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Metabolic heterogeneity in human calf muscle during maximal exercise

(³¹P magnetic resonance spectroscopy/muscle fiber types/muscle metabolism/magnetic resonance imaging)

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ABSTRACT **Human skeletal muscle is composed of various muscle fiber types. We hypothesized that differences in metabolism between fiber types could be detected noninvasively with ^{31}P nuclear magnetic resonance spectroscopy during maximal exercise. This assumes that during maximal exercise all fiber types are recruited and all vary in the amount of acidosis. The calf muscles of seven subjects were studied. Two different coils were applied: an 11-cm-diameter surface coil and a five-segment meander coil. The meander coil was used to localize the ^{31}P signal to either the medial or the lateral gastrocnemius. Maximal exercise, consisting of rapid plantar flexions, resulted in an 83.7% ± 7.8% decrease of the phosphocreatine pool and an 8-fold increase of the inorganic phosphate (P_i) pool. At rest the P_i pool was observed as a single resonance (pH 7.0). Toward the end of the first minute of exercise, three subjects showed three distinct P_i peaks. During the second minute of exercise the pH values stabilized at 7.12 ± 0.12, 6.63 ± 0.15, and 6.27 ± 0.23. The same pattern was seen when the signal was collected from the medial or lateral gastrocnemius. In four subjects only two distinct P_i peaks were observed. The P_i peaks had differing relative areas in different subjects, but they were reproducible in each individual. This method allowed us to study the appearance and disappearance of the different P_i peaks, together with the changes in pH. Because multiple P_i peaks were seen in single muscles they most likely identify different muscle fiber types.**

Human muscle is composed of different muscle fiber types (1). They are usually referred to as slow oxidative, fast oxidative–glycolytic, and fast glycolytic fibers, according to their contractile speed and the extent of their oxidative and glycolytic metabolism (2). As their ability to produce and sustain work varies greatly, the relative proportion of the different fiber types is a major determinant of exercise capacity (3). The conventional method to assess muscle fiber types, however, requires an invasive muscle biopsy (4).

^{31}P magnetic resonance spectroscopy (MRS) is a noninvasive method used to study oxidative metabolism of skeletal muscle (5, 6). Through the shift of the P_i peak with change in pH, Park *et al.* (7) observed splitting of the P_i peak during exercise in the wrist flexor muscles and attributed the peaks to correspond to the slow- and fast-twitch fibers. Achten *et al.* (8) confirmed these results on the calf muscles. Since the concept of fiber typing by NMR is of such potential importance, we studied the nature of the multiple P_i peaks more closely. We hypothesized that the difference in metabolism between the fiber types could be detected noninvasively by the splitting of the P_i peak during maximal exercise. This assumes that during maximal exercise all fiber types are recruited maximally and vary in the amount of acidosis produced. Spectra were localized to a single muscle, namely the medial or lateral gastrocnemius, with a "superficial looking" coil (meander coil). Using localization, we eliminated the possibility that the observed heterogeneity is due to different muscle groups. Proton imaging was used to identify the muscles contributing to the ^{31}P signal.

METHODS

Subjects. The subjects were seven healthy men and women (ages 23–40), all regularly engaged in low to moderate aerobic exercise.

^{31}P MRS Measurements. All subjects were placed on a platform in a 1.8-T, 1-m-bore magnet, such that the belly of the calf muscle was centered over an 11-cm-diameter surface coil. A 0.05-msec pulse at 1500 W was used to produce a nominal "45°" pulse. The sweep width was 3000 Hz and 2048 complex data points were used. To collect ^{31}P signal of the medial or lateral gastrocnemius muscle only, a five-segment meander coil (10 × 10 cm) was used (9). The meander coil was constructed by using a 3-mm insulated wire that conformed to the shape of the calf. The physical dimensions of each segment were 1.1 cm × 10 cm.

The B1 field penetration of the meander coil was calibrated in two ways. ^{31}P spectra were collected from a 1.5-cm-thick Petri dish containing 1 M NaH_2PO_4 placed at various distances from the coil. The space between the Petri dish and the coil contained water. Ten percent of the signal was obtainable from the Petri dish 1.5 cm from the coil. At 3.0 cm distance, less than 5% of the signal was obtained.

Gradient echo surface coil proton images were collected to determine the relative contribution of the gastrocnemius muscle to the ^{31}P signal. The meander coil was positioned over either the medial or lateral part of the gastrocnemius. The proton tip angles were equal to the corresponding ^{31}P angles, and pulse repetition times were adjusted to produce the same partial saturation (H = 0.47 sec, P = 2 sec). The total signal intensity of both gastrocnemius muscles was measured and expressed as a percentage of the total muscle intensity, after subtraction of background noise. According to the image intensities, 87% and 90% of the ^{31}P signal was collected from the medial and lateral gastrocnemius, respectively. In comparison, images collected with the 11-cm coil and 45° pulse showed that only 67% of the signal came from both the medial and lateral gastrocnemius together. Thirty-three percent of the signal was obtained from the soleus, the other flexor muscles, and the extensor compartment.

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Abbreviations: MRS, magnetic resonance spectroscopy; PCr, phosphocreatine; PME, phosphomonoesters; NMLE, near-maximum-likelihood estimation.

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Both the 11-cm-diameter surface and the meander coils were double tuned to proton (78.2 MHz) and phosphorus (31.66 MHz) frequencies. Pulse repetition time was 2 sec and data were averaged into 10-sec bins. Spectra of phosphorus-containing metabolites were obtained at rest (1 min), during maximal exercise (2 min), and during recovery (5 min).

Exercise Protocol. The subject's foot was placed on a pedal that was attached to a variable number of elastic bands appropriate to the work required. Exercise consisted of repeated plantar flexions performed for 2 min against a resistance equal to 30% of a maximal single lift. The plantar flexions were executed as rapidly as possible and relative work was recorded in some subjects. A system of straps around shoulders, waist, knee, and ankle was used to immobilize the body.

Data Analysis. Intracellular pH was calculated from the chemical shift of P_i based on the equation $pH = 6.75 + \log[(\delta - 3.27)/(5.69 - \delta)]$, where δ equals the chemical shift of the P_i peak in ppm (10). Quantification of ^{31}P signals was carried out by using a time domain analysis (M.P.) termed near-maximum-likelihood estimation (NMLE). It models the signal as a sum of complex exponentials in additive gaussian white noise. As no user input was required, a potential source of systematic bias and/or error was eliminated. Calculation of the disappearance rates of the different P_i peaks required 10-sec averages, which do not give a good enough signal-to-noise ratio to quantify the individual P_i areas with the NMLE program. For this calculation we therefore used a Marquardt algorithm, which is a least-squares fit as implemented by Bevington (11). The operator phased the spectrum, entered a noise threshold for the peak-picking algorithm, and identified the peaks of interest.

Concentrations of the phosphorus compounds are given as millimoles per liter of intracellular H_2O (mM), using a value of 0.67 ml of intracellular H_2O per g of muscle wet weight (4). A value of 8.2 mM adenosine triphosphate (ATP) was assumed for resting muscle [5.5 mmol/kg wet weight (12)], and all concentrations were calculated on this basis. Peak areas were corrected for saturation effects using fully relaxed resting spectra collected with a pulse repetition time of 30 sec. ATP concentration was calculated from the area of the β-phosphate peak. Because of the relatively poor signal-to-noise ratio of the ATP peaks, it was quantified on spectra derived from signals collected over 30 sec.

Statistical Analysis. The paired *t* test was used to examine the significance of the pH drop, difference in P_i disappearance rates, and changes in the concentration of the phosphate metabolites. Analysis of regression was used to determine changes in the concentration of the phosphate metabolites during recovery. Significance was assumed at P values < 0.05.

RESULTS

All seven subjects completed the 2 min of "maximal" exercise. In the beginning of exercise typical plantar flexions traversed an angle of 60° and were performed at a rate of three per sec. During the protocol both a decrease in the angle (to approximately 20°) and a decrease in the rate (to approximately two per sec) were observed.

Time Sequence of P_i Peak Splitting. In all subjects the P_i pool was observed as a single resonance at rest [P_i/phosphocreatine (PCr) = 0.11 ± 0.02 (mean ± SEM)]. Splitting of the P_i peak was observed within 20–40 sec of exercise (Fig. 1). This initially resulted in two P_i peaks in all seven subjects. Toward the end of the first minute of exercise (40–50 sec) three subjects showed three distinct P_i peaks. Peak differentiation became more pronounced during the second minute of exercise. The split P_i peaks finally stabilized during the last 30 sec. In accordance with their final pH we will refer to them as high-, intermediate-, and low-pH peaks.

In four of the subjects the P_i peak did not resolve into three separate peaks. The P_i peak did, however, split into two distinct P_i peaks. These two peaks were either a high- and intermediate-pH peak (Fig. 2*A*) or an intermediate- and low-pH peak (Fig. 2*B*). In two of the subjects the linewidth of the intermediate peak was broad enough to account for the merger of two peaks.

Changes in pH During Exercise and Recovery in the Subjects with Three P_i Peaks. The changes in pH during exercise and

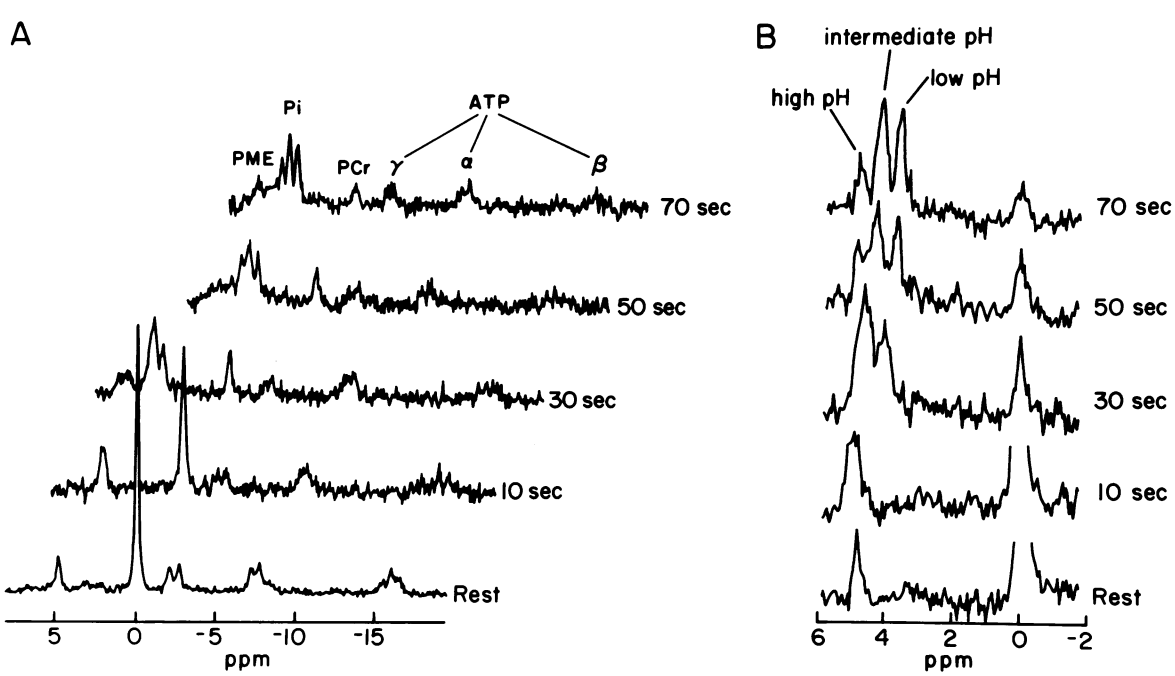

FIG. 1. (*A*) Representative ^{31}P spectra at rest and during exercise of the calf muscles of subject I acquired with an 11-cm-diameter surface coil. Spectra were obtained in 10-sec bins and subsequently recorded with 1-Hz line broadening. PME, phosphomonoesters. (*B*) Expanded view of the P_i peaks. Note splitting of the P_i resonance into two and three P_i peaks at 30 and 50 sec, respectively.

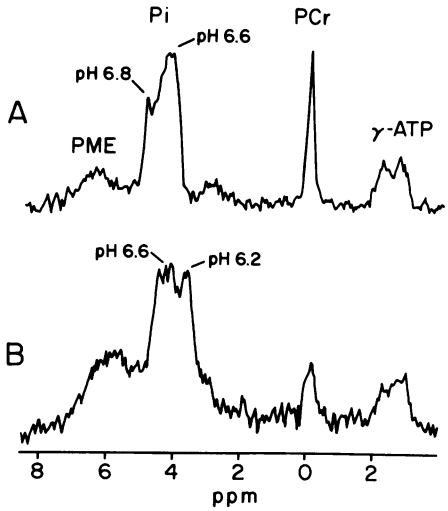

FIG. 2. In four subjects only two P_i peaks were observed. One example each of subjects who showed an intermediate- and high-pH peak (*A*) or an intermediate- and low-pH peak (*B*) is shown.

recovery in the subjects with three P_i peaks are shown in Fig. 3. After a transient alkalotic phase at the onset of exercise, we observed a high-pH peak which remained at a constant pH (7.12 ± 0.12) throughout exercise. The intermediate- and the low-pH peaks dropped their pH very fast and stabilized at pH values of 6.63 ± 0.15 and 6.27 ± 0.23 toward the end of exercise. The initial slopes of the pH drop (of mean data) in the intermediate- and low-pH peak during exercise were 0.56 unit/min and 1.43 units/min, respectively. No significant pH drop was seen in any of the P_i peaks at the end of exercise.

In the subjects with only two distinct P_i peaks patterns similar to those of the corresponding P_i peaks in the pH during exercise as well as recovery were seen.

P_i Disappearance. In all the subjects the P_i peaks lost most of their area during the first 2 min of recovery. The high-pH peak disappeared in 10–30 sec, the intermediate pH peak after 60–80 sec, and the low-pH peak after 80–120 sec into the recovery. The initial disappearance rates of the P_i areas were calculated for all seven subjects (Fig. 4). The high- and intermediate-pH peaks had disappearance rates of 15.2 ± 3.4 mM/min (mean ± SEM) and 18.1 ± 1.2 mM/min, respectively. The low-pH peak disappeared at 1/5th the rate (3.2 ± 0.8 mM/min).

P_i Areas. In different subjects the P_i peaks had different relative areas, which were reproducible in each individual.

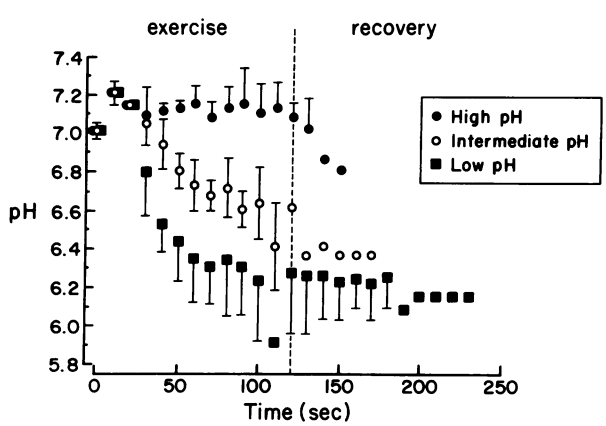

FIG. 3. pH changes in the three different P_i peaks during exercise and recovery. The values are mean ± SD collected from the three subjects who showed three distinct P_i peaks. The data points without error bars are data from one subject.

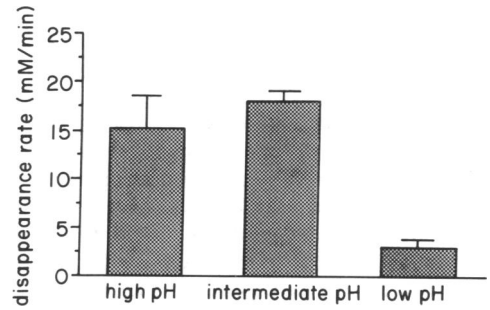

FIG. 4. Initial rate of disappearance of the three different P_i peaks. The low-pH peak had a much slower recovery (3.2 ± 0.8 mM/min) than the high- (15.2 ± 3.4) and intermediate- (18.1 ± 1.2 mM/min) pH peaks. Data from all seven subjects were included in this figure (mean ± SEM).

For example, in subject I the high-pH peak, the intermediate-pH peak, and the low-pH peak contributed 16%, 63%, and 21% to the total area, respectively. In contrast the spectrum in Fig. 2*B* showed 62% for the intermediate- and 38% for the low-pH peak. The integrals of the P_i peaks were determined with the NMLE program. To achieve sufficient signal-to-noise ratio spectra were collected during the last 30 sec of exercise.

Reproducibility. To determine the reproducibility of the protocol the experiment was repeated five times on one subject. The average SDs of all the data points during exercise and recovery on the pH of the high-pH, intermediate-pH, and low-pH peaks were 0.05, 0.08, and 0.12 unit, respectively. The mean SD of the P_i areas was 5.7%. Two other subjects were also tested repeatedly, and the results were similar.

Localized Spectra. Because of the shallow penetration of the signal, experiments were performed on a subject with a very small amount of subcutaneous fat (skin + fat = 3 mm). In both the medial and lateral gastrocnemius three distinct P_i peaks were observed during maximal exercise (Fig. 5). In the medial gastrocnemius the three P_i peaks stabilized at pH values of 7.0, 6.7, and 6.1, respectively. In the lateral gastrocnemius the P_i peaks stabilized at pH values of 7.2, 6.9, and 6.5.

Phosphate Metabolites During the Exercise and Recovery. Maximal exercise resulted in a fast decrease in PCr and increase in P_i (Fig. 6). The initial slope of the PCr decrease was 56.2 ± 9.9 mM/min (mean ± SEM) and that of the P_i increase was 31.7 ± 1.9 mM/min. By the end of exercise the PCr pool decreased to 16 ± 2% of its resting value. The P_i pool showed an 8-fold increase from 4.3 ± 0.4 mM at rest to 33.4 ± 3.2 mM during exercise. Both PCr and P_i recovered immediately after exercise was stopped. PCr recovery was multiphasic with an initial rapid phase (29.3 ± 2.8 mM/min), followed by a much slower rise (4.1 ± 0.6 mM/min). At the end of the recovery period, the PCr values in all subjects were greater than or equal to resting PCr values. The initial rate of P_i disappearance (29.7 ± 3.9 mM/min) was similar to the initial PCr appearance rate.

In all of the subjects the concentration of ATP decreased markedly ($P < 0.01$) within the first 30 sec of exercise. The ATP signal decreased to 43 ± 2.6% of its initial value (Fig. 6). There was a gradual increase in the PME peak (2.9 ± 0.3 mM/min; $P < 0.01$) during exercise and early recovery (Fig. 6). The total levels of adenine nucleotide phosphate (sum of PME and ATP peaks) decreased significantly with the onset of exercise and remained low throughout exercise (Fig. 6). During recovery a gradual decrease in the PME concentration (1.17 ± 0.02 mM/min; $P < 0.01$) was observed, but no significant ATP repletion was seen. Even though the PCr concentration returned to resting values, the total phosphate

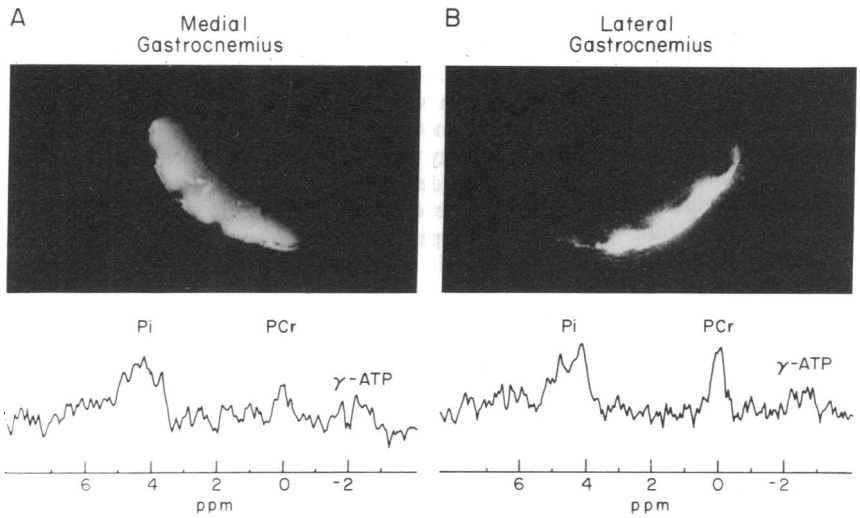

FIG. 5. Localized proton images (*Upper*) and ^{31}P spectra (*Lower*) of the medial (*A*) and lateral (*B*) gastrocnemius were collected with the meander coil. According to the image intensities 87% and 90%, respectively, of the ^{31}P signal was collected from the medial or lateral gastrocnemius. The spectra were acquired during the last 30 sec of exercise.

level (adenine nucleotide phosphate, PCr, and P_i) dropped significantly (from 79.1 ± 3.7 mM to 60.8 ± 3.1 mM) during exercise and did not return to resting values during the recovery period (7).

DISCUSSION

Multiple P_i Peaks Due to Fiber Heterogeneity. The identification of two or three P_i peaks during maximal exercise represents metabolic heterogeneity. We were able to identify three distinct P_i peaks during maximal exercise. The three P_i peaks were separated by the amount of acidosis. Different levels of acidosis can be explained either by different muscles working at different intensities or by different metabolic responses. While with the 11-cm-diameter surface coil a large contribution of other muscles, including the soleus, was observed, the meander coil unquestionably localized to a single muscle. As three distinct P_i peaks were seen in both the medial and lateral gastrocnemius during exercise with the meander coil, this demonstrates that multiple P_i peaks can originate from one single muscle. This result demonstrates that the split P_i peaks are due to metabolic differences rather than to muscles working at different intensities.

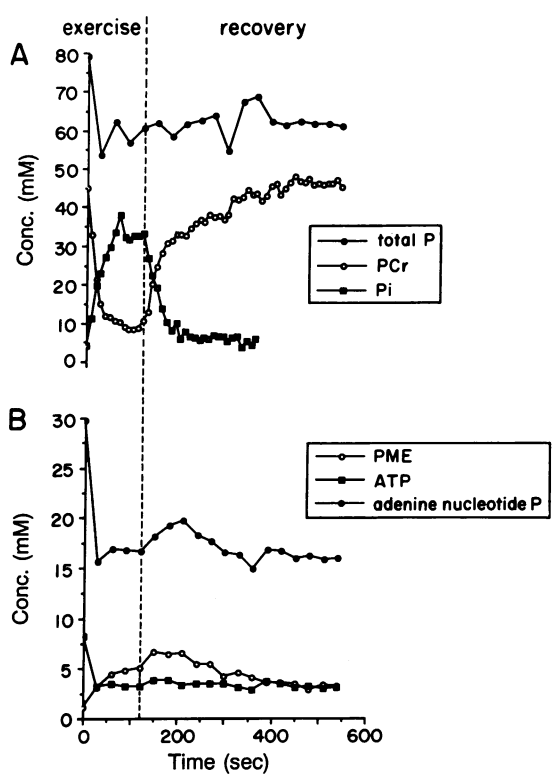

FIG. 6. (*A*) Mean concentrations of P_i, PCr, and total phosphate in the calf muscles at rest (time = 0 sec) and during exercise and recovery (n = 7). Note that the PCr concentration recovered to its initial resting value after exercise. (*B*) Mean concentrations of ATP, PME, and adenine nucleotide phosphate in the calf muscles during rest, exercise, and recovery. The data were determined from 30-sec spectra.

The metabolic differences represented by the split P_i peaks seen during maximal exercise are most simply explained by the different types of muscle fibers. Skeletal muscle is a heterogeneous tissue and consists of fibers with different metabolic and contractile characteristics. Three main groups of fibers exist in human skeletal muscle: slow oxidative, fast oxidative–glycolytic, and fast glycolytic (13). The high-pH peak would represent the slow oxidative fibers which have low myosin ATPase activity and high oxidative capacity. They should produce only small amounts of lactate and protons during high-intensity exercise, consistent with the high-pH peak (pH 6.9–7.1). The intermediate-pH peak would represent the fast oxidative–glycolytic fibers, which have high myosin ATPase rates and high oxidative capacities. These fibers produce lactate and protons and should drop their pH during high-intensity exercise. The low-pH peak would be consistent with the fast glycolytic fibers. These fibers have a high myosin ATPase activity and low oxidative capacity (14). They will produce large amounts of lactate and protons during high-intensity exercise and therefore have the largest pH shift.

The rates of pH drop in the different P_i peaks agree well with the glycolytic capacities of the different fiber types as found in muscle biopsy. Assuming that the rate of acidosis reflects the rate of anaerobic glycolysis, we found a 2.5 times higher glycolytic activity in the low-pH peak than in the intermediate-pH peak, while the high-pH peak had the lowest glycolytic activity. Phosphofructokinase and lactate dehydrogenase activities, indicators of glycolytic capacity, are approximately 1.5 times higher in the fast glycolytic fibers than in the fast oxidative–glycolytic fibers and 2–4 times higher than in the slow oxidative fibers (15, 16). The rates of acidosis in the different P_i peaks are consistent with the glycolytic capacities of the different fiber types of human skeletal muscle, as found in muscle biopsies.

The rates at which the P_i peaks disappear agree well with the oxidative capacities of the different fiber types, as found in muscle biopsies. Using the initial P_i disappearance rate in recovery as an indicator of oxidative capacity, the high- and intermediate-pH peaks had similar oxidative capacities,

which were approximately 5 times higher than the oxidative capacity of the low-pH peak. Biopsy data from human skeletal muscle (15–17) have shown that the oxidative capacity in the slow oxidative fibers is slightly higher than in the fast oxidative–glycolytic fibers and 3–4 times higher than in the high glycolytic fibers.

Different subjects had differing relative areas of the three P_i peaks. These differences in the P_i peaks were reproducible in each individual. Some subjects showed P_i peaks consistent with a large percentage of fast oxidative–glycolytic fibers, some had a large proportion of fast glycolytic fibers, while others showed a mixture of all three types. While the number of subjects tested was small, the variation between different subjects is consistent with the expected fiber type variation, as found in biopsies of normal subjects (18).

Alterations of Phosphate Compounds. Two minutes of maximal exercise was associated with a loss of approximately half of the ATP signal in 30 sec. The observed loss of ATP is a real loss of ATP content and not due to signal degradation, as the PCr values regained and even overshot their initial levels during recovery. ATP loss with strenuous exercise has been reported previously (7, 19). Other studies have found that fast fibers exhibit a large depletion of ATP, while slow fibers maintain their ATP content near normal levels during intense stimulation conditions that produce rapid fatigue (20).

The repletion of ATP after strenuous exercise has been reported to be very slow. Taylor *et al.* (19) and Harris and Snow (21) found no recovery of ATP in skeletal muscle 30 min after the end of exercise in which more than 40% of the ATP was lost. As the PME concentration decreased during recovery, while the ATP level remained unchanged, it seems likely that, under the conditions of our experiment, purine depletion occurred. Purine depletion results in adenine nucleotide phosphate being catabolized to nonphosphorylated metabolites that are lost from the muscle. Release of adenosine, inosine, and hypoxanthine from contracting muscle has been reported (22).

Difficulties of Using ^{31}P MRS as a Method for Fiber Typing. The main difficulty in this test is that all muscle fibers should be recruited equally and maximally, so that the different levels of acidosis seen in this protocol are due to different metabolic states and not to different levels of activity. We used a short-term maximal exercise in an attempt to minimize differences in activity between the different fiber types. Poorly localized spectra may increase the potential of collecting signal from muscles with different levels of activity. Therefore localization techniques, such as the meander coil, are desirable.

It is difficult to determine the percentage of the different fiber types on the basis of the area of the P_i peaks, even though identification of the fiber types is clear. This difficulty is due to the fact that the creatine kinase reaction is not the sole source or sink for P_i. For example, the P_i concentration is influenced by phosphorylation and dephosphorylation of proteins (23), ATP depletion, and P_i "leakage" from the cell (24). These mechanisms do not necessarily affect the different fiber types equally. Indeed, an unequal contribution from ATP (1–3 P_i per ATP) is expected. Fast oxidative–glycolytic and fast glycolytic fibers exhibit a larger depletion of ATP than the slow oxidative fibers and produce, therefore, an extra amount of P_i (20). Loss of P_i has been noticed in conditions of severe exercise with acidosis, which is most likely to occur in the fast fibers (24). Thus the correct algorithm for quantitation of the amounts of the three fibers is yet to be determined.

During our experiments we found up to three distinct P_i peaks during maximal exercise. Three P_i peaks have not been reported previously in ^{31}P MRS studies, to our knowledge. Localization with the meander coil showed that these peaks are due to metabolic heterogeneity. Even though metabolic heterogeneity in one muscle is seen, it is currently not possible to determine fiber type composition by using ^{31}P MRS. However, by following pH decrease during exercise and P_i disappearance during recovery it is possible to study the metabolic characteristics of the different fiber types.

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