Adaptation of human skeletal muscle to endurance training of long duration

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(Received 9 December 1981; accepted 8 October 1982)

Summary. The aim of this study was to examine the adaptive response of skeletal muscle to dynamic training of long duration and low intensity. The response of m. triceps brachii, (TB) and m. quadriceps femoris, vastus lateralis (VL) to training of long duration at an intensity corresponding to approximately 45% of $\dot{V}_{O_2}$ max, has been studied in six subjects. Muscle specimens were taken during training (18.5 miles (30 km)), which involved skiing with a backpack, (6 days/week, 8 weeks), as well as during a subsequent period of detraining (33 weeks). Increases in oxidative enzyme activities and capillarization during training were limited to the TB. There were parallel increases in citrate synthase (CS) and malate dehydrogenase (MDH) during the first 3 weeks of training (30%). CS then continued to rise (to 90% above pretraining level), while the increase in MDH levelled off. The activity of 3-hydroxyacyl-CoA-dehydrogenase (HAD) and the number of capillaries per fibre increased by 50% and 40% respectively. A selective decrease (12%) in area of fibre type IIA was noted during training after a 2-week period of energy deficiency. During the first 6 weeks of detraining, the decrease in CS and HAD (30%) was greater than the decrease in capillaries per fibre (14%).

It is concluded that prolonged duration of training is not, in itself, sufficient to elicit an adaptive response in the leg muscles, while a modest training effect was seen for the arm muscles. The finding emphasizes the importance of adequate intensity in physical training programmes.

Introduction

Pronounced adaptive changes occur in skeletal muscle in response to endurance training. This has been well documented both in animals and man (Holloszy & Booth, 1976; Salmons & Henriksson, 1981). In humans, the most pronounced response involving marked increases in mitochondrial enzyme activities and capillarization, has been reported from training at intensities demanding 70–90% of the subjects' maximal

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0144-5979/83/0400-0141 \$02.00 © 1983 Blackwell Scientific Publications

oxygen uptake ($\dot{V}_{O_2}$ max) and with a duration of up to 1 h per session (Varnauskus *et al.*, 1970; Gollnick *et al.*, 1973; Andersen & Henriksson, 1977; Bylund *et al.*, 1977; Henriksson & Reitman, 1977; Ingjer, 1979). However, less is known about the effect on skeletal muscle of training at a lower intensity but longer duration. To study this, we monitored the response of a postural, and therefore moderately trained, muscle (vastus lateralis, VL, m. quadriceps femoris) and of an untrained muscle (m. triceps brachii, TB) to two months of skiing with a backpack on at an average exercise intensity corresponding to 45 % of $\dot{V}_{O_2}$ max for 5.5 h per day. Oxidative and glycolytic enzyme activities, capillary supply and muscle fibre areas were determined. These variables were also monitored during a detraining period lasting 33 weeks.

Subjects and methods

Subjects. Six healthy men, 25 years (range 23–30), mean height 183 cm (range 175–197 cm) and mean weight 76 kg (range 67–84 kg) participated in the study. They were informed about the procedure and risks involved in the experiments before they volunteered. The subjects had performed endurance training on a non-competitive basis on an average of 3×45 min per week in the years preceding the study, but only two subjects had exercised regularly in the months preceding the study. No subject had performed any arm-training in the 8 months prior to the study. The triceps brachii muscle could therefore be described as truly 'untrained'. During detraining, their normal training habits were recommenced.

Training. Training consisted of 1500 km of skiing with backpacks on (25 kg) in the Swedish mountains. The distance was covered in 50 effective days of skiing, with 7 days of rest evenly interspersed. Thus, the group skied about 30 km per day using about 5.5 h of effective exercise time a day. Integrated EMG (surface electrodes) during skiing with backpack, in relation to the EMG activity during submaximal and maximal voluntary isometric contractions (MVC), showed that during dynamic skiing (flat and uphill skiing), EMG activity was greater in the triceps brachii (50–75 % of the activity at MVC) than in the vastus lateralis (40–60 %). The work intensity during skiing was estimated on the basis of each subjects' heart rate, recorded with a portable tape recorder (SRA AB, Stockholm, Sweden) during the 2nd, 5th and 8th week of training. The relationship between oxygen uptake and heart rate during bicycle ergometer exercise was determined for the different subjects in the laboratory before and after the training period (see below). In addition, the oxygen uptake/heart rate relationship during skiing with backpack was determined in one subject at the start (10–15 min) and after 2 h of skiing. The heart rate at a given oxygen uptake in skiing at 40–60 % of $\dot{V}_{O_2}$ max was identical to, or a few beats lower than in bicycling. Thus estimated, the subjects' mean work intensity in skiing averaged 45 % (range 40–50) of $\dot{V}_{O_2}$ max. During 75 % of the effective exercise, work intensity was within 35–55 % of $\dot{V}_{O_2}$ max. From the relationship between heart rate and oxygen uptake, exercise energy output during a day was estimated at 14,000 kJ (week 2), 18,000 kJ (week 5) and 17,800 kJ (week 8). Assuming an average oxygen uptake and R-value of $0.35 \text{ litres min}^{-1}$ and

0.76, respectively during non-skiing hours, the energy output during 24 h was estimated at 21,800 kJ (week 2), 25,600 kJ (week 5) and 25,300 kJ (week 8). During weeks 2 and 3, the diet consisted of standard army rations of 18,000 kJ per day. During the other 6 weeks, diets were chosen *ad lib*. During week 7, its energy content was calculated from the nutritional declarations at 24,000 kJ per day. Thus, the rations were inadequate during weeks 2 and 3 which the subjects also experienced.

Oxygen uptake and heart rate determinations. These were made before and after training at three submaximal work loads on a bicycle ergometer (Monark, Sweden). Maximal oxygen uptake was achieved in running uphill on a treadmill. For criteria, see Åstrand & Rodahl (1977). For oxygen uptake determinations, expired air was collected in Douglas bags, measured for volume in a balanced spirometer and analyzed for CO₂ and O₂ content using a mass spectrometer (MGA 200, UK).

Muscle biopsy analyses. Muscle biopsies were taken from the middle portion of the vastus lateralis of quadriceps femoris (VL) muscles of both the left and right leg and from the middle-lower portion of the medial head of the left m. triceps brachii (TB) using the needle biopsy technique (Bergström, 1962). Sampling was performed before training, after the 3rd week of training, immediately after training and after 6 weeks of detraining. Additional muscle samples were taken from the arms after 33 weeks of detraining. Samples were taken about 48 h (week 3:24 h) after the last exercise session. Samples for enzyme assays were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Samples for histochemical analysis were stained for myofibrillar ATPase to identify fibre types (Brooke & Kaiser, 1969, 1970; Dubowitz & Brooke, 1973) and with the amylase-PAS method to visualize capillaries (Andersen, 1975). Cross-sectional areas of the muscle fibres were measured from the amylase-PAS sections with a grid method (Edström & Torlegård, 1968, 1969). The area of an average of 55 (19–119) type I, 33 (6–64) type IIA and 14 (1–41) type IIB fibres in VL and 44 (7–112) type I and 34 (6–61) type IIA fibres in the TB were measured for each individual at each sampling occasion. Due to a small percentage of type IIB fibres, only pretraining area in VL will be stated. Capillary counts were performed as described by Andersen & Henriksson (1977). The number of capillaries per mm² (cap × mm⁻²) was counted for an average cross-sectional area of 0.44 ± 0.08 mm² (TB) and 0.53 ± 0.04 mm² (VL) containing 55 ± 9 (TB) and 92 ± 9 (VL) muscle fibres, respectively. The capillary per fibre ratio corresponds to the total number of capillaries divided by the total number of fibres within a given area. The mean number of capillaries in contact with a single fibre of each type (CC) and CC/1000 μm² fibre type area were calculated by counting all the capillaries around each type I and type IIA fibre. The number of fibres included in calculating cap × fibre⁻¹, CC, and CC/fibre type area was, on the average, 18% (6–41) larger than that used for calculating cap × mm⁻².

Enzyme assays. Freeze-dried muscle samples were weighed and homogenized (1:100) in ice-cooled 0.1 M potassium phosphate buffer, pH 7.3, with 0.05% BSA. For MDH analysis, this homogenate was further diluted 1:5. The assays were performed at 25°C. Citrate synthase (CS, EC 4. 1. 3. 7), 3-hydroxyacyl-CoA dehydrogenase (HAD,

EC 1.1.1.35), 6-phosphofructokinase (PFK, EC 2.7.1.11) and lactate dehydrogenase (LDH, EC 1.1.1.27.) were determined fluorometrically. CS was determined as described by Shepherd & Garland (1969), HAD at pH 7.0 and PFK at pH 7.6 with the methods described by Lowry *et al.* (1978). LDH was determined spectrophotometrically (Bass *et al.*, 1969). Glyceraldehydephosphate dehydrogenase (GAPDH, EC 1.2.1.12) and malate dehydrogenase (MDH, EC 1.1.1.37) were determined spectrophotometrically (Bücher, Luh & Pette, 1964). To avoid the danger of including part of the lactate dehydrogenase activity in the MDH assay (due to breaking down of oxaloacetate to pyruvate) un-neutralized oxaloacetate not older than 2 h was used.

Statistics. Results will be stated as means and standard errors of the mean. A two-way analysis of variance (ANOVA) of no difference in means was applied to the absolute values of the data on TB and VL respectively. When the ANOVA indicated an overall significance between the sampling sessions, comparisons among means (Snedecor, 1956) was applied. When one or two values were missing for some variables, substitute values were calculated with standard formulas. The degrees of freedom were adjusted accordingly. A three-way ANOVA was applied to the pooled data on fibre type IIA area in the TB and VL. An orthogonal comparison was performed on the decrease (w.8–w.14) in $\text{cap} \times \text{fibre}^{-1}$ versus that of HAD and CS activities. Wilcoxon's rank sum test for paired data was used in other comparisons. The variances in fibre type area at w.0 and w.8 were compared with an F-test of variances. Only differences with a significance probability level less than 0.05 has been designated in Figures and Tables or commented upon in the text. Otherwise, this has been stated.

Results

MAXIMAL OXYGEN UPTAKE

The pretraining maximal oxygen uptake of 4.7 litres min^{-1} , or 61 l $\text{kg}^{-1} \text{min}^{-1}$, did not change during training.

CAPILLARY SUPPLY

The values for the pretraining capillary supply are given in Table 1. In m. triceps brachii (TB), the number of capillaries per fibre increased during training by 41 %. During the

Table 1. Values for the different measures of the capillary supply at pretraining in m. triceps brachii and vastus lateralis (mean $\pm$ SE)

	$\text{cap} \times \text{fibre}^{-1}$	CC type I	CC type IIA	$\text{cap} \times \text{mm}^{-2}$	CC $\times$ type I fibre area $^{-1}$	CC $\times$ type IIA fibre area $^{-1}$
m. triceps brachii	2.45 $\pm$ 0.20	5.7 $\pm$ 0.4	6.2 $\pm$ 0.5	295 $\pm$ 23	0.97 $\pm$ 0.07	0.65 $\pm$ 0.04
vastus lateralis	2.08 $\pm$ 0.15	5.8 $\pm$ 0.5	5.4 $\pm$ 0.4	357 $\pm$ 26	1.11 $\pm$ 0.03	0.93 $\pm$ 0.05

Fig. 1. Changes (mean $\pm$ SE) during training and detraining in relation to pretraining values for capillaries per fibre (cap $\times$ fibre $^{-1}$) and capillaries in contact with (CC) fibre type I and IIA in the triceps brachii. Significant differences ($P < 0.05$) between two consecutive sampling occasions are designated with an a between the values. Differences between w.0 and w.8, as well as between w.8 and w.41, are designated with an asterisk at the values of w.8 and w.41 respectively.

Fig. 2. Changes (mean $\pm$ SE) during training and detraining in relation to pretraining values for capillaries per mm 2 (cap $\times$ mm $^{-2}$) and capillaries in contact with (CC) fibre type I and IIA per 1000 μ m 2 fibre type area in the triceps brachii. (For designations of significance, see the legend for Fig. 1).

first 6 weeks of detraining, it decreased by 14% and at 33 weeks, after discontinuation of training, it was approximately the same as the pretraining value (Fig. 1). Similar changes were noted in capillaries in contact with (CC) type I and type IIA fibres, capillaries per mm² and capillaries in contact with each fibre type (CC) per 1000 μm^2 (Figs. 1, 2). However, due to decreased fibre areas, the values at 33 weeks of detraining were higher than the pretraining values for the two latter variables (Fig. 2). There were no significant changes in the capillary supply of vastus lateralis (VL) during training or detraining.

Table 2. Pretraining enzyme activities in m. triceps brachii and vastus lateralis, expressed as mkatal $\times$ kg dry weight⁻¹ (mean $\pm$ SE)

	CS	HAD	MDH	PFK	GAPDH	LDH
m. triceps brachii	0.24 $\pm$ 0.03	0.51 $\pm$ 0.06	9.3 $\pm$ 1.2	0.76 $\pm$ 0.10	12.0 $\pm$ 1.0	2.19 $\pm$ 0.31
vastus lateralis	0.61 $\pm$ 0.10	1.03 $\pm$ 0.14	15.4 $\pm$ 2.2	0.84 $\pm$ 0.18	11.6 $\pm$ 1.6	2.41 $\pm$ 0.33

Fig. 3. Time course of the changes (mean $\pm$ SE) during training and detraining in relation to pretraining values for CS, HAD and MDH activities in the triceps brachii. (For designations of significance, see the legend for Fig. 1.)

OXIDATIVE ENZYME ACTIVITIES

The pretraining enzyme activities are given in Table 2.

M. triceps brachii (Fig. 3). The activities of CS, HAD and MDH increased during training by 92%, 51% and 44% respectively. During the first 6 weeks of detraining, CS, HAD and MDH activities decreased by 34%, 29% and 8% (NS), respectively. Pre-training enzyme levels were noted at 33 weeks after discontinuation of training. The MDH/CS activity ratio did not change during the first 3 weeks of training, but decreased by $23 \pm 10\%$ from week 3 to the end of training. During the first 6 weeks of detraining, it reverted to its pretraining value. The HAD/CS activity ratio decreased by $19 \pm 5\%$ during training and was back at its pretraining level by the 33rd week of detraining.

Vastus lateralis. No significant changes in enzyme activities or enzyme activity ratios were noted during training or detraining.

GLYCOLYTIC ENZYME ACTIVITIES

The pretraining enzyme activities are given in Table 2.

M. triceps brachii. The activities of PFK, GAPDH and LDH as well as the LDH/GAPDH-, LDH/PFK- and GAPDH/PFK-activity ratios did not change significantly during training or detraining.

Fig. 4. Changes in the area of fibre type I and IIA in *m. triceps brachii*, and *m. quadriceps femoris*, *vastus lateralis*, during training and detraining. Each subject is designated by a specific symbol. The mean fibre type IIB area in VL at pretraining was $5000 \pm 700 \mu\text{m}^2$ ($n = 5$).

Table 3. Mean fibre area and fibre type IIA/I area ratio in m. triceps brachii and vastus lateralis (mean $\pm$ SE)

	Pretraining	3 week-training	End of training	6 week-detraining	33 week detraining
<i>m. triceps brachii</i>					
Mean fibre area (μm^2)	8200 $\pm$ 1000	7400 $\pm$ 1000	8000 $\pm$ 400	8300 $\pm$ 500	6700 $\pm$ 600
Fibre type IIA/I area ratio	1.64 $\pm$ 0.06	1.40 $\pm$ 0.03	1.46 $\pm$ 0.05	1.56 $\pm$ 0.13	1.56 $\pm$ 0.10
<i>vastus lateralis</i>					
Mean fibre area (μm^2)	5400 $\pm$ 500	5300 $\pm$ 600	5500 $\pm$ 300	5600 $\pm$ 800	—
Fibre type IIA/I area ratio	1.14 $\pm$ 0.05*	0.93 $\pm$ 0.04*	1.14 $\pm$ 0.09	1.08 $\pm$ 0.04	—

*Significant differences ($P < 0.05$) between sampling occasions.

Vastus lateralis. PFK activity decreased by $32 \pm 7\%$ during training. No other change in enzyme activities or the LDH/GAPDH-, LDH/PFK- and the GAPDH/PFK-activity ratios was noted during training or detraining.

MUSCLE FIBRE AREAS

Mean fibre area in the TB and VL did not change significantly during training or detraining (Fig. 4, Table 3). The only significant change in fibre type area was a 20% decrease in the type IIA area of the TB between the 6th and 33rd week of detraining. However, when the TB and VL were pooled, a significant decrease in type IIA areas of 12%, but not of the type I areas, was noted during the first 3 weeks of training. This resulted in a significantly decreased type IIA/I area ratio in the VL and, although non-significantly, in the TB of all subjects. During training, the range of the individual means for the fibre type areas narrowed in both type I and IIA in the TB and in type I in the VL. Expressed as the relative spread around the mean fibre type area, the range decreased by an average of 60%. The significance of these changes was tested with the F-test of variances. No significant difference in variance was noted, but in type I in the VL it tended to be smaller after training ($P < 0.1$). However, the F-test is insensitive with small materials. Since these changes were observed independently in both muscles, they most probably reflect significant changes.

Discussion

The observed changes in oxidative enzyme activities and capillary supply, in addition to the lack of change in glycolytic enzyme activities, are consistent with the findings of previous studies comprising training of shorter duration and higher intensity (for ref., see Introduction). These changes were seen in the previously untrained muscle (TB), but not in the trained muscle (VL), suggesting that increased exercise duration/week was not alone enough for an adaptive response.

Enzyme activities. The CS and MDH activity increased in parallel during the first 3 weeks of training with no further increase in MDH-activity and thus decreased

MDH/CS ratio during the latter part of training (Fig. 5). Parallel changes in MDH and CS have not been reported in studies of shorter duration and more trained muscles (Holloszy *et al.*, 1970, 1975; Benzi *et al.*, 1977). The latter studies have shown that the increase in MDH-activity with training normally is less than in other TCA cycle enzymes.

The MDH assay used includes both mitochondrial and cytoplasmic MDH. Both fractions catalyze reactions in the malate-aspartate shuttle. Holloszy *et al.* (1975) have provided evidence showing that the malate-aspartate shuttle is the only shuttle system whose capacity may increase in response to endurance training. An explanation to the present apparently two-phase adaptation with respect to the MDH/CS activity ratio may therefore be that the energy-yielding processes were initially highly dependent on 'aerobic glycolysis' and, thus, on a high malate-aspartate shuttle capacity, whereas fat oxidation played a greater role at a later stage of the adaptation, resulting in less dependence on this shuttle mechanism. This explanation is compatible with the observed time course of change in the fatty acid beta-oxidation marker HAD (Fig. 3).

Muscle fibre areas. The decreased variation in fibre areas during training is consistent with a study on extensive bicycling by Gollnick *et al.* (1973). Less variation in fibre areas was also noted in endurance trained subjects compared to untrained subjects (Brodal, Ingier & Hermansen, 1977). It may indicate the existence of optimal fibre type areas for endurance exercise. The selective decrease in fibre type IIA area during the first 3 weeks of training coincided with a period of energy deficit (see Methods). Energy

Fig. 5. Changes in MDH/CS activity ratio in m. triceps brachii (TB) and m. quadriceps femoris, vastus lateralis (VL), during training and detraining. b above VL values at w.0 and w.14 indicate significant differences compared to the corresponding TB values. (Other designations of significance as described in the legend for Fig. 1.)

deficiency has been shown to selectively affect the fast-twitch fibres (Goldspink & Ward, 1979). Thus, energy deficiency may have been the cause of the present changes in type IIA area.

Capillarization. Klausen *et al.* (1981) found that both capillary supply and enzyme activities reverted to pretraining levels within 8 weeks of detraining. The present results indicate, however, a significantly ($P < 0.05$) slower rate of retrogression of capillaries per fibre, as compared to CS and HAD activities. During the first 6 weeks of detraining, the relative rate of retrogression were $14 \pm 7\%$ ($\text{cap} \times \text{fibre}^{-1}$), $34 \pm 6\%$ (CS) and $29 \pm 3\%$ (HAD) respectively. After 6 weeks of detraining, the number of capillaries per fibre was still significantly higher ($26 \pm 8\%$) than the pretraining value, whereas CA and HAD activities were not. It should, however, be pointed out that the increases during training in CS (98%) and HAD activities (51%) were greater than the increase in the number of capillaries per fibre (40%), although this difference was not significant.

Acknowledgment

The authors gratefully acknowledge Ms Barbara Altmejd, Marlena Grynfarb, Ann-Chatrine Samuelsson, Anette von Schewelov and Berit Sjöberg for technical assistance as well as Ulla Siltberg and Anne Marie Gullström for secretarial assistance.

This study was supported by grants from the Swedish Medical Research Council (4554), the Research Council of the Swedish Sports Federation and from the Swedish National Defense Research Institute.

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