Enzyme activity and fiber composition
in skeletal muscle of untrained and trained men

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GOLLNICK, P. D., R. B. ARMSTRONG, C. W. SAUBERT IV, K. PIEHL, AND B. SALTIN. *Enzyme activity and fiber composition in skeletal muscle of untrained and trained men.* J. Appl. Physiol. 33(3): 312-319, 1972.—Succinate dehydrogenase (SDH) and phosphofructokinase (PFK) activities and the histochemical identification of fiber types and localization of oxidative activity were determined on biopsy samples from the vastus lateralis and deltoid muscles of 74 untrained and trained men. SDH activities were highest in the muscles of the groups participating in endurance training. Highest activities existed in the muscles that were extensively engaged in the endurance work. Only minor differences existed for PFK activities. Only slow twitch (ST) and fast twitch (FT) fibers were identified in the muscle samples. ST fibers predominated in the muscles of the endurance athletes. A wide variety of fiber populations existed in all groups. The percent distribution of a fiber was found to be indicative of the relative area that the fiber occupied in the muscle. Oxidative capacity of both fiber types was greater in the endurance athletes than in the other groups. Muscle glycogen was highest in the trained subjects. No consistent pattern for glycogen storage in the two fiber types existed.

succinate dehydrogenase; glycogen storage; fiber types; phosphofructokinase; human skeletal muscle

AT LEAST THREE DIFFERENT FIBER types have been identified in the skeletal muscle of most species. Several systems have been proposed for the identification of these fiber types (3, 5, 10, 24). In human muscle most investigators have identified only two distinctly different fiber types. These have been classified as type I and type II (7, 16) or red and white (9) fibers. Edström and Nyström (9) have reported differences in the percent distribution of these fibers both between different muscles and within the same muscles comparing different subjects.

The interconvertibility of fiber types has been demonstrated in animals following cross-innervation (8). Barnard and co-workers (3) have also reported that physical training resulted in an increase in the percentage of red fibers at the expense of white fibers in guinea pig muscle.

In animals, endurance training can alter the metabolic characteristics of skeletal muscle by increasing the activity of some Krebs cycle enzymes, mitochondrial protein concentration, and the ability to oxidize fat (11, 12, 14). Some of these changes have been observed in human skeletal muscle after training (15, 26). However, the metabolic

characteristics and fiber composition in human muscle of trained and untrained subjects and how these relate to the activity of specific muscle groups and to performance characteristics have not been studied. The purpose of this investigation was to approach this problem by studying groups of subjects who had been engaged in various types and intensities of training for prolonged periods of time.

SUBJECTS AND METHODS

Nine groups of men were employed in the study (Table 1). These subjects were selected to represent different age groups and states of physical fitness and to encompass men who were participating in a variety of sport activities that used different muscle groups and training programs. Altogether 74 men between the ages of 17 and 58 years were studied.

Samples of the vastus lateralis and deltoid muscles were obtained with the needle biopsy technique (4) and divided into three parts. One portion of the sample was immediately frozen in liquid nitrogen and stored in Dry Ice for subsequent histochemical analysis. The remaining two parts were weighed and used to determine succinic acid dehydrogenase (SDH) and phosphofructokinase (PFK) activities at 25 C with the methods of Cooperstein et al. (6) and Shonk and Boxer (23), respectively. On some occasions a second biopsy was taken to provide enough tissue for the different measurements. In such cases the biopsy was taken at the same sampling site as the first one.

The samples frozen for histochemical analysis were examined under a dissecting microscope in a cold room at −25 C to determine fiber orientation. They were then cooled to the temperature of liquid nitrogen, placed on specimen holders in OCT embedding medium (Ames Tissue-Tek) at near 0 C, and immediately immersed in liquid nitrogen. Serial sections, 10 μ thick, were cut in a cryostat at −20 C and mounted on cover glasses for staining. Myosin adenosine triphosphatase (ATPase), reduced diphosphopyridine nucleotide-diaphorase (DPNH-diaphorase), and alpha-glycerophosphate dehydrogenase activities were estimated with the methods of Padykula and Herman (20), Novikoff and associates (18), and Wattenberg and Leong (27), respectively. The distribution of glycogen in one of the serial sections (16 μ thick) was estimated from the periodic acid-Schiff (PAS) reaction (21). Photographs of the slides were taken and 20 x 25 cm black-and-white

TABLE 1. *Physical characteristics, fiber populations, enzyme activities, and $\dot{V}_{O_2 \, max}$ of groups studied*

Groups, Age, No.	Height, Weight	Type of Work (Arm-Leg)	% ST Fiber	Enzyme Activities, μmoles/g min^{-1} SDH	Enzyme Activities, μmoles/g min^{-1} PFK	$\dot{V}_{O_2 \, max}$, ml/kg min^{-1}
Untrained, 27 (24–30) yr, n = 12	179 cm (166–187)	A	46.0 ± 6.8 (14.3–59.8)	3.6 ± 0.4 (2.1–6.0)	21.6 ± 3.7 (19.8–28.6)	
	76.5 kg (66.4–105.5)	L	36.1 ± 5.0 (13.0–50.8)	4.3 ± 0.6 (2.7–6.5)	25.3 ± 2.1 (17.7–38.0)	42.9 ± 1.9 (32–53)
Untrained, 39 (31–52) yr, n = 14	178 cm (168–191)	A	45.2 ± 2.7 (33.5–58.3)	3.5 ± 0.4 (1.9–6.6)	23.0 ± 1.9 (16.5–32.3)	
	75.6 kg (63.2–88.6)	L	43.9 ± 4.8 (24.0–72.9)	4.4 ± 0.3 (2.3–6.5)	25.2 ± 1.9 (14.3–36.6)	41.4 ± 1.2 (35–49)
Trained,* 25 (17–30) yr, n = 12	179 cm (165–189)	A	54.8 ± 3.4 (45.5–66.2)	4.0 ± 0.5 (2.7–5.3)	19.1 ± 1.8 (13.3–24.6)	
	68.8 kg (59.6–76.0)	L	52.3 ± 6.8 (26.0–62.5)	6.0 ± 0.3 (4.0–8.0)	19.9 ± 3.6 (14.3–28.3)	57.1 ± 1.3 (51–65)
Bicyclists, 24 (18–33) yr, n = 4	182 cm (175–189)	A	50.7 ± 4.4 (39.5–63.8)	6.1 ± 0.2 (5.5–6.7)	24.0 ± 4.0 (19.4–32.0)	
	74.5 kg (74.0–75.0)	L	61.4 ± 5.9 (47.9–73.2)	11.0 ± 1.0 (8.2–12.4)	23.9 ± 1.4 (21.4–32.0)	68.2 ± 2.2 (64–75)
Canoeists, 26 (25–27) yr, n = 4	181 cm (179–186)	A	58.4 ± 3.8 (48.2–65.9)	7.9 ± 0.6 (7.1–9.2)	25.0 ± 6.8 (19.0–42.0)	54.9 ± 2.0 (51–60)
	74.0 kg (71.0–79.0)	L	61.4 ± 6.2 (45.0–72.4)	5.8 ± 0.9 (3.3–8.0)	22.2 ± 4.7 (11.3–31.5)	56.8 ± 0.8 (55–58)
Runners, 23 (19–33) yr, n = 8	177 cm (168–185)	A		4.2†		
	69.5 kg (59.1–80.8)	L	58.9 ± .3.7 (52.7–70.0)	6.4 ± 0.5 (4.3–8.2)	20.1 ± 2.5 (10.3–26.5)	72.4 ± 1.9 (61–80)
Swimmers, 21 (18–23) yr, n = 5	181 cm (175–189)	A	74.3 ± 5.7 (65.9–85.3)	8.6 ± 0.7 (6.2–9.9)	22.7 ± 0.4 (21.0–23.2)	
	78.3 kg (70.0–78.6)	L	57.7 ± 9.3 (37.2–79.7)	7.6 ± 0.5 (5.5–8.5)	29.3 ± 0.4 (28.0–29.7)	79.9 ± 1.0 (69–74)
Weight lifters, 25 (23–29) yr, n = 4	171 cm (159–186)	A	52.6 ± 7.7 (42.6–66.7)	2.6 ± 0.4 (2.0–3.2)	21.1 ± 4.8 (12.1–29.0)	33.5 ± 3.06 (27–40)
	81.3 kg (52.0–107.0)	L	46.1 ± 10.5 (25.3–59.7)	3.0 ± 0.3 (2.5–3.7)	24.7 ± 1.7 (20.8–29.4)	40.1 ± 6.4 (32–55)
Orienteers, 52 (47–58) yr, n = 11	176 cm (161–184)	A	63.1 ± 5.1 (31.0–98.2)	4.1 ± 0.5 (1.8–6.6)		
	72.7 kg (59.5–88.0)	L	68.8 ± 5.2 (46.5–95.7)	5.7 ± 0.3 (3.9–7.9)		50.7 ± 2.2 (37–62)

Values are means ± se. Values in parentheses are highest and lowest observations.   *This group is composed of athletes competing in several different events.   †Average of 3 values.

prints made for the purpose of classifying fibers and estimating the distribution of oxidative and glycolytic capacity in the different fibers.

Total glycogen in some samples was determined with the method described by Karlsson et al. (13). Ribonucleic acid (RNA) was determined in the muscle samples of some groups with the modified Schmidt-Thannhauser method described by Munro and Fleck (17). Yeast RNA was used as the standard.

Maximal oxygen uptake ($\dot{V}_{O_2 \, max}$) during leg work was determined for all subjects either while running on a treadmill or pedaling a bicycle. In some of the groups whose athletic specialty involved extensive use of the arms, $\dot{V}_{O_2 \, max}$ was also measured while cranking a specially adapted bicycle with the hands and arms (25). Expired air was collected in Douglas bags and its volume was determined with a wet spirometer. Gas analysis was performed with the Haldane technique.

The area of the muscle fibers from some subjects was determined by planimetry from the photographs of the sections used to determine DPNH-diaphorase activity. A total of 40 randomly selected cells, including 20 of each fiber type, were measured for each subject. Cell diameters were calculated from the area.

RESULTS

The intent of this investigation was to study subjects of different ages and states of physical training, some of whom were participating in various sport activities. The effectiveness of subject selection in meeting the fitness criteria is illustrated by the $\dot{V}_{O_2 \, max}$ during leg work (Table 1) which varied from a mean value of 41 (untrained groups and weight lifters) to 75 (young cross-country runners) ml/kg min^{-1}. The lowest individual value was 27 and the highest 80 ml/kg min^{-1}. These values are similar to those previously reported for sedentary individuals and champion athletes (22). It has previously been shown that $\dot{V}_{O_2}$ during maximal arm work is only about 70% of that during maximal leg work (1, 25). For the groups of subjects tested in the present study these values were 79 and 97% for weight lifters and canoeists, respectively. This illustrates the importance of total muscle mass involved in the work and of training a specific muscle group.
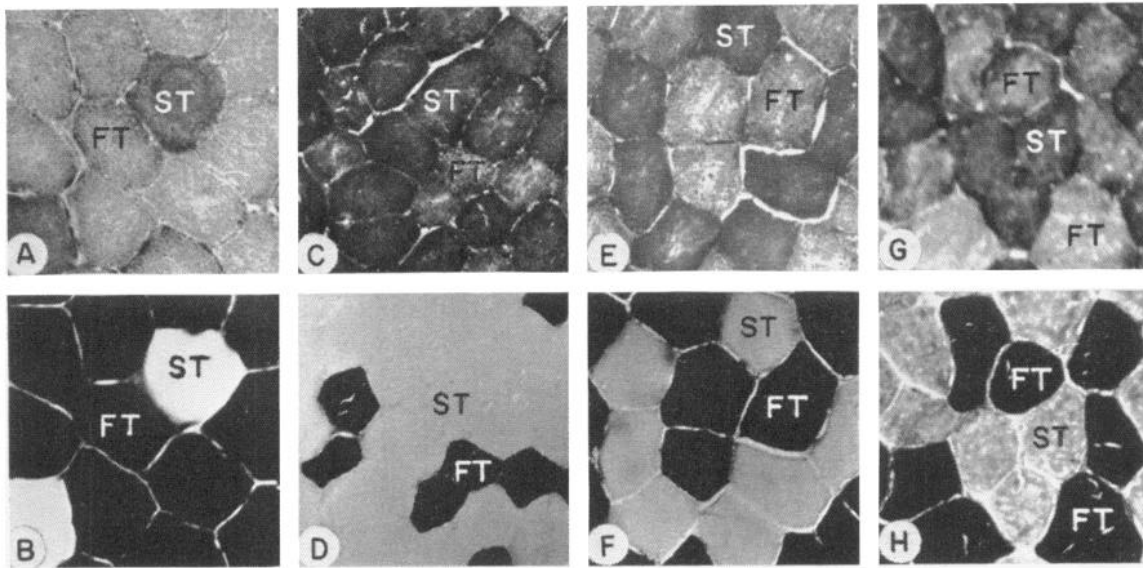
FIG. 1. Serial sections from vastus lateralis muscle stained for DPNH-diaphorase (*top*) and myosin ATPase (*bottom*) from four subjects. *A* and *B:* untrained middle-aged subject (*BA*). Note relatively large fibers and light DPNH-diaphorase stain in both FT and ST fibers. *C* and *D:* distance runner (*DM*). FT fibers are smaller than ST fibers in this sample. Both fiber types have intense DPNH-diaphorase stain, particularly the subsarcolemmal region of the ST fibers. *E* and *F:* sprinter (*NP*). Both FT and ST fibers exhibit a relatively low oxidative potential. *G* and *H:* orienteer (*CB*). Intensity of the stain for DPNH-diaphorase in some of the FT fibers approaches that of the ST fibers (×185).

Two fiber types were identified in the muscle samples examined in this study (Fig. 1). One possesses high, and the other low, myosin ATPase activity at alkaline pH (9.4). According to Barnard et al. (2), a high myosin ATPase activity under the conditions of our assay occurs in muscle fibers with fast twitch characteristics and a low activity in muscle fibers with slow twitch characteristics. We have thus designated the two fiber types as fast twitch (FT) and slow twitch (ST). As indicated by alpha-glycerophosphate dehydrogenase activity, FT fibers always have higher glycolytic capacity than ST fibers. The FT fibers generally have a lower oxidative capacity than ST fibers but it is apparent from DPNH-diaphorase activity that a continuum of oxidative capacity exists in both fiber types. In the center of this continuum there is a region where the oxidative capacity of the two fiber types overlaps. As will be shown subsequently, it appears that with training the oxidative capacity of both fiber types is enhanced. Thus, any classification system based either partially or solely on oxidative capacity would be difficult to apply objectively to all muscle samples.

A third fiber type having high myosin ATPase activity at alkaline pH and high oxidative capacity has been identified in skeletal muscle of various species (2). This fiber type has been called red (2), C (24), FT (5), and $\alpha\beta$ (10). We have not seen this fiber type in the muscle samples studied. Ogata and Murata (19), however, have identified three fiber types in human intercostal muscle on the basis of SDH and sudan black staining. These investigators, however, did not consider contractile characteristics as indicated by myosin ATPase activity.

The average fiber distribution in the samples of the deltoid muscle was 46% ST and 45% ST (% FT fibers = 100-ST fibers) for the untrained young and middle-aged groups, respectively (Table 1). These samples contained from 14 to 60% ST fibers in the young subjects and from 34 to 58% ST fibers in the middle-aged group. The average distribution of fibers in the lateral portion of the vastus lateralis muscle was comparable to that of the deltoid with the young subjects having 36% ST and the middle-aged group 44% ST fibers. The range of fiber populations of this muscle was similar to that of the deltoid, 13–73% ST fibers, for both the young and middle-aged groups. Edström and Nyström (9) have reported a similarly wide distribution of fiber types in human skeletal muscle.

The mean percentage of ST fibers in the muscles of the subjects who participated in endurance training was higher than in the sedentary groups. Although there were some exceptions, the highest percent of ST fibers was consistently found in the muscle which was most extensively engaged in the endurance work. The mean value for the leg muscle was about 60% ST fibers. The range of both ST and FT fibers within any specific group of endurance athletes was similar to that of the untrained groups. However, the low and high values were greater than those of the sedentary groups. ST and FT fibers were about equally distributed in both the deltoid and vastus lateralis muscles of the weight lifters.

Fiber diameters and areas and the relative areas occupied by each fiber type in a muscle were determined on 25 samples that included 15 from the leg and 10 from the arm (Table 2). These samples represented all of the groups and included six subjects from which measurements were made on both the arm and leg. The average area of the ST fibers for all samples measured was 5,423.6 and 5,406.5 μ^2 for the vastus lateralis and deltoid, respectively. FT fiber areas were about 20% larger than ST areas in both vastus lateralis (6,379.8 μ^2) and deltoid (6,295.7 μ^2). When comparisons were made for subjects where data were available from both leg and arm, ST fiber areas were 5,771.8 and 5,295.7 μ^2, respectively. In these subjects the ST fibers of

TABLE 2. *Fiber sizes, populations, and contribution to muscle area of several subjects*

Subj	Sample Site (Arm or Leg)	Group	Fiber Diam, μ		Area, μ^2		% ST Fibers	% Area ST Fibers
			ST	FT	ST	FT		
PG	L	Untrained	75.2 ± 2.9 (47.1–96.1)	85.8 ± 2.0 (69.3–101.7)	4,567.5 ± 343.2 (1,740–7,250)	5,843.0 ± 273.9 (3,770–8,120)	34.0	28.7
CS	L	Untrained	80.3 ± 3.3 (54.4–112.2)	93.2 ± 2.2 (71.9–110.4)	5,234.5 + 440.3 (2,320–9,860)	6,902.0 + 329.9 (4,060–9,570)	30.0	24.5
MKS	L	Untrained	63.4 ± 2.4 (47.1–79.4)	67.7 ± 2.4 (47.1–86.0)	3,057.5 ± 273.6 (1,740–4,930)	3,683.0 ± 250.0 (1,740–5,800)	34.0	30.0
GK	A	Untrained	63.6 ± 2.0 (38.0–76.0)	67.3 ± 1.7 (53.8–79.2)	3,234.0 ± 181.2 (1,135–4,540)	3,594.0 ± 177.8 (2,270–4,918)	48.3	45.7
	L		72.2 ± 3.5 (43.9–98.2)	75.0 ± 1.4 (65.9–87.8)	4,275.0 ± 382.1 (1,513–7,566)	4,445.0 ± 169.1 (3,405–6,053)	48.6	47.6
NP*	L	Sprinter	79.5 ± 2.6 (54.4–98.0)	89.4 ± 2.1 (74.4–101.7)	5,060.5 ± 314.0 (2,320–7,540)	6,336.5 ± 284.2 (4,350–8,120)	26.0	21.9
DM*	L	Distance runner	67.1 ± 1.7 (54.4–83.3)	58.0 ± 1.3 (47.1–69.3)	3,581.1 ± 186.2 (2,320–5,510)	2,668.0 ± 1,220 (1,740–3,770)	75.0	80.1
DS	L	Distance runner	85.1 ± 3.5 (57.7–105.3)	105.2 ± 2.5 (81.5–124.6)	5,858.0 ± 445.8 (2,610–8,700)	8,776.1 ± 403.2 (5,220–12,179)	70.0	60.9
DF*	L	Middle-dist runner	95.5 ± 3.2 (74.4–121.6)	87.9 ± 3.4 (47.1–115.3)	7,307.8 ± 499.6 (4,350–11,599)	6,235.0 ± 448.2 (1,740–10,439)	55.0	58.9
RP	L	Middle-dist runner	59.2 ± 2.7 (27.2–79.2)	71.6 ± 2.5 (50.9–98.0)	2,856.5 ± 231.8 (580–4,830)	4,118.0 ± 295.9 (2,030–7,540)	47.0	38.1
BA*	L	Former wt lifter	107.1 ± 3.8 (83.8 135.9)	108.9 ± 3.4 (92.2 160.8)	9,199.1 ± 656.7 (5,510 14,499)	9,482.9 ± 666.9 (6,670 20,299)	24.0	23.5
MH	L	Weight lifter	85.6 ± 4.9 (47.1–113.7)	110.8 ± 3.0 (86.0–135.9)	6,035.6 ± 629.4 (1,740–10,149)	9,758.1 ± 516.6 (5,800–14,499)	25.3	23.5
	A		83.5 ± 2.4 (60.8–98.0)	105.0 ± 3.3 (74.4–135.9)	5,553.5 ± 303.8 (2,900–7,540)	8,917.2 ± 543.1 (4,350–14,499)	48.4	36.9
JR*	A	Bicyclist	83.1 ± 1.4 (71.9–96.1)	96.2 ± 1.9 (76.9–108.7)	5,467.0 ± 187.9 (4,060–7,250)	7,337.0 ± 273.7 (4,640–9,280)	52.1	48.6
	L		104.6 ± 2.3 (86.0–127.5)	112.2 ± 2.2 (98.0–137.2)	8,651.5 ± 763.9 (5,800–12,759)	9,946.6 ± 401.5 (7,540–14,789)	51.3	44.0
BL	A	Canoeist	101.9 ± 2.6 (86.3–129.5)	102.9 ± 2.3 (88.6–118.2)	8,244.0 ± 570.3 (5,850–13,162)	8,391.0 ± 361.9 (5,484–10,968)	57.9	74.6
	L		90.5 ± 2.7 (68.2–107.9)	80.3 ± 1.5 (68.2–91.6)	6,544.0 ± 387.5 (3,656–9,140)	5,100.0 ± 190.1 (3,656–6,581)	69.9	57.5
SH	A	Swimmer	88.0 ± 1.7 (71.6–101.2)	91.0 ± 2.4 (77.8–105.7)	6,124.0 ± 233.9 (4,022–8,043)	6,552.0 ± 263.2 (4,753–8,774)	85.3	84.4
	L		79.0 ± 2.0 (61.9–91.6)	93.6 ± 1.9 (74.8–105.7)	4,954.0 ± 237.6 (2,925–6,581)	6,928.0 ± 266.9 (4,387–8,774)	79.7	73.7

Values are means ± SE. Values in parentheses are highest and lowest observations.   *See Table 4 for performance data.

the leg were 9% larger and the FT fibers 8% larger than those of the arm. Marked variations did exist between different muscles from the same subject and between subjects. For several subjects (*DM, BL,* and *DF*) ST fibers were larger than FT fibers (Table 2, Fig. 1*C*).

With several exceptions, both fiber types of trained subjects were larger than those of the sedentary individuals. The type of training also seemed to have an influence on the relative size of the two fiber types. In *subject MH* (weight lifter) the fibers of the leg were only about 9% larger than those of the arm. However, in both the arm and the leg FT fibers were more than 60% larger than the ST fibers. In contrast, the ST and FT fibers in the arm of canoeist *BL* were 26 and 64% larger, respectively, than in the leg. In *subject SH*, a swimmer, the ST fibers of the arm were 24% larger than those of the leg, whereas the FT fibers of the arm were 5% smaller than those of the leg. In this subject both fiber types in the arm were about equal in size, but in the leg FT fibers were 40% larger than ST fibers. These examples illustrate the effects of extensive training of the arm on fiber size. In *subject JR*, a bicyclist, ST and FT fibers in the arm were 37 and 26% smaller, respectively, than

those of the leg. FT fibers in the arm were 34% larger than ST fibers, but in the leg this difference was only 15%. In all of these examples, the area of the fibers in the trained subjects was larger than that of the untrained subjects.

The relative area of a muscle occupied by a given fiber type was quite different in nonendurance as compared to endurance athletes. In the untrained subjects the area composed of ST fibers ranged from 24.5 to 53.2%. Low values in nonendurance athletes included 17.3% in the leg of a weight lifter and 21.9% in the leg of a sprinter. In contrast, the area occupied by ST fibers was 80.1% in the leg of a distance runner (*DM*), 84.4 and 73.7% in the arm and leg of a swimmer (*SH*), respectively, and 74.5% in the arm of a canoeist (*BL*).

From the data presented above and in Table 2 it is apparent that for most individuals FT fibers are larger than ST fibers. It also appears that with some types of training a preferential enlargement of either fiber type can occur. Under these conditions the percent distribution of the two fiber types in a muscle may not be indicative of the relative area occupied by a given fiber type. The relationship between the percentage of ST fibers and the relative area
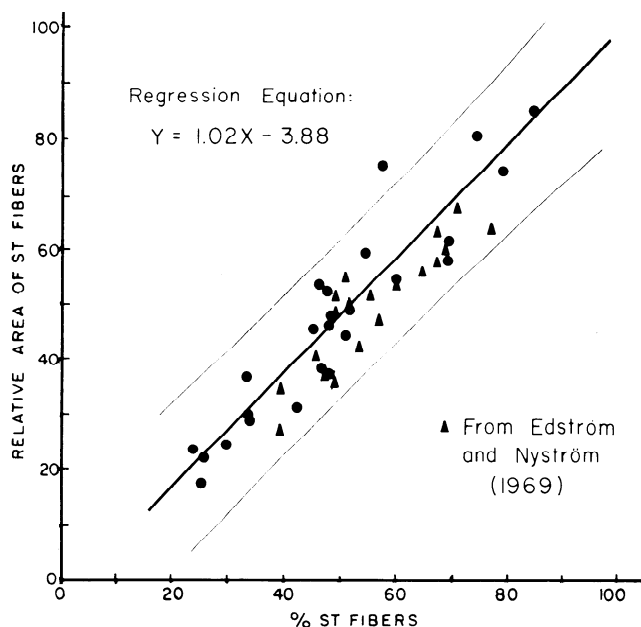
FIG. 2. Relationship between percentage of ST fibers in a muscle sample and relative area occupied by these fibers. Regression line and 95% confidence limits were determined from data collected in this study. Data from Edström and Nyström are also plotted.

they occupy in a muscle sample was determined from all of the data available in the present study. As shown in Fig. 2, a linear relationship existed between ST percent distribution and relative area. This was true even though samples containing vastly different fiber populations and size relationships were included. Data previously published by Edström and Nyström (9) reporting the percent distribution and relative area of the two fiber types from several different muscles from men and women are also included in Fig. 2. Close agreement exists between the results of the present study and those reported by Edström and Nyström. All the values from both studies except one fall within the 95% confidence limits of the regression line. Thus the percentage composition of a muscle in terms of fiber types is indicative of the relative area that each fiber type contributes to the total muscle.

SDH activities in the untrained subjects, both young and middle-aged, were about 3.5 and 4.4 μmoles/g min^{-1} for the arm and leg muscles, respectively (Table 1). The SDH activities of the arm and leg muscles of the weight lifters were 30% less than those of the untrained subjects. SDH activities of the arm and leg muscles of the remaining trained groups were from 20 to 150% higher than the means of the sedentary groups. The highest individual SDH activities were found in the leg muscle of a bicyclist (12.4 μmoles/g min^{-1}) and arm muscle of a swimmer (9.9 μmoles/g min^{-1}).

SDH activities were highest in the most active muscles both for the trained and untrained subjects. In the untrained groups the SDH activity of the leg muscle was 25% greater than that of the arm. In those groups whose training involved leg work the SDH activities of the deltoid muscle average 15–20% more than that of the untrained subjects. In these same subjects, SDH activity in the leg muscles was 55% higher than that of the arms. In the group of middle-

aged orienteers the activity of the vastus lateralis was 40% higher than that of the deltoid muscle. Even greater differences existed between the untrained groups and those subjects whose sport event involved extensive use of the leg or arm muscles. Differences between muscles in the same subject also became more pronounced in the well-trained groups. In the case of the bicyclists, the SDH activity of the leg muscle was 2.5-fold greater than that of the sedentary subjects. The activity in the arms of these subjects was 70% higher than the untrained subjects, but only 55% that of the legs. SDH activities of the deltoid muscle of the canoeists and swimmers were 2.2- and 2.4-fold higher, respectively, than those of the untrained groups. In these subjects SDH activities were higher in the arm than leg muscles. These findings suggest a specific localized training effect.

Although PFK activities were not determined for all groups, there was no difference among groups or between the muscles within a given group. Average values were from 19 to 29 μmoles/g min^{-1}, with the lowest mean found in the group of subjects engaged in different sport activities and the highest mean value found in the leg of the swimmers (Table 1).

The importance of variation in the biopsy site on enzyme activity and fiber composition was also investigated by sampling the vastus lateralis at different sites in 18 subjects (including trained and untrained). The standard sampling site used in these experiments was 12–16 cm above the patella at a depth of 4 cm in the muscle. Alternate sites were 4 cm above or below this point. The standard deviation of the differences between these sampling sites was 0.46 and 3.86 μmoles/g min^{-1} for the SDH and PFK activities, respectively, and 4.6 for the percent distribution of the fibers. Although this represents a small degree of variability, it does illustrate the need for a standard sampling site. Variations in depth of the biopsy might also affect enzyme activities and fiber size and distribution. However, this was not determined in the present investigation.

RNA in the deltoid and vastus lateralis muscles was determined only in the bicyclists, weight lifters, middle-aged orienteers, and swimmers. The mean values for these groups were 1.5 and 1.7 μg/g for the arm and leg, respectively. No significant differences existed among groups or between the two muscles studied. Since these groups included both a wide range of ages (17–58 years) and different sport activities, it appears that only minor differences may be attributed to these factors.

Muscle glycogen concentrations of the trained subjects were generally higher than those of the sedentary individuals (Table 3). It was also apparent that for a single subject the glycogen content was highest in the muscle that was used most extensively in the sport activity. Examples are *subject JR* (bicyclist), where the highest glycogen content existed in the leg muscle, and *subjects SH* and *BL* (swimmer and canoeist), where the pattern was reversed. Based on PAS staining, it appears that no uniform pattern for the storage of glycogen in the two fiber types in skeletal muscle exists. Examples of some of the patterns observed in this study are presented in Fig. 3. In some instances it was impossible to distinguish differences in glycogen concentration between the two fiber types (Fig. 3, *A* and *C*). The glycogen content of the tissue in Fig. 3*A* was low (84 mM

glucose units/kg), and that in Fig. 3C high (178 mM glucose units/kg). In other examples (Fig. 3, E and G) the pattern varied with either ST or FT fibers staining more intensely. In many muscles the pattern alternated (Fig. 3G). The ability of ST fibers to accumulate glycogen is illustrated by *subject DM* whose muscle contained 75% ST fibers and a high glycogen content (127 mM glucose un.ts/kg).

DISCUSSION

The identification of only two fiber types in human muscle in the present study agrees with several earlier reports. (7, 9, 16). In contrast to these earlier observations we have chosen to identify fibers on the basis of contractile characteristics.

The higher SDH activity in the muscles of the trained groups agrees with the findings reported by Varnauskas and co-workers (26) and Morgan et al. (15). The DPNH-diaphorase activity in the FT and ST fibers of the muscles

TABLE 3. *Glycogen concentration in arm and leg muscle of untrained subjects and of representative subjects from trained groups*

| Subj | Event | Glycogen, mM Glucose units/kg | |
		Arm	Leg
Untrained		93.3* (75–120)	80.7† (53–111)
JR	Bicyclist	90	153
SH	Swimmer	223.0	122
BL	Canoeist	105	79
DM	Dist runner		127
DS	Dist runner		173
DF	Mid-dist runner		178

Values in parentheses are high and low observations. *Mean of 3 subjects. †Mean of 10 subjects.

from the trained subjects was higher than that of the sedentary subjects (Fig. 1). This is evidence of the adaptability of oxidative capacity in both fiber types. In some of the trained groups (Fig. 1), the DPNH-diaphorase activity of the FT fibers appeared to have been as great or greater than that of the ST fibers in the muscles of the untrained groups. The ST fibers in the muscles of the highly trained subjects also appeared to have been more oxidative than the ST fibers from the untrained subjects. This was characterized by a dark subsarcolemmal DPNH-diaphorase stain. In the subjects with the highest SDH activity the DPNH-diaphorase activity of the FT fibers approached that of the ST fibers.

The question arises as to whether the percentage distribution of a specific fiber type in skeletal muscle can be altered by training. Morgan et al. (15) have reported that in man the frequency of red fibers increased following training. Barnard and associates (3) also reported that the percentage of red fibers in guinea pig muscle increased after training. It should be pointed out that the conversion of fibers types as reported by Barnard et al. did not require any change in contractile properties. Furthermore, Morgan and co-workers identified fibers as red or white only on the basis of SDH activity. Since training produces a change in SDH activity (3, 11) and because it has been shown in the present study that the oxidative potential of FT fibers can vary dramatically, it would be easy to identify FT as ST fibers after training from only a histochemical determination of SDH activity. However, Guth and Yellin (10) have reported a progressive decrease in high myosin ATPase staining fibers in the rat soleus during compensatory hypertrophy. Although the question stated above cannot be answered by the data from this study, we cannot exclude the possibility that such a conversion of fibers can occur in response to training. The wide range of fiber compositions
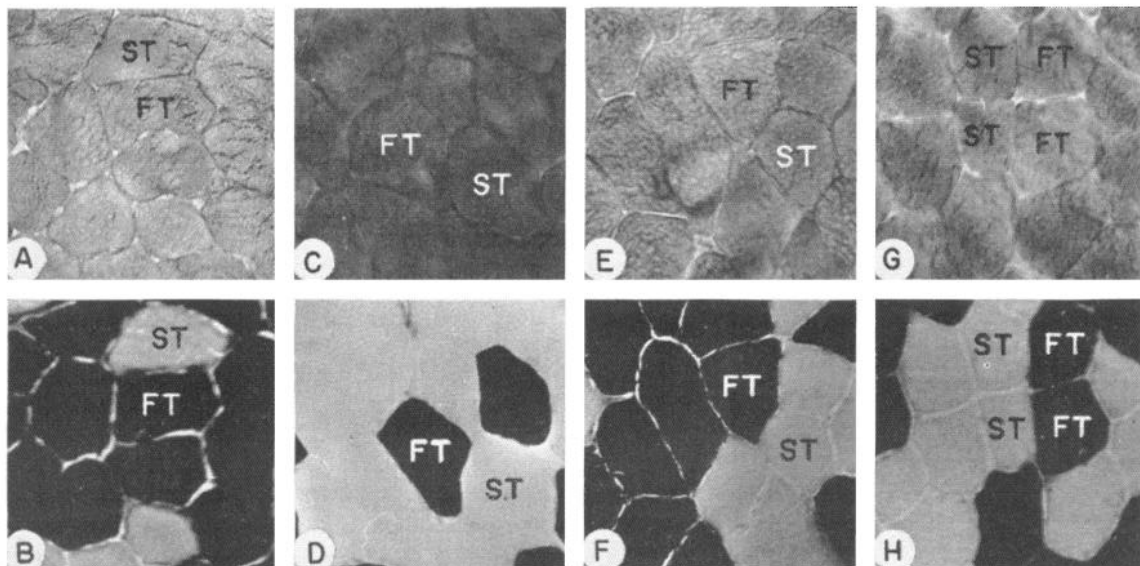

FIG. 3. Serial sections from vastus lateralis muscle showing PAS stain for glycogen (*top*) and stain for myosin ATPase (*bottom*) for four subjects. A and B: untrained middle-aged subject (*PG*). Both FT and ST fibers stained lightly for glycogen. C and D: middle-distance runner (*DF*). Both FT and ST fibers show an intense stain for glycogen. E and F: untrained young subject (*CS*). ST fibers appear to have a higher glycogen concentration than FT fibers in this sample. G and H: distance runner (*DC*). Illustration of inconsistent staining of fibers within the same muscle (×185).

TABLE 4. *Fiber population, enzyme activities, $\dot{V}O_{2\,max}$, and performance characteristics of several subjects*

Subj—Age, Ht, Wt	Sample Site (Arm or Leg)	% ST Fibers	Enzyme Activities μmoles/g min^{-1}		$\dot{V}O_{2\,max}$, liter/min	Comments on Performance
			SDH	PFK		
JR—33 yr, 175 cm, 75 kg	A	51.3	6.73	19.36		Bicyclist; 5 Swedish championships
	L	52.1	12.38	21.37	5.50	
CS—26 yr, 189 cm, 74 kg	A	39.5	5.49			Bicyclist; third in world championship, 1969; 5 Swedish championships
	L	72.5	8.21		4.80	
RP—27 yr, 186 cm, 79 kg	A	48.2	9.24	22.98	4.77	Canoeist; Olympic Gold Medal, 1964; Olympic Silver Medal, 1968; world champion 5 times—last 1971
	L	45.0	5.65	31.45	4.55	
AB—19 yr, 175 cm, 70 kg	A	71.8	9.60	20.97		Swimmer; 400 m—4:05.8; 1,500 m—16:33.4
	L	66.4	8.06	28.83	5.20	
DM—20 yr, 170 cm, 62 kg	A					Distance runner; 4th in NCAA Cross-Country Championships, 1971; 28:15—6 mile
	L	75.0	8.03	15.07	4.95	
DF—21 yr, 178 cm, 69 kg	A					Middle-distance runner; 1:51—880 yd; 4:04—mile
	L	55.0	5.14	26.53	4.78	
NP—22 yr, 165 cm, 61 kg	A					Sprinter; 9.3—100 yd
	L	26.0	3.95	28.34	3.59	
BA—31 yr, 191 cm, 87 kg	A					Former weight lifter; somewhat active in recreational sports
	L	24.0	4.67	23.12	4.39	

within the various groups would argue against such a conversion of fibers from one type to another. On the other hand, some of the endurance athletes studied did have a relatively high percentage of ST fibers. This could be due to a selection of these activities by individuals possessing the natural endowment. Additional studies are needed to clarify this point.

Wide variations existed in muscle fiber composition and enzyme activity, and performance characteristics of some of the best athletes studied. Several examples of this are presented in Table 4. A comparison of *subjects JR* and *RP* with *DM* indicates that each had high SDH activity and $\dot{V}O_{2\,max}$ but a strikingly different muscle fiber composition. *Subject RP*, a world champion canoeist, and *subject JR*, an exceptional bicyclist, both had approximately equal distributions of the two fiber types in the leg and arm muscles, whereas *subject DM*, a distance runner, had a predominant number of ST fibers (75%) in the vastus lateralis muscle. The high levels of SDH activity in the muscles of *subjects JR* and *RP* were the result of a high oxidative capacity in both fiber types as indicated by the DPNH-diaphorase activity. Another difference between these athletes was their ability to perform sprint work. Both *subjects RP* and *JR* possessed high capacity for sprint work. *Subject RP* has performed best in relatively short-distance events, whereas *subject DM* is best at long distances and has limited sprint capability. *Subject JR* has been able to perform prolonged endurance work and then to sprint near the end of the competition. *Subject DF*, with 55% ST fibers, is a middle-distance runner (4:04 mile and 1:51 half-mile). *Subject NP* (74% FT fibers) is a sprinter (9.3-sec 100 yd). The differences in the fiber composition of their muscles may be responsible, at least in part, for these performance characteristics. It is also interesting to note that the $\dot{V}O_{2\,max}$ of *subject RP* was greater during arm than leg work. This was no doubt related to the extensive training of his arm for canoeing. Similar comparisons can also be made between *subject AB*, a swimmer competing in 400- and 1,500-m events, and *subjects JR* and *RP*.

Another interesting example is *subject CS*, who in 1969

had a $\dot{V}O_{2\,max}$ of 5.6 liters/min and finished third in the world championship bicycle competition. Due to an inability to continue top training his $\dot{V}O_{2\,max}$ had declined to 4.8 liter/min at the time of this study. However, his vastus lateralis contained 73% ST fibers and had an SDH activity of 8.2 μmole/g min^{-1}. These characteristics are indicative of a high capacity for aerobic work. In *subject BA*, a retired weight lifter who is still somewhat active in other sports, FT fibers in the leg were dominant (76%), whereas SDH activity was only average (4.47 μmoles/g min^{-1}).

The muscle samples from some of the untrained subjects possessed percentages of ST fibers equal to or higher (70–85%) than the endurance athletes even though $\dot{V}O_{2\,max}$ and SDH and DPNH-diaphorase activities were much lower. In every case the highly trained endurance athletes possessed high SDH and DPNH-diaphorase activities. These two characteristics appear to be well related. Since the SDH activity and DPNH-diaphorase activities can vary dramatically in both fiber types, and athletes with high percentages of FT fibers can still possess extremely high oxidative capacities, it would appear that the adaptability of the fibers for aerobic metabolism may be a more important factor in training and performance than the basic composition of the muscle.

One question currently being redebated is whether the oxidative capacity of the muscles or the transport capacity of the cardiovascular system is the limiting factor for $\dot{V}O_{2\,max}$. This question can be approached from the standpoint of the oxidative potential of the muscles based on SDH activities. For such a consideration it should be pointed out that SDH activities were measured at 25 C in the present study, and that at a normal body temperature of 37 C there is a 2.2-fold increase in activity (6). If it is assumed that the $\dot{V}O_2$ capacity of skeletal muscle is equal to the conversion rate for succinate, 1 kg of skeletal muscle, such as the vastus lateralis from the sedentary groups, could consume 0.6 liter O_2/min. For the bicyclists (SDH activity of 11.0 μmoles/g min^{-1}), this value would be 1.6 liters O_2/kg min^{-1}. Under these conditions total body oxygen consumption during maximal work could occur in 6–7 kg of muscle for

the untrained group and from 3–3.5 kg of muscle for the bicyclist. This would suggest that both in the sedentary and trained subjects the oxidative capacity of the muscle mass normally used in leg exercise would exceed reported values for $\dot{V}o_{2\ max}$. These calculations are based on the assumption that all of the components of the oxidative pathway function at the rate of the SDH reaction, which is unlikely. However, it has been demonstrated (12) that most components of the Krebs cycle and electron transport system increase in proportion to each other during training. Furthermore, SDH activity is representative of total mitochondrial protein. Based on these considerations and the SDH activities of this study, the oxidative capacity of the leg muscle of the bicyclists is 2.5-fold greater than that of the sedentary group. $\dot{V}o_{2\ max}$ of these highly trained athletes, however, was not 2.5-fold greater than that of the untrained subjects. These considerations seem to point to the cardiovascular system as imposing an upper limit on $\dot{V}o_{2\ max}$.

What then is the importance of the large aerobic potential of the skeletal muscle of the trained individuals? It would seem to relate specifically to the increase in oxidative capacity of the FT fibers, with the result that these fibers would be able to better utilize their glycogen stores aerobically and to oxidize fatty acids. In this manner the onset of fatigue in these fibers would be delayed. This increased oxidative capacity of the FT fibers may also contribute to the reduction in lactate production during submaximal work that occurs after training.

As stated above, it seems unlikely that the capacity of the body to utilize oxygen during maximal work is limited by the oxidative capacity of the muscle cell. In spite of this, profound differences were observed between trained and untrained muscle groups. The endurance athlete was characterized by a high percentage of ST fibers in his trained muscles. The oxidative capacity of these fibers and the FT fibers was significantly higher than in his less active muscles or in muscles of untrained persons. The extent to which the regular training has contributed to this observed difference cannot be settled by the present study. The observed results suggest that physical conditioning in man does not influence fiber composition as much as it does the oxidative capacity of the individual muscle fibers.

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