A "functional biopsy" of muscle properties in sprinters and distance runners

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ABSTRACT

CROWTHER, G. J., S. A. JUBRIAS, R. K. GRONKA, and K. E. CONLEY. A "functional biopsy" of muscle properties in sprinters and distance runners. *Med. Sci. Sports Exerc.*, Vol. 34, No. 11, pp. 1719–1724, 2002. **Purpose:** Fast- and slow-twitch human muscle fibers exhibit large (two- to threefold) differences in metabolic enzyme activities and contractile economy. We asked whether comparable flux differences are evident in the muscles of athletes specializing in extremely different (i.e., sprint and long-distance) running events. **Methods:** We took an *in vivo* "functional biopsy" of the ankle dorsiflexor muscles of 17 members of a university track team by using ^{31}P magnetic resonance spectroscopy. Ten sprinters (SPR) and seven distance runners (DIS) performed rapid isometric dorsiflexions against the resistance of a plastic foot holder. The contractile cost of exercise and glycolytic flux were calculated from changes in pH, [PCr], and [P_i] during ischemic exercise, and oxidative capacity was calculated from PCr recovery kinetics after aerobic exercise. **Results:** Contractile costs were 47% higher in SPR than in DIS, whereas oxidative capacities were 52% higher in DIS than in SPR. Surprisingly, glycolytic ATP production was similar in the two groups. **Conclusion:** The muscles of SPR and DIS exhibit clear differences in energetic properties, but these differences are smaller than the two- to three-fold variations seen in the properties of individual muscle fibers. **Key Words:** SPRINT-TRAINED, ENDURANCE-TRAINED, ENERGY METABOLISM

Fast- and slow-twitch muscle fibers are often thought to represent the extremes of muscle specialization, with fast-twitch (Type II) fibers designed for brief but intense bursts of activity and slow-twitch (Type I) fibers designed for much longer exercise bouts. Consistent with this view, a number of metabolic traits have been found to vary with fiber type in human muscles, among them glycolytic enzyme activity (11,12), mitochondrial density (16), and contractile economy (14,25). When comparing Type I ("slow oxidative") fibers with Type IIX ("fast glycolytic") fibers, these differences often are on the order of two- to three-fold.

Are such large metabolic differences also evident among intact human muscles specialized for different tasks? This question can be addressed by examining the muscles of highly trained sprinters and distance runners, because sprinters train for events lasting less than 1 min, whereas distance runners compete in races of 8 min or longer. In the classic biopsy study of international-caliber athletes by Costill et al. (7), sprinters were found to have about twice the glycolytic enzyme activity of distance runners, but the activity of succinate dehydrogenase (SDH; a mitochondrial enzyme) was only ~40% higher in the muscles of the distance runners. These data suggest that differences in muscle properties may be smaller for the intact muscles of "extreme" athletes (sprinters vs distance runners) than for the extreme muscle fiber types (Type I vs IIX).

A limitation of biopsy assays is that they generally measure the flux capacities of individual enzymes, often under unphysiological conditions, rather than *in vivo* fluxes through multistep pathways. Fortunately, such *in vivo* flux measurements can now be made using magnetic resonance spectroscopy (MRS). These measurements monitor intracellular metabolite and pH changes during exercise and yield quantitative estimates of the oxidative ATP supply, glycolytic ATP supply, and contractile ATP demand in muscle (4–6). The result is a "functional biopsy" that measures metabolic fluxes directly *in vivo* rather than inferring them from marker enzyme activities.

The goal of this study was to determine whether the functional properties of track athletes' muscles exhibit the

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full range of differences apparent in different fiber types. Muscle-specific fluxes *in vivo* have not been carefully compared in these two "extreme" groups, aside from McCully et al.'s (20) finding of slow oxidative recoveries from exercise in sprinters relative to distance runners. We therefore quantified the extent to which sprinters and distance runners differ in their rates of ATP production (by glycolysis and oxidative phosphorylation) and ATP consumption during exercise. We studied the ankle dorsiflexor muscles because the tibialis anterior (the largest muscle in this group) is highly active during running, according to electromyography (EMG) data (22,24).

METHODS

Subjects. Ten sprinters (6 men and 4 women) and seven distance runners (4 men and 3 women), all between 18 and 22 yr of age, were recruited from the track and field team of a large NCAA Division I university. The sprinters competed in events of 100–400 m, the long jump, and/or the pole vault. (Long jumpers and pole vaulters were considered sprinters because their events require them to sprint down the jumping runway.) The distance runners competed in events of 3,000–10,000 m. Subjects were studied at the end of their spring track season. The experimental protocols were approved by the Institutional Review Board of the University of Washington, and voluntary, written informed consent was obtained from each subject.

Experimental setup. Each subject lay supine with the right leg and foot in the bore of a 4.7-T magnet. The leg and foot were held in place with a plastic holder, and a strain gauge measured force exerted by the ankle dorsiflexors. A surface coil placed over the anterior compartment of the leg was used to acquire ^{31}P magnetic resonance spectra of the dorsiflexors.

Magnetic resonance methods. A high-resolution control spectrum of the resting muscle was acquired under conditions of fully relaxed nuclear spins (interpulse delay: 16 s). Sequential spectra were then obtained under partially saturating conditions (interpulse delay: 1.5 s) throughout the experimental protocols described below. The spectrum for each time point consisted of four summed acquisitions taken over 6 s. Free-induction decays (FIDs) were summed, apodized with an exponential filter matched to the half-height line width of PCr (10–25 Hz), and Fourier-transformed into spectra. Peak areas of fully relaxed spectra were integrated using Omega software (GE Medical Systems, Waukesha, WI), whereas partially saturated spectra were analyzed with the "Fit-to-Standard" program (15). Relative areas of the PCr and P_i peaks were converted to absolute concentrations assuming [ATP] to be 8.2 mM in human muscle (13). The chemical shift of the P_i peak relative to PCr was used to calculate muscle pH (26).

Exercise protocols. Each subject performed isometric exercise under both ischemic and aerobic conditions. The ischemic exercise protocol began with the inflation of a blood pressure cuff around the thigh, which was followed by 300 s of ischemic rest and 90–120 s of ischemic exercise. The aerobic exercise protocol consisted of 60 s of aerobic rest, 420 s of aerobic exercise, and 288 s of aerobic recovery. All exercise bouts consisted of a series of isometric ballistic contractions to ~50% of maximal voluntary contraction (MVC) force, which achieve full recruitment of all muscle fibers (8–10). Subjects used a metronome to maintain the desired contraction frequency and used visual feedback from a light-emitting diode (LED) display to achieve the desired peak force. They each completed one ischemic exercise bout at a contraction frequency of 0.5 Hz and several aerobic bouts at frequencies ranging from 0.33 to 1.33 Hz.

Calculations. Contractile cost was quantified as the rate of [PCr] decline during pH alkalinization at the start of ischemic exercise (4). This rate of ATP use should not be contaminated by glycolytic or oxidative ATP synthesis, because there is little glycolysis during alkalinization (1) and no oxidative phosphorylation due to the ischemia. As expressed in mM ATP·s^{-1}, cost is an intensive property that does not depend on muscle size. However, costs in mM ATP·s^{-1} were divided by the fraction of MVC force at which each subject worked (54 ± 2%) to correct for the fact that this fraction differed slightly among subjects.

Glycolytic ATP production was calculated from changes in muscle pH, [PCr], and [P_i] during ischemic exercise, as previously described (5). In brief, H^+ generation by glycolysis equals the observed change in [H^+] plus the H^+ consumed in the breakdown of PCr:

$$\Delta H^+_{glycol} = \Delta pH \times \beta_{tot} + (-\gamma) \times \Delta[PCr] \quad (1)$$

where ΔpH is the change in muscle pH, β_{tot} is the total muscle buffer capacity (5), γ is the proton stoichiometric coefficient of PCr hydrolysis (18), and Δ[PCr] is the change in [PCr]. Glycolytic H^+ production can then be converted to glycolytic ATP production by using a stoichiometry of 1.5 ATP per H^+ (5), as is appropriate when glycogen (rather than free glucose) is the primary substrate for glycolysis. Because some subjects fatigued toward the end of the ischemic exercise bout, each subject's glycolytic flux was calculated for the period of time over which he/she was able to perform the ballistic contractions correctly.

Oxidative capacity was estimated using PCr data during recovery from aerobic steady-state exercise—"steady-state" meaning that [PCr] did not decline significantly during the final 2 min of exercise. Each set of PCr recovery data was fit to a monoexponential curve (21). The reciprocal of the time constant of this curve was taken to be the oxidative recovery rate constant k_{PCr} (23,28), which is independent of end-exercise [PCr] within the range of PCr depletion (30–60%) encountered in these trials (21,27). Oxidative capacity was then calculated as:

$$\text{oxidative capacity} = k_{PCr} \times [PCr]_{rest} \quad (2)$$

as previously described (6), where $[PCr]_{rest}$ is the [PCr] at rest (i.e., before exercise). Because each subject completed several aerobic trials, the mean k_{PCr} of all aerobic steady-state trials was used in this calculation. Muscle pH remained

close to 7 in all subjects during these steady-state aerobic trials.

Statistics. Values reported are means ± SEM. Two-tailed paired *t*-tests were used to evaluate potential differences between means. *P*-values below 0.05 were considered statistically significant.

RESULTS

Sprinters (SPR) and distance runners (DIS) did not differ in their resting PCr/ATP or P_i/ATP ratios. PCr/ATP ratios were 4.24 ± 0.07 for SPR and 4.14 ± 0.06 for DIS; P_i/ATP ratios were 0.40 ± 0.01 for SPR and 0.43 ± 0.03 for DIS.

Our "functional biopsy" of muscle properties included measures of contractile cost, oxidative capacity, and glycolytic flux. We assessed these properties in SPR and DIS by quantifying chemical changes in muscle during and after bouts of ischemic and aerobic exercise. PCr and pH changes during ischemic exercise are shown in Figure 1, whereas PCr recoveries after maximum steady-state aerobic exercise are shown in Figure 2. These data suggest that SPR break down PCr more quickly and resynthesize it more slowly than DIS, as would occur if SPR have increased contractile costs and decreased oxidative capacities relative to DIS. Consistent with these expectations, the contractile costs of exercise were greater for SPR than for DIS (Fig. 3), whereas oxidative capacities were greater for DIS than for SPR (Fig. 4). Glycolytic fluxes during ischemic exercise were similar for the two groups (Fig. 5). In fact, glycolytic ATP production was actually greater for DIS than for SPR when expressed as a percentage of contractile ATP use (Fig. 6).

DISCUSSION

The purpose of this study was to use a "functional biopsy" to determine whether the muscle properties of sprinters and distance runners represent the full range of properties found in Type I and Type II muscle fibers. Magnetic resonance spectroscopy was used to measure ATP production by glycolysis and oxidative phosphorylation as well as ATP consumption by contraction. We found that glycolytic fluxes during exercise were equal in the two groups (Fig. 5), whereas contractile costs were 47% higher in the sprinters (Fig. 3) and oxidative capacity was 52% higher in the distance runners (Fig. 4). These differences (1.5-fold at most) are smaller than the two- to three-fold metabolic differences between fast- and slow-twitch fibers. Thus, although sprinters and distance runners are commonly thought of as "fast-twitch" and "slow-twitch" athletes, respectively, their muscles are not as metabolically divergent as these labels would imply.

The finding of higher contractile costs in the sprinters is in accord with the fact that fast-twitch muscle fibers have higher tension-specific costs than slow-twitch fibers. The cost of producing a given amount of isometric tension can vary by 2.5- to 3.5-fold between the fastest (Type IIX) and slowest (Type I) fibers (14,25). The smaller 1.5-fold difference observed between the two groups in this study probably reflects the facts that (1) virtually all human muscles are a mixture of fast- and slow-twitch fibers and that (2) the uneconomical IIX fibers are quite rare even in the muscles of sprinters (2).

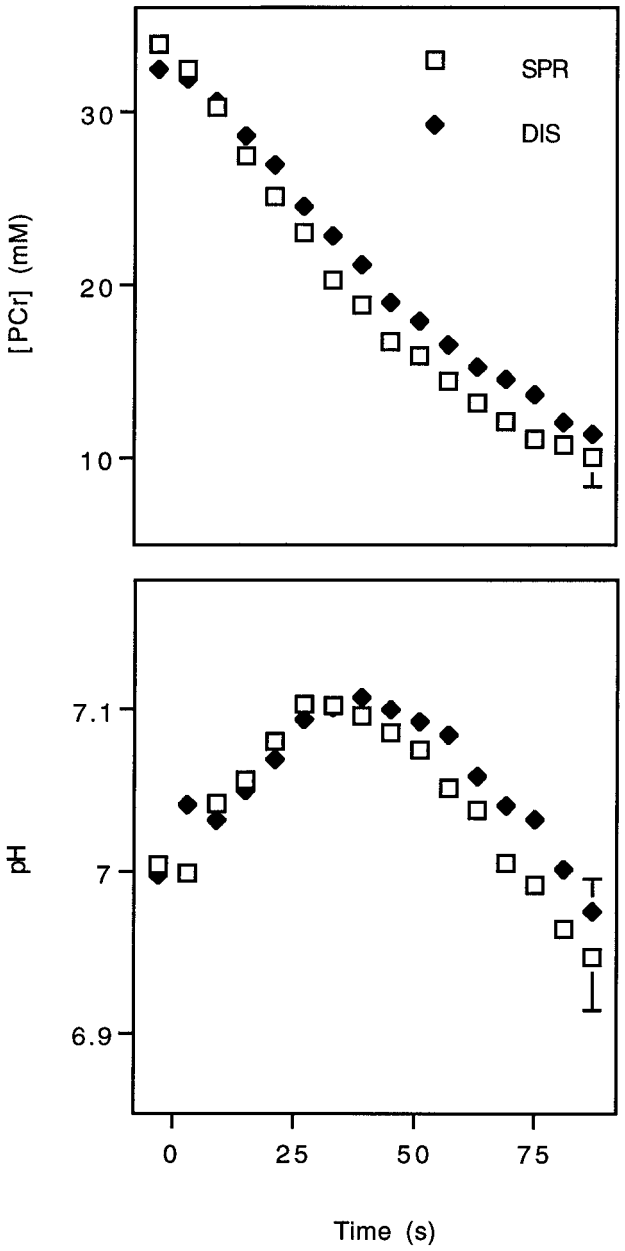

FIGURE 1—Summary data showing PCr and pH changes during ischemic exercise. Time 0 corresponds to the start of exercise. Data shown are mean values for SPR (N = 10) and DIS (N = 7). Standard errors of the mean (SEM) are shown for the first and last points; some error bars are contained within the width of the points.

Our finding of a ~50% higher oxidative capacity in distance runners relative to sprinters was similar to Costill et al.'s (7) finding of ~40% higher SDH activity in distance runners but less dramatic than McCully et al.'s (20) finding of a twofold difference in the oxidative capacities of sprinters and distance runners. The causes of this apparent discrepancy are not obvious but may reflect differences in the sprinter populations of the two studies, because those in the

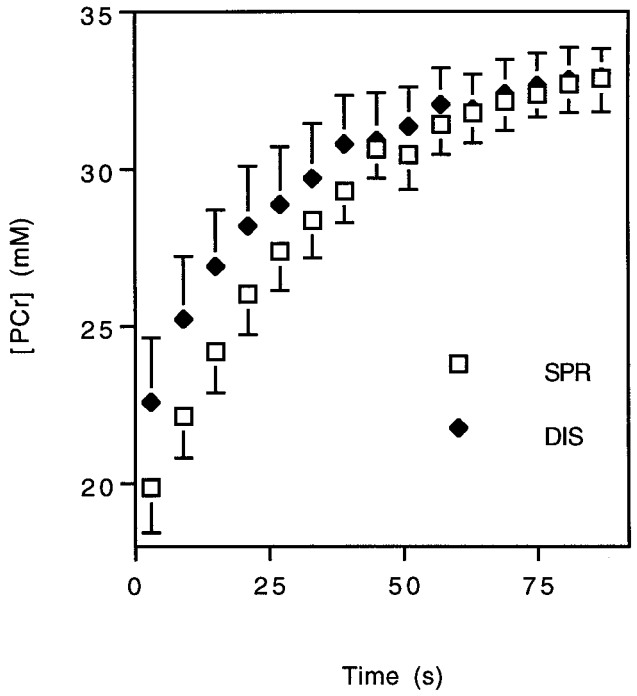

FIGURE 2—PCr recovery after maximum steady-state aerobic exercise. Data for SPR (N = 9) and DIS (N = 7) are shown as means ± SEM. One SPR subject was omitted from this analysis because we were unable to obtain any data on him after steady-state aerobic exercise.

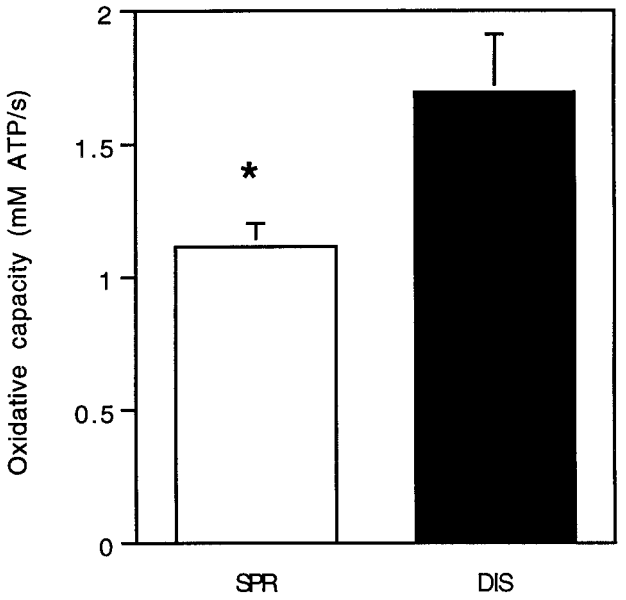

FIGURE 4—Muscle oxidative capacity is greater for SPR than for DIS. One SPR subject was omitted from this analysis because we were unable to obtain any data on him after steady-state aerobic exercise.

Costill study had more SDH activity than control subjects, whereas those in the McCully study had a (nonsignificantly) lower oxidative capacity than control subjects. The present study did not include sedentary subjects, but the average k_{PCr} of our sprinters ($0.032 \cdot s^{-1}$), whereas less than that of our distance runners ($0.050 \cdot s^{-1}$), is similar to that of the control subjects ($0.030 \cdot s^{-1}$) in a similar study (17). It should also be mentioned that, by the criterion of race times, both the athletes studied by Costill et al. and those studied by McCully et al. were of somewhat higher caliber than our subjects. Our failure to replicate others' findings of higher PCr/ATP and PCr/P_i ratios in sprinters than in distance runners (3,19) may likewise reflect the performance gap between the university-level athletes of the present study and the more accomplished athletes studied previously.

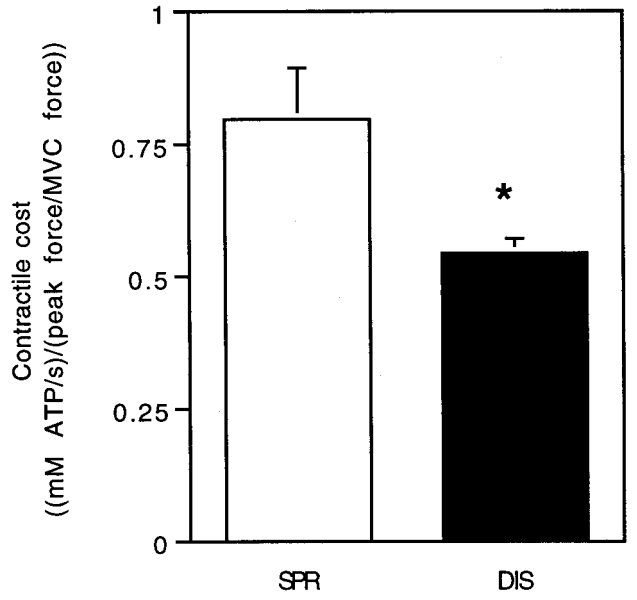

FIGURE 3—The contractile cost of exercise is greater for SPR than for DIS. Data shown here were calculated by dividing the contractile cost of ischemic exercise by the fraction of MVC force at which each subject worked. One SPR subject was omitted from this analysis because his MVC force could not be accurately determined. Asterisks here and in subsequent figures denote statistically significant differences.

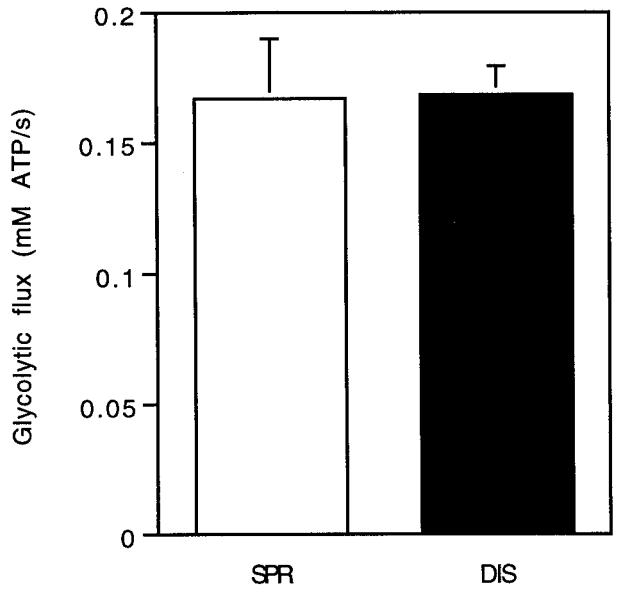

FIGURE 5—Glycolytic flux (quantified as glycolytic ATP production per second) during ischemic exercise is similar for SPR (N = 10) and DIS (N = 7).

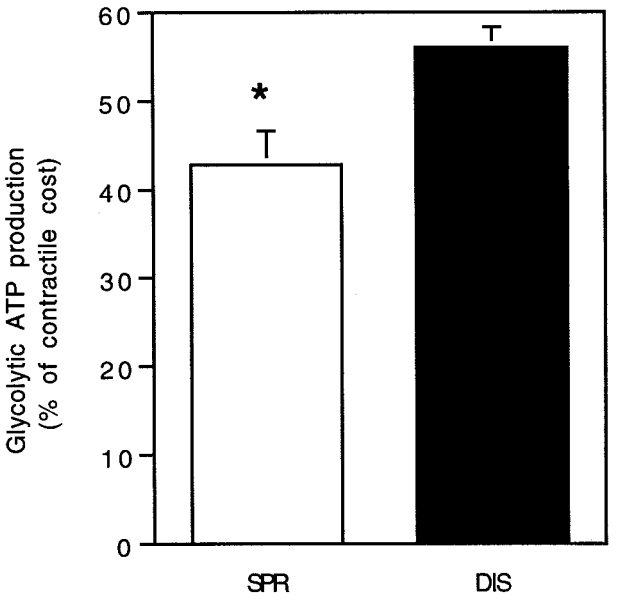

FIGURE 6—Glycolytic ATP production as a percentage of contractile ATP consumption is greater for DIS ($N = 7$) than for SPR ($N = 10$) during ischemic exercise.

The finding of approximately equal glycolytic fluxes in the sprinters and distance runners runs counter to Costill et al.'s report of higher glycogen phosphorylase and lactate dehydrogenase activity in sprinters (7). These contrasting data could reflect the difficulties of inferring flux through a multistep pathway (e.g., glycolysis) from assays of isolated enzymes. Alternatively, they could result from differences in training among the athletes studied a quarter-century ago and those of the present study. Costill et al. (7) do not describe the training habits of the athletes they studied, but our distance runners' training included two sessions per week of high-speed 200- to 1200-m intervals, which may have stimulated adaptation of the glycolytic pathway.

In conclusion, a functional biopsy of the energetic properties of the ankle dorsiflexor muscles revealed that sprinters and distance runners exhibit clear differences in metabolism. Nevertheless, the magnitude of these differences is modest relative to the two- to three-fold variation seen in the metabolic properties of individual muscle fibers, indicating that even the muscles of athletes trained for extreme forms of exercise do not reflect the extremes of the energetic properties of individual muscle fibers. The relatively small energetic differences between sprinters and distance runners indicate that the mixed-fiber nature of muscle is retained in university-level athletes trained for very different events. This work illustrates the ability of MRS to quantify muscle properties in a manner analogous to that of a conventional biopsy. However, unlike traditional biopsies, MRS does not require the removal of samples from the subject and allows metabolic fluxes to be measured in exercising muscle *in vivo*.

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