, 1994.—The metabolic response to graded rhythmic forearm exercise determined by phosphorus-31 nuclear magnetic resonance (³¹P-NMR) spectroscopy was evaluated with respect to recruitment pattern of muscles and to fiber type composition of the nondominant arm in 14 untrained subjects. Rectified smoothed surface electromyography (rsEMG) over finger and wrist flexor muscles increased similarly and progressively at 30, 45, and 60% of maximal exercise intensity. On the basis of the ³¹P-NMR response pattern during exercise, subjects were separated into three groups: H group ($n = 4$), with a single high-pH P_i peak; M group ($n = 7$), with two P_i peaks reflecting two pH components; and L group ($n = 3$), with a single low-pH P_i peak. The H group demonstrated a faster recovery of P_i and phosphocreatine than the L group ($P < 0.05$). The M group displayed both types of P_i recovery and an intermediate resynthesis rate of phosphocreatine. The M group had an even distribution of slow- and fast-twitch fibers in the flexor carpi radialis muscle, whereas subjects in the H and L groups possessed a predominance of slow- and fast-twitch fibers, respectively. The results suggest that during rhythmic forearm exercise the interindividual NMR variation in energy metabolism is related closely to fiber type composition of the muscle.

fast-twitch fibers; forearm exercise; inorganic phosphate; muscle pH; phosphocreatine; slow-twitch fibers

BY THE USE OF phosphorus-31 nuclear magnetic resonance (³¹P-NMR) spectroscopy, reduction of phosphocreatine (PCr) and increase in P_i during exercise differ among individuals (4, 23, 29). In some subjects, forearm (28, 31, 37) and calf muscle exercise (1, 40) cause the appearance of muscle pH compartmentation in the form of a high- and a low-pH P_i peak. This interindividual variation in the NMR response during exercise may reflect different acquisition from active and inactive fibers and/or muscles. Squeezing a rubber ball with one hand results in a reduction of PCr and muscle pH as well as an increase in P_i that is greater in finger than in wrist flexor muscles (37). NMR imaging on the finger and wrist flexor muscles demonstrates a difference in transversal relaxation time, possibly due to variation in exercise-induced water accumulation between handgrip and wrist flexion (13–15). Furthermore, within the finger flexor muscles, regional differences exist depending on which fingers are mainly used during exercise (16, 19). However, a mixture of active and inactive fibers imposed by partial neuromuscular blockade does not provoke muscle pH compartmentation during forearm exercise (26, 28).

Alternatively, interindividual differences in PCr and P_i

as well as in end-exercise muscle pH may reflect the metabolic characteristics of the two major fiber types and their recruitment pattern (1, 4, 5, 21, 26, 31, 40). Human skeletal muscle possesses a mixture of slow-twitch (ST; type I or oxidative) and fast-twitch (FT; type II or glycolytic) fibers (11, 20, 35). The relative occurrence of ST fibers is normally distributed in healthy individuals (35). However, neither muscle pH compartmentation nor energy metabolite dynamics during exercise and recovery have been related to the relative proportion of ST and FT fibers in the muscle on which NMR spectroscopy was carried out.

The aim of this study was to evaluate whether interindividual variability in NMR-detected energy metabolism during forearm exercise is related to a different involvement of finger and wrist flexor muscles as examined by the rectified smoothed surface electromyographic activity (rsEMG) and/or to the histochemically determined fiber type composition estimated in surgical biopsies from wrist flexor muscles.

METHODS

The nondominant arm of 12 male and 2 female healthy subjects (age 20–25 yr, weight 56–77 kg, height 171–193 cm) was studied. None had participated in regular training requiring forearm exercise. Subjects were informed about the nature and possible risks of the experiments before they gave written consent to participate in this study, which was approved by the Local Ethics Committee.

Exercise Protocol

Subjects were familiarized with the experimental procedures two to five times separated by ≥ 1 wk. Rhythmic forearm muscle contractions were performed with a modified pneumatic ergometer involving both handgrip and wrist flexion (33). The dampening system made the ergometer handle return automatically to the resting position. Thus, it allowed the forearm flexor muscles to perform only concentric contractions. The contraction time was 0.8–1 s, and one contraction was performed every 6th s.

Maximal rhythmic forearm exercise capacity was defined as the maximal intensity (in W) that could be maintained with 10 contractions/min for ~ 2 min and was determined by a step-incremental procedure. The initial 3-min exercise intensity was 0.6 W for the males and 0.2 W for the females. Exercise intensity was increased every 2 min by 0.4 W for the males and by 0.2 W for the females. In six subjects the final increment was 0.1 W. The stepwise incremental test was repeated on a separate day, and the maximal forearm exercise capacity was the same. Maximal voluntary contraction (MVC) force was determined using the same ergometer and is presented in newton-meters. Forearm circumference was measured corresponding to one-

fourth of the distance between the olecranon and the styloid process.

In NMR experiments, graded rhythmic exercise was performed at 30% of maximal intensity for 3 min, at 45% for 2 min, and at 60% for 3 min followed by 15 min of recovery. Immediately after exercise, the intensity of effort was rated subjectively. In this rating, "2" signifies a minor effort, "7" a major effort, and "10" a maximal effort.

NMR Spectroscopy

A Vivospec spectrometer (Otsuka Electronics) and an 80-cm-long 2.9-T horizontal superconducting magnet with a 26-cm bore (MagneX Scientific) were used. A 4-cm-diam two-turn surface coil was placed over the wrist flexor muscles centered at one-fourth of the distance between the olecranon and the styloid process. Thus, the coil was expected to cover primarily musculus (m.) flexor carpi radialis and also portions of m. palmaris longus and m. flexor carpi ulnaris, as well as m. flexor digitorum superficialis and m. flexor digitorum profundus.

Homogeneity of the magnetic field was optimized by shimming on the proton signal. Line width before experiments was <0.5 parts per million. The ³¹P spectra were obtained at 49.83 MHz by single-pulse excitations (65 μs), and pulse power and width were optimized by the height of the PCr peak. Data were collected in 2K points over 205 ms at a 6-s interpulse delay. Data acquisition was performed for 1.5 min at rest and during exercise, continuously in blocks of 30 s at the end of exercise and during the first 2 min of recovery, and in blocks of 1 min during the remaining 13 min of recovery. In ³¹P spectra (16 scans) obtained at rest, the signal-to-noise ratio ranged from 12 to 16.

Spectral Analysis

All spectra were analyzed by the same operator without knowledge of subjects' fiber type composition. Applying exponential line broadening of 5 Hz to each spectrum, muscle pH was calculated according to Taylor et al. (37). The chemical shift value at the maximum height(s) of P_i resonance(s) was used for this calculation.

The peak areas of P_β, PCr, and β-ATP were measured by a curve-fitting program of the Vivospec spectrometer software (Otsuka Electronics), assuming Lorentzian line shape and applying a line broadening of 5 Hz. Baseline correction was performed automatically after manual choosing of eight points at the same chemical shifts in all spectra. The program subsequently identified the peaks of phosphomonoester, P_β, PCr, and ATP. When splitting of the P_i peak was not identified by the program, even a broadened signal was considered as a single peak.

The peak areas were corrected for partial saturation. Saturation factors, determined as the ratio of the peak area in spectra obtained with 6- and 30-s interpulse delays, were 1.44 for P_β, 1.41 for PCr, and 1.15 for β-ATP. During the course of the experiments, the total ³¹P signal (phosphomonoester + P_β + PCr + β-ATP) was constant within 10%. The coefficient of variation in double determinations of the peak area was 1.6% for high-pH P_β, 1.3% for low-pH P_β, 2.8% for PCr, and 1.8% for β-ATP. In all spectra the total P_i peak area was used for calculating the P_i/PCr ratio. The area of PCr and β-ATP was expressed as percentage of the resting value and fold change was used for P_i. The kinetics of PCr resynthesis and P_i decrease during recovery were evaluated by fitting data to a monoexponential function applying least square regression. The half time (t_{1/2}) of recovery was calculated from the fitted curve.

rsEMG

Two surface electrodes (5-mm diameter) (Blue sensor type N-10-A, Medicotest) were placed 3 cm apart over the flexor digitorum superficialis and profundus (finger flexor muscles) and the flexor carpi radialis and ulnaris (wrist flexor muscles) (8) on the area defined by the position of the NMR coil. The rsEMG was registered by a DISA-EMG amplifier (Copenhagen, Denmark) with a built-in mean voltage unit kit (type 15 C 01) and a multichannel recorder (Elema-Schönander, Stockholm, Sweden). This activity is reported in millivolts.

The rsEMG experiments were performed outside the NMR instrument. With a 1-wk interval, either the flexor digitorum superficialis and the flexor carpi radialis or the flexor digitorum profundus and the flexor carpi ulnaris were studied. First, the rsEMG was recorded during MVC involving both handgrip and wrist flexion. Second, subjects were asked to perform handgrip MVC. Thereafter, wrist flexion MVC was performed with extended fingers. Finally, the rsEMG was recorded during 30, 45, and 60% of maximal rhythmic exercise intensity corresponding to NMR measurements.

Muscle Biopsy

Seven subjects accepted the muscle biopsy protocol. Surgical biopsies were taken from the flexor carpi radialis of the non-dominant arm. The sampling site was the superficial part of the muscle at a location defined by the center of the NMR coil. Approximately 30 mg of muscle were mounted in an embedding medium (O.C.T. Compound, Miles) and frozen in isopentane precooled with liquid nitrogen. Samples were stored at -80°C until analyses were performed. Serial transverse sections (10 μm thick) were cut at -20°C by using a Cryo-cut Cryostat (American Optical) and incubated for myofibrillar adenosine-triphosphatase reaction at pH 9.4 (30). After preincubations at pH 4.3, 4.6, and 10.3, the muscle fibers were classified under light microscopy as ST, FTa, or FTb (6). The relative occurrence of fiber types was determined on an average of 338 (range 276-592) fibers in each biopsy.

Statistics

Values are expressed as means ± SD. Comparisons between related observations and between independent samples were performed by Friedman's two-way analysis of variance by rank and by the Kruskal-Wallis one-way analysis of variance by rank, respectively (36). When significant differences were detected, these were located by the Wilcoxon's matched-pairs signed-ranks test for related observations or the Mann-Whitney U test for independent samples. The interdependence of observations was expressed by the Spearman rank correlation coefficient. A 5% level was considered significant for two-tailed tests.

RESULTS

Separation of Subjects

At the end of rhythmic forearm exercise with 60% of maximal intensity, subjects could be separated into three groups according to the appearance of 1) a single P_i peak with a high pH (H group; n = 4), 2) two P_i peaks with a high and a low pH (M group; n = 7), or 3) a single P_i peak with a low pH (L group; n = 3) (Table 1, Fig. 1). At the end of exercise, muscle pH of 6.6 was the lowest value observed in the high-pH P_i peak for the M group. This value was used for dividing subjects who showed a single P_i peak into either the H or L group.

TABLE 1. Physiological characteristics and data obtained from ³¹P-NMR spectra in three groups of subjects

	Group		
	H	M	L
<i>n</i>	4	7	3
Forearm circumference, cm	25.1±2.0	25.5±1.0	26.2±1.7
MVC, N·m	28.1±8.6	26.4±4.7	30.8±2.2
Maximal exercise, W	1.44±0.58	1.56±0.44	1.58±0.12
Resting P _i /PCr	0.12±0.02	0.13±0.04	0.10±0.02
Resting muscle pH	7.03±0.04	7.06±0.06	7.04±0.09
End-exercise (60%) muscle pH			
High-pH P _i	6.78±0.12	6.71±0.07	
Low-pH P _i		6.19±0.14*	6.34±0.08*

Values are means ± SD. NMR, nuclear magnetic resonance; H group, single high-pH P_i peak at end of exercise; M group, 2 P_i peaks reflecting 2 different pH components; L group, single low-pH P_i peak; MVC, maximal voluntary contraction; PCr, phosphocreatine. * Significantly different from high-pH P_i in both H and M group ($P < 0.05$).

Forearm circumference, MVC force, and maximal rhythmic exercise intensity did not differ significantly between the three groups (Table 1). Subjective ratings at the end of exercise with maximal and 60% intensity were 8–10 and 5–7, respectively, with no difference between the three groups. At rest, no significant difference in the P_i/PCr ratio or muscle pH was found between the three groups.

Energy Metabolite Dynamics

Forearm exercise. In the M group, two P_i peaks were already evident at 30% of maximal exercise intensity, differentiating a high- (6.87 ± 0.08) and a low-pH peak (6.50 ± 0.15) ($P < 0.05$; Fig. 1). At this work intensity, the high-pH P_i peak had increased 4.6 ± 2.1-fold relative to the resting P_i peak ($P < 0.01$) and remained fairly constant at 45% (4.6 ± 2.4-fold) and 60% (4.4 ± 2.3-fold) of maximal exercise intensity. In contrast, the low-pH P_i peak increased progressively from 2.4 ± 1.6-fold at 30% to 3.3 ± 1.8-fold at 45% intensity and reached 4.3 ± 1.6-fold at 60% intensity ($P < 0.01$). At the end of exercise, muscle pH was 6.71 ± 0.07 for the high-pH and 6.19 ± 0.14 for the low-pH P_i peaks. An additional P_i peak with intermediate pH (triple P_i peak splitting) was noted in two subjects of the M group who had resulting end-exercise pHs of 6.40 and 6.58, respectively.

In the H and L group subjects who displayed a single P_i peak throughout exercise and recovery, the response patterns were similar to that of the high- and the low-pH P_i peaks in the M group, respectively (Fig. 1). During 30% exercise intensity, muscle pH was lower in the L (6.63 ± 0.20) than in the H group (6.89 ± 0.10) ($P < 0.05$). In the H group at 30% exercise intensity, the P_i peak already reached maximum (5.6 ± 1.1-fold) and remained fairly constant until the end of exercise (45%, 5.6 ± 1.0-fold; 60%, 5.7 ± 1.3-fold). In contrast, in the L group there was a progressive increase in P_i peak at 30% (9.2 ± 1.3-fold) and 45% (10.5 ± 0.9 fold) exercise intensities, and the maximal value was observed at 60% exercise intensity (11.3 ± 1.0-fold). The end-exercise P_i peak was twofold higher in the L than in the H group ($P < 0.05$).

The P_i/PCr ratio at each exercise intensity was approximately fourfold lower in the H than in the L group ($P < 0.01$; Fig. 2). For the M group this ratio displayed an intermediate level ($P < 0.05$). At the end of exercise, the reduction in PCr was greatest in the L group (86.9 ± 1.8%), medium in the M group (69.1 ± 11.1%), and smallest in the H group (60.7 ± 10.6%) ($P < 0.05$). ATP at the end of exercise was 73.5 ± 16.6% of the resting value, and no difference was observed between the three groups.

Recovery. In the M group, the $t_{1/2}$ for recovery of the high-pH P_i peak was 0.48 ± 0.24 min, four times faster than that of the low-pH P_i peak, 2.01 ± 0.94 min ($P < 0.05$). Thereafter, the high-pH P_i peak remained below the resting value until 5 min of recovery. After 6 min of recovery only a single P_i peak could be identified, which had returned to the resting value within the 15 min of recovery (1.3 ± 0.3-fold), whereas muscle pH was still slightly lower than at rest (6.97 ± 0.03) ($P < 0.05$).

The $t_{1/2}$ for recovery of the P_i peak was shorter in the H (0.64 ± 0.08 min) than in the L group (1.00 ± 0.17 min) ($P < 0.05$). The P_i peak in the H group reached the resting value already at 2 min of recovery and remained below the resting value until 10 min of recovery. In contrast, the P_i peak in the L group was higher than in the H group until 10 min of recovery ($P < 0.05$) and did not decrease below the resting value. At 8 min of recovery muscle pH was still lower in the L (6.65 ± 0.08) than in the H group (7.00 ± 0.06) ($P < 0.05$). In the H and L groups, both P_i (1.1 ± 0.3) and muscle pH (7.01 ± 0.04) at 15 min of recovery returned to the resting value.

The $t_{1/2}$ for resynthesis of PCr was 1.14 ± 0.14 min in the H group, 1.97 ± 0.42 min in the M group, and 3.02 ± 0.38 min in the L group ($P < 0.05$). After 15 min of recovery, PCr had reached an average of 93.8 ± 5.9% of the resting value, with no significant difference between the groups.

rsEMG

During MVC, rsEMG was similar in the flexor digitorum profundus, the flexor carpi radialis, and the flexor carpi ulnaris (0.50 ± 0.10 mV), whereas the flexor digitorum superficialis showed 17% larger activity (0.60 ± 0.10 mV) ($P < 0.05$). Compared with MVC, all flexor muscles showed a reduction in rsEMG during separated isometric contraction of either handgrip or wrist flexion ($P < 0.01$). However, during the isometric handgrip contraction, a higher rsEMG was observed in the flexor digitorum superficialis and profundus (finger flexor muscles; 0.40 ± 0.07 mV) than in the flexor carpi radialis and ulnaris (wrist flexor muscles; 0.12 ± 0.05 mV) ($P < 0.01$). In contrast, during the isometric contraction with only wrist flexion, rsEMG was higher in the wrist (0.42 ± 0.09 mV) than in the finger flexor muscles (0.16 ± 0.06 mV) ($P < 0.01$).

When subjects repeated the graded rhythmic exercise used in the NMR experiment, rsEMG was similar in the finger and wrist flexor muscles at each exercise intensity (Fig. 3). Furthermore, no difference in rsEMG was found between the three groups of subjects. The mean rsEMG of the four flexor muscles was 0.31 ± 0.05 mV for 30%

FIG. 1. Fold changes in P_i peak(s) relative to resting value and muscle pH during 30, 45, and 60% intensity of rhythmic forearm exercise followed by 15 min of recovery in 3 groups of subjects. H group, single high-pH P_i peak at end of exercise ($n = 4$); M group, 2 P_i peaks reflecting 2 different pH components ($n = 7$); L group, single low-pH P_i peak ($n = 3$). Values are means $\pm$ SD.

exercise intensity and increased slightly but significantly during 45% (0.35 ± 0.06 mV) and 60% exercise intensity (0.38 ± 0.07 mV) ($P < 0.05$). No correlation was found between rsEMG from the flexor carpi radialis during 60% of maximal exercise intensity and the end-exercise muscle pH as observed in all subjects.

Fiber Type Composition

The relative occurrence of ST fibers in the flexor carpi radialis ranged from 15 to 65% (Table 2). When subjects were classified into the three groups on the basis of their NMR response, the M group showed an even distribution of ST and FT fibers (Fig. 4). The highest value of relative occurrence of ST fibers was found in one subject from the H group, whereas the two lowest values were obtained from two subjects in the L group.

A correlation was found between the end-exercise

muscle pH and the relative occurrence of ST fibers in the flexor carpi radialis ($P < 0.05$; Fig. 5). Furthermore, the P_i /PCr ratio at each exercise intensity and the $t_{1/2}$ for resynthesis of PCr were also correlated inversely to the proportion of ST fibers (Fig. 6). However, no correlation was found between the $t_{1/2}$ for recovery of P_i and the fiber type composition.

DISCUSSION

This study addresses the cause(s) of muscle pH compartmentation in the form of a high- and a low-pH P_i peak frequently observed during ³¹P-NMR spectroscopy of exercising muscle (1, 28, 31, 37, 40). Splitting of the P_i peak may reflect the difference in lactate production, accumulation, turnover, and/or removal between ST and FT fibers (12, 26, 38) rather than a difference in muscle buffering capacity (27) and therefore may be an indica-

FIG. 2. P_i /phosphocreatine (PCr) ratio at 30, 45, and 60% exercise intensity in H, M, and L groups. Values are means $\pm$ SD. Values for M group were calculated as (high-pH + low-pH P_i peak)/PCr peak. * Significantly different from other 2 groups ($P < 0.05$). ** Significantly different from H group ($P < 0.01$).

tion of the relative occurrence of the two major muscle fiber types (1, 31, 40). Alternatively, P_i peak splitting may reflect differences in recruitment pattern of fibers and/or muscle groups, not necessarily related to fiber type composition (13–16, 19, 37).

The present study combined histochemical fiber type evaluation, rsEMG, and ³¹P-NMR spectroscopy, and the findings support the former interpretation. Muscles that possessed a larger oxidative potential as expressed by a dominance of ST fibers showed less change in P_i and PCr during exercise and a faster resynthesis of PCr during recovery compared with muscles with a large glycolytic capacity estimated by a dominance of FT fibers (Figs. 4 and 6). Accordingly, a muscle with an even distribution of ST and FT fibers showed an intermediate response. Furthermore, a correlation was demonstrated between the NMR-observed muscle pH at the end of exercise and the relative proportion of ST fibers in the flexor carpi radialis (Fig. 5). Between the three groups, however, no difference was found in rsEMG at each exercise intensity. This makes it unlikely that interindividual differences in recruitment of various muscle groups could explain the variability in NMR-detected energy metabolism during rhythmic forearm exercise.

These findings were obtained in subjects who were not involved in any type of training requiring forearm exercise. Likewise, between the three groups of subjects, no difference was found in forearm circumference, MVC, maximal work capacity, or in the degree of perceived exhaustion (Table 1). Thus, we demonstrated a close relationship between the NMR-observed metabolic response

FIG. 3. Rectified smooth surface EMG (rsEMG) from m. flexor digitorum superficialis (DS) and profundus (DP) (finger flexor muscles) and m. flexor carpi radialis (CR) and ulnaris (CU) (wrist flexor muscles) during 30 (A), 45 (B), and 60% (C) exercise intensity in H, M, and L groups. Values are means $\pm$ SD.

and histochemically determined fiber type composition of the muscle.

Involvement of Finger and Wrist Flexor Muscles

The rsEMG data confirmed observations by NMR imaging technique demonstrating functional differences between the finger and wrist flexor muscles (13, 14). During handgrip, the flexor digitorum superficialis and profundus (finger flexor muscles) were mainly recruited, whereas the flexor carpi radialis and ulnaris (wrist flexor muscles) were the most active during wrist flexion. How-

TABLE 2. Relative occurrence of ST, FTa, and FTb fibers

Group	Subject	Relative Fiber Occurrence, %		
		ST	FTa	FTb
H	A	65.0	27.3	7.7
	B	51.6	41.2	7.2
	C	46.6	7.5	45.9
	D	46.1	28.4	25.5
	E	40.9	20.9	38.2
L	F	21.5	43.6	34.9
	G	14.6	39.0	46.4
All groups combined				
Mean $\pm$ SD		40.9 $\pm$ 17.4	29.7 $\pm$ 21.8	29.4 $\pm$ 16.6

ST, slow twitch; FTa and FTb, fast twitch a and b, respectively. Relative occurrence was determined in surgical biopsies from the superficial part of m. flexor carpi radialis at a location defined by NMR coil.

FIG. 4. ³¹P-nuclear magnetic resonance (NMR) spectra obtained during 60% of maximal exercise intensity and relative occurrence of 2 major fiber types [slow twitch (ST) and fast twitch (FT)] determined in surgical muscle biopsies from m. flexor carpi radialis in H group (subj A), M group (subj C), and L group (subj G). Exponential line broadening of 5 Hz was used for each spectrum. ppm, Parts per million.

FIG. 5. End-exercise muscle pH related to relative occurrence of ST fibers in m. flexor carpi radialis. Values for muscle pH in M group are expressed as mean of high and low pH. $y = 7.58 \times 10^{-3}x + 6.14$ [Spearman rank correlation coefficient (r_s) = 0.75; $P < 0.05$].

ever, during graded rhythmic exercise, rsEMG did not show any change in relative involvement of the finger and wrist flexor muscles. Both muscle groups were equally recruited at the three applied exercise intensities, and rsEMG was intensified to the same extent with an increase in work rate (Fig. 3). Furthermore, no difference in rsEMG was found between the three groups of subjects. These findings suggest that the interindividual variability in NMR metabolic response is not explained by different placement of the NMR coil and/or by differing involvement of finger and wrist flexor muscles.

Fiber Type Characteristics

Human skeletal muscle consists of a heterogeneous mixture of ST and FT fibers, including antigravity (20,

FIG. 6. P_i/PCr ratio during 30 (A), 45 (B), and 60% (C) exercise intensity and half time ($t_{1/2}$) for resynthesis of PCr during recovery (D) related to relative occurrence of ST fibers in m. flexor carpi radialis. A: $y = -0.06x + 5.08$ ($r_s = 0.93$, $P < 0.01$); B: $y = -0.11x + 8.28$ ($r_s = 0.93$, $P < 0.01$); C: $y = -0.12x + 8.23$ ($r_s = 0.89$, $P < 0.01$); D: $y = -0.035x + 3.67$ ($r_s = 0.86$, $P < 0.05$).

35) and respiratory muscles (25). This was also the case for the flexor carpi radialis in which the relative occurrence of ST fibers ranged from 15 to 65% and was similar to values obtained from the flexor digitorum profundus (20). Whether the fiber type composition of the intact wrist flexor muscles is faithfully reflected by one small muscle biopsy was not addressed in the present study. Because a larger sample only slightly increases the reliability of fiber type composition, multiple biopsies are required to obtain more accurate data (22).

In resting human skeletal muscle, intracellular pH and P_i/PCr ratio appear to be similar regardless of fiber type composition (4, 21). However, slightly higher values are found in leg muscles rich in FT fibers (9, 21). Results from animals show distinct differences between the two major fiber types. In skeletal muscle of rat and cat, resting intracellular pH and P_i/PCr ratio determined by ³¹P-NMR spectroscopy are lower in ST than in FT fibers (2, 7, 24). Thus, the metabolic difference between the skeletal muscle fiber types is apparently smaller in humans (10, 34, 39) than in animals. In contrast to resting muscles, the difference in energy metabolite dynamics during forearm exercise among the subjects in the H and the L group (Fig. 1) was consistent with results obtained from rat and cat muscles during electrical stimulation in which ST fibers show less change in P_i , PCr, and pH than FT fibers (2, 7).

Muscle pH Compartmentation

Because the evaluation of fiber type composition in this study was performed in combination with NMR examination, it could be shown that intercellular pH compartmentation correlates with the relative occurrence of

ST and FT fibers (Fig. 4). Vandenborne et al. (40) reported that, during calf muscle exercise, three of seven subjects showed three P_i peaks, which were suggested to reflect ST and the two subgroups of FT fibers. In the present study, four subjects (subs D, E, F, and G) possessed an equal proportion of FTa and FTb fibers (Table 2), whereas two subjects in the M group (subs D and E) showed triple P_i peaks during rhythmic forearm exercise.

After intense static (38) and dynamic exercise (12) of thigh muscles, lactate concentration determined by quantitative analyses of single fibers is approximately twofold higher in FT than in ST fibers. The difference in lactate concentration between the two fiber types corresponds to a calculated pH difference of 0.05–0.27, which is small compared with the NMR-observed difference between the high- and the low-pH P_i peak in the present study (pH 0.50) and those reported by others (pH 0.70) (1, 31, 40). This discrepancy is not understood at present. However, an inability of the muscle biopsy technique to correctly preserve the in vivo distribution of accumulated lactate between different intra- and intercellular compartments may play a significant role.

Fiber Type Recruitment Pattern

On the basis of NMR data, the recruitment pattern of two fiber types has been proposed in forearm flexor (31) and calf muscles (1). In the present study the subjects who possessed an even distribution of the two fiber types (M group) showed an increase in both P_i peaks at the onset of exercise (Fig. 1). Although the larger change in the high-pH P_i component reached a plateau, the low-pH P_i component continued to increase after elevated exercise intensities. Furthermore, the subjects with a predominance of either ST or FT fibers showed a time course of the P_i response similar to that of the high- or low-pH P_i peak, respectively. This temporal pattern of the P_i change is in accordance with observations of histochemical studies on glycogen depletion pattern in human limb muscles with mixed fiber types (17, 35). Assuming that the high-pH P_i peak reflects the ST fibers and the low-pH P_i peak the FT fibers, the present results indicate that there is a primary recruitment of ST fibers during low-intensity rhythmic exercise, followed by a progressive involvement of FT fibers on an elevated intensity.

Recovery of PCr and P_i

In the present study, the recovery of PCr and P_i after exercise showed significant differences between the three groups of subjects. In particular, in the M group, which displayed P_i peak splitting, the high-pH P_i component was characterized by a fourfold faster $t_{1/2}$ for recovery than the low-pH P_i component. Similarly, the recovery of P_i and PCr was two- and threefold faster in the H than in the L group, respectively. These differences in P_i and PCr kinetics during recovery are not necessarily a reflection of the observed difference in fiber type composition but may in part be explained by an effect of the different postexercise muscle pH, as demonstrated by Barbiroli et al. (3). After exercise, resynthesis of PCr requires ATP produced through mitochondrial oxidative phosphorylation (32), and, furthermore, the rate of resynthesis is

correlated to the activity of mitochondrial oxidative enzymes (18). Similarly the present results demonstrate a correlation between the $t_{1/2}$ for recovery of PCr and the proportion of ST fibers in the muscle (Fig. 6).

In conclusion, our results support the notion that during rhythmic forearm exercise the interindividual NMR variation in energy metabolism is related to the relative proportion of the two major fiber types in the working muscle. Furthermore, this study suggests that fiber type heterogeneity can be detected by the dynamic changes of the chemical shift of the P_i pool(s) during exercise and recovery by using ³¹P-NMR spectroscopy.

This study was supported by States Sundhedsvidenskabelige Forskningsråd Grants 5.1.7.5.2.14, 12 9789, and 12 9793 (to B. Quistorff) and by grants from Direktor E. C. Hertz Fond (to B. Quistorff) and Direktor J. and H. O. Madsens Fond (to M. Mizuno).

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Received 6 August 1992; accepted in final form 1 September 1993.

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