

# Effect of training on enzyme activity and fiber composition of human skeletal muscle

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GOLLNICK, P. D., R. B. ARMSTRONG, B. SALTIN, C. W. SAUBERT IV, W. L. SEMBROWICH, AND R. E. SHEPHERD. *Effect of training on enzyme activity and fiber composition of human skeletal muscle.* J. Appl. Physiol. 34(1): 107-111. 1973.—The effect of a 5-month training program on the oxidative and anaerobic capacity, fiber composition, fiber area, and glycogen concentration of multiple biopsy samples from the vastus lateralis muscle of six subjects was studied. The training program consisted of pedalling a bicycle ergometer 1 hr/day 4 days a week at a load requiring from 75 to 90% of the subjects maximal aerobic power. Mean maximal oxygen uptake increased 13% (range 3.6-25%), and mean succinate dehydrogenase and phosphofructokinase activities increased 95 and 117% ( $P < 0.01$ ), respectively, after training. Oxidative potential (DPNH-diaphorase activity) increased in both fiber types whereas glycolytic capacity (alpha-glycerophosphate dehydrogenase activity) appeared to have increased only in the fast twitch fibers. No change occurred in the percentages of slow or fast twitch fibers as identified from myosin ATPase activity. Slow twitch fibers were larger after training as compared to before training. The relative area the slow twitch fibers occupied in the muscle was also higher after training. Muscle glycogen was 2.5-fold higher after as compared to before training.

muscle glycogen; succinate dehydrogenase; phosphofructokinase; human fiber types; fiber areas

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A RECENT STUDY revealed that the skeletal muscles of highly trained endurance athletes possessed higher oxidative capacities and percentages of slow twitch fibers than those of untrained men (14). The high oxidative potential in the muscles from endurance athletes was consistent with earlier reports on the effect of training on the skeletal muscle of animals and man (1, 3, 16-18, 21, 29). From the previous investigation it was not possible to determine the extent that the oxidative capacity and fiber distribution in human muscle could be altered by strenuous endurance training.

The purpose of this study was to determine the effects of a 5-month endurance training program on succinate dehydrogenase (SDH) and phosphofructokinase (PFK) activities and fiber composition in skeletal muscle of man. The distribution of aerobic and anaerobic capacity and glycogen concentration in the two fiber types of human muscle before and after training was also estimated.

## MATERIALS AND METHODS

Six healthy male subjects were studied (see Table 1 for physical characteristics). All were active in recreational sports but none had engaged in endurance training for at least 2 years prior to this study. Before training maximal oxygen consumption ( $\dot{V}O_{2\max}$ ) was determined and muscle samples were taken from two different sites in the lateral portion of the vastus muscle with the needle biopsy technique (4).

The subjects proceeded to train for 5 months by pedaling a cycle ergometer 1 hr/day 4 days a week at a load requiring 75% of their maximal aerobic power. Initially the subjects could not tolerate this load for the full hour and it was reduced to about 65% of  $\dot{V}O_{2\max}$  during a portion of the exercise bout. After about 2 weeks all subjects were able to complete the basic training load for 1 hr. Thereafter each subject attempted to increase the work load to the maximum that could be tolerated for the 1-hr period. Midway through the training program  $\dot{V}O_{2\max}$  tests were repeated to ensure that all subjects were still working at the prescribed load. At the end of the training program most of the subjects were working for 1 hr at 85-90% of their  $\dot{V}O_{2\max}$ .

Three to five days after the final exercise bout multiple biopsy samples were taken from the vastus lateralis muscle at sites near those of the initial sampling.  $\dot{V}O_{2\max}$  was determined at the end of the study.

Maximum oxygen uptake tests were done on the cycle ergometer using the leveling-off criteria. Expired air was collected in a 600-liter gasometer and analyzed with a Beckman E2 oxygen analyzer (8). The accuracy of the analyzer was verified with the Scholander microtechnique.

The muscle samples were divided into three parts. One portion was examined under a dissecting microscope to determine fiber orientation, mounted onto a specimen holder in OCT embedding medium (Ames Tissue-Tek), and frozen in isopentane cooled to the temperature of liquid nitrogen. This sample was used for histochemical analysis. The remaining parts of the sample were weighed and used for the estimation of SDH and PFK activities at 25 C with the methods of Cooperstein and associates (7) and Shonk and Boxer (27), respectively. In some cases a second biopsy sample was taken at the same site to provide enough tissue for all analyses.

TABLE 1. *Physical characteristics, enzyme activities, and muscle glycogen concentrations*

Subj	Age	Ht, cm	Wt, kg	$\dot{V}O_2 \text{ max}$ , liters/min		SDH Activity, $\mu\text{moles g}^{-1} \text{ min}^{-1}$		PFK Activity, $\mu\text{moles g}^{-1} \text{ min}^{-1}$		Muscle Glycogen, mmoles glucose units/kg	
				B	A	B	A	B	A	B	A
WLS	29	183	106.0	3.7	4.4	2.72	9.77	38.99	66.71		179
PDG	37	187	85.5	3.9	4.2	6.23	10.21	36.58	51.95	84	192
CWS	28	180	72.7	3.7	4.3	5.06	7.05	20.90	50.64	79	165
MKS	40	168	63.6	2.8	2.9	4.88	7.19	28.54	64.51		196
RBA	31	191	91.0	4.4	5.5	4.29	10.91	23.52	60.28	53	189
RES	30	187	91.0	5.0	5.4	4.72	9.20		37.18		173
Mean				3.9	4.5*	4.65	9.06†	27.13	58.82†	72	182†

B = before training; A = after training. \* Before vs. after training  $P < 0.05$ . † Before vs. after training  $P < 0.01$ .

TABLE 2. *Effect of training on distribution and area of fibers of human skeletal muscle*

Subj	Total Fibers Counted		% ST fibers		Area of Fibers, $\mu^2 \times 10$				% Relative Area of ST fibers	
	B	A	B	A	ST		FT		B	A
					B	A	B	A		
WLS	88	1,193	18	27	5,597	6,492	7,221	6,328	14.5	27.7
PDG	387	2,239	35	35	4,568	7,193	5,844	6,029	29.6	39.2
CWS	182	1,408	34	38	5,235	6,820	6,902	5,910	28.3	40.5
MKS	342	1,113	38	37	3,058	5,552	3,683	4,313	33.7	42.9
RBA	199	1,691	27	36	9,199	8,895	9,483	7,596	26.4	40.0
RES	163	2,726	41	42	5,313	5,716	6,694	6,656	35.3	38.0
Mean	226	1,728	32	36	5,495	6,778*	6,638	6,139	27.9	38.1†

B = before training; A = after training. \* Before vs. after training  $P < 0.05$ . † Before vs. after training  $P < 0.01$ .

Serial sections  $10 \mu$  thick were cut in a cryostat at  $-20 \text{ C}$  and mounted on cover glasses for histochemical analysis. Myosin adenosine triphosphatase (ATPase), reduced diphosphopyridine nucleotide-diaphorase (DPNH-diaphorase), and alpha-glycerophosphate dehydrogenase activities were estimated with the methods of Padykula and Herman (23), Novikoff et al. (22), and Wattenberg and Leong (30), respectively. The distribution of glycogen in one of the serial sections ( $16 \mu$  thick) was estimated from the periodic acid-Schiff (PAS) reaction as described by Pearse (24). The tissue remaining after the sections were cut for histochemical analysis was removed from the embedding medium and analyzed for total glycogen with the method described by Karlsson et al. (19).

Muscle fibers were identified as slow twitch (ST) or fast twitch (FT) on the basis of myosin ATPase activity as described previously (14). The area of the two fiber types was determined by direct planimetry on photographs made of the DPNH diaphorase sections.

## RESULTS

Individual values are presented in Tables 1 and 2.  $\dot{V}O_2 \text{ max}$  increased an average of 13% during training ( $P < 0.05$ ). The largest increase in  $\dot{V}O_2 \text{ max}$  was 1.1 liters  $\text{min}^{-1}$  (25%) and the smallest, 0.1 liter  $\text{min}^{-1}$  (3.6%).

Mean SDH activity in the vastus lateralis muscle increased 95% ( $P < 0.01$ ) with training. The smallest pre-

and posttraining difference in SDH activity for a single subject was 40%. The largest individual difference, 256%, occurred in the subject with the lowest initial value. Mean PFK activity increased 117% ( $P < 0.01$ ) during training with a similar change occurring in all subjects.

The percentage of ST and FT fibers in the muscle samples from the vastus lateralis was not significantly altered by the training program. In four of the six subjects the pre- and posttraining fiber compositions of this muscle were nearly identical. In the other two subjects there were about 9% more ST fibers in the posttraining samples.

Oxidative capacity, as indicated histochemically by DPNH-diaphorase activity, appeared to have increased in both fiber types (Fig. 1) following training. In contrast, anaerobic capacity, as indicated histochemically by alpha-glycerophosphate dehydrogenase activity, appeared to have increased only in the FT fibers (Fig. 1).

Muscle samples were available for the determination of total glycogen from only three subjects prior to training. The average glycogen content of these samples was 72 mmoles glucose units  $\text{kg}^{-1}$  wet wt. This value is similar to that of other nontrained subjects (14). Following training the glycogen concentration of the vastus lateralis muscle was 182.0 and 182.3 mmoles glucose units  $\text{kg}^{-1}$  wet wt for the three subjects and total groups, respectively. The difference in glycogen concentration before and after training was also evident from the PAS staining of the muscle sections (Fig. 1). There was no clear pattern of a higher glycogen content in one or the other of the two fiber types either before or after training.

The mean ST fiber area prior to training was  $5,495 \mu^2$  as compared to  $6,638 \mu^2$  for the FT fibers ( $P < 0.01$ ). After training the area of the ST fibers was  $6,778 \mu^2$  ( $P < 0.05$ ) and that of the FT fibers  $6,139 \mu^2$ . The ratio of the ST to FT fiber areas increased from 0.82 before to 1.11 ( $P < 0.01$ ) after training. Considerable variation existed in the areas of the two fiber types among subjects. This was less pronounced after training.

## DISCUSSION

An increase in  $\dot{V}O_2 \text{ max}$  was observed in all subjects. These were within the limits of several earlier reports (10). The largest individual change of 25% was only slightly lower than the 37% observed by Saltin and co-workers (25) in sedentary subjects following a 55-day training pro-

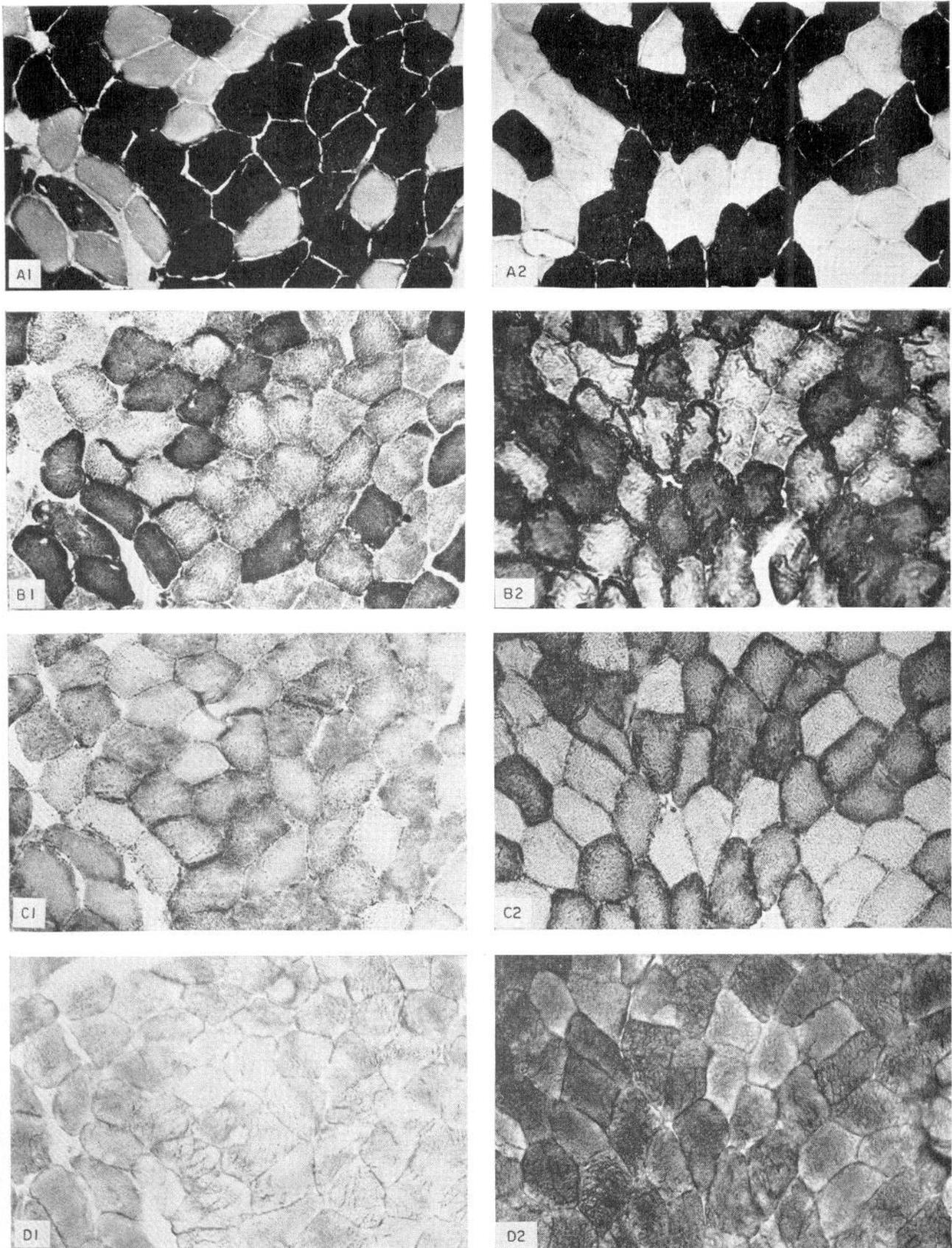


FIG. 1. Photomicrographs of serial sections ( $\times 130$ ) from one subject before (1) and after (2) training. A, B, C, and D are serial sections stained for myosin ATPase, DPNH-diaphorase, alpha-glycerophosphate dehydrogenase, and glycogen (PAS), respectively.

gram of running. The difference between the changes in  $\dot{V}O_{2\text{ max}}$  of the subjects in this study and that of Saltin et al. can probably be attributed to the initial fitness of the subjects and the type of training employed.

The magnitude of the increases in muscle SDH activity following training was similar to the increased oxidative capacity reported by Holloszy and associates (16–18) for endurance-trained rats. It was larger than that observed by Morgan and co-workers (21) and Varnauskas et al. (29) for men following training. However, the training program used in this study was longer and more strenuous than previously used for studying metabolic adaptations in the skeletal muscle of man. The SDH activities following training approached those in well-trained bicyclists and were greater than those seen in endurance runners (14). These high activities probably reflect a more extensive use of the vastus lateralis muscle in cycling than during running. This conclusion was reached from EMG determinations (J. Hendriksson, personal communications). Bicycle exercise was chosen for the training in the present study specifically for this reason. The extensive use of this muscle during bicycle exercise explains the high enzyme activity even though the  $\dot{V}O_{2\text{ max}}$  per kilogram for well-trained bicyclists and the subjects of the present study is lower than that of well-trained endurance runners.

The small increase in  $\dot{V}O_{2\text{ max}}$  contrasted with the large change in oxidative potential of the skeletal muscle supports the thesis that the aerobic capacity of muscle does not limit total body  $\dot{V}O_{2\text{ max}}$  (14). As suggested by Holloszy and co-workers (16, 17), these changes in oxidative capacity are probably more important during submaximal than maximal work. They may contribute to a greater oxidation of fat and the lower lactate production seen during exercise after training.

The increase in PFK activity suggests a greater glycolytic capacity of the vastus lateralis muscle after training. Eriksson and co-workers (11) have also observed increases in PFK activity in the vastus lateralis muscle of 11- to 13-year-old boys following training with bicycle work. This finding is in contrast to that of Holloszy et al. (18) in the rat following endurance training. Recently, however, Baldwin and associates (2) have reported elevated activities of some glycolytic enzymes, including PFK, in the soleus but not quadriceps muscle of the trained rat. The predominate fiber type of the rat soleus possesses low myosin ATPase and high oxidative activity (9). In some ways these fibers are similar to the ST fibers of human muscle. This might suggest that the ST fibers had increased their glycolytic capacity after training. However, on the basis of alpha-glycerophosphate dehydrogenase activity, it appears that an increase in glycolytic capacity occurred mainly in the FT fibers. Since the work load in the present experiment required a large consumption of muscle glycogen (26), an increase in PFK activity in either or both fiber types would aid in the degradation of glycogen to supply energy for muscular contraction by both the aerobic and anaerobic pathways.

The increases in enzyme activities in this study appear to be the result of the training and not a response to a single work bout. This conclusion is based on the relative magnitude of the change as compared to other studies with man and rats (1, 3, 11, 16–18, 21, 29) and from an earlier study

by Eriksson et al. (11), in which SDH and PFK activities in the skeletal muscle of 10- and 11-year-old boys were unchanged after 2 weeks but significantly increased after 6 weeks of training.

The elevation in muscle glycogen is consistent with studies on trained and untrained animals and man (14, 15). The posttraining glycogen concentrations in this experiment were similar to those of well-trained distance runners (14). These changes may have been due to the normal supercompensation of glycogen seen after a single heavy work bout and not due to training. This is particularly true since a time period of 3–5 days elapsed between the final exercise session and taking the posttraining muscle biopsy. The glycogen concentrations were, however, higher than those usually seen after a single work bout (6) but similar to those produced by exercise and dietary modification (5, 19). These changes are compatible with the observation that the glycogen synthetase activity of human skeletal muscle is increased by training (28).

In five of the six subjects ST fiber size increased following training, whereas FT fiber area decreased in four subjects. However, the decrease in FT fiber area was quite small (about 7%) as compared to the increase in ST fiber area (23%). The relative area of the muscle composed of ST fibers increased from 28 to 38% ( $P < 0.01$ ) following training. This change with training is evidence that the ST fibers may have been used more extensively during the training program than the FT fibers. In all cases the relationship between the percent of ST fibers and the relative area they occupied in the muscle both before and after training was within the 95% confidence limits of the regression line published previously (14). The changes with training resulted in moving all points toward the upper confidence limit. We have previously observed larger ST than FT fibers in the muscles of some endurance athletes.

Reports have appeared in the literature in which increases in the percentage of "red" as compared to "white" fibers occurred in the muscles of animals and man following training (3, 12, 13, 21). In all cases these studies have employed oxidative capacity as measured by SDH or DPNH-diaphorase activity to classify fibers. On the basis of the large change in oxidative capacity as identified either by SDH activity of homogenates or histochemically by DPNH-diaphorase activity, it is easy to see how such an interpretation could be made. However, we have concluded that the apparent ease with which the oxidative capacities of both fiber types change with training renders any classification system based solely on this characteristic tenuous. Furthermore, Baldwin et al. (1) have shown that the increase in oxidative capacity of all three fiber types of rat skeletal muscle was approximately twofold following training. Thus the relative oxidative potential of the fast-twitch white fiber was unchanged by training. These data suggest that the relative fatigability of both fiber types in human muscle, and perhaps also the recruitment pattern during exercise, would not be significantly altered by training. In no instance has a change in fiber characteristics as determined by myosin ATPase been demonstrated.

Based on the criterion of myosin ATPase, the present data suggest that fiber types are not altered by prolonged endurance training in adult man. We interpret the approxi-

mately 9% increase in the ST fibers of two subjects as a normal variation in the sampling procedure and not one that reflects a biological change. This conclusion is based on previous data (14) which showed that with multiple biopsy samples the standard deviation between samples from the same subject was about 5%.

A close relationship existed between the percentage fiber composition determined from the relatively small number of fibers in the pretraining samples and the larger number of fibers from the posttraining samples. This suggests a fairly homogeneous composition of the vastus lateralis muscle and supports the reliability of data obtained from the biopsy samples both in this and previous studies.

The results of this study clearly demonstrate the large local adaptability of skeletal muscle to training. They also

indicate that the basic fiber types of skeletal muscle are probably not altered by physical training in adult man. The area of a given fiber type may, however, change in response to training. These findings point to the importance of training in the metabolic characteristic, but not fiber distribution, of human skeletal muscle. They cannot answer the question of whether the changes in enzyme activity (along with increases in mitochondria) produce the metabolic difference between untrained and trained men during exercise.

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