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CAPILLARY SUPPLY OF THE QUADRICEPS FEMORIS MUSCLE OF MAN: ADAPTIVE RESPONSE TO EXERCISE

BY PER ANDERSEN AND JAN HENRIKSSON
From the August Krogh Institute, University of Copenhagen, Copenhagen, Denmark

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SUMMARY

1. Five subjects trained for 8 weeks on a bicycle ergometer for an average of 40 min/day, four times a week at a work load requiring 80% of the maximal oxygen uptake ($\dot{V}_{O_2\,max.}$). $\dot{V}_{O_2\,max.}$ determinations were performed, and muscle biopsies from the quadriceps femoris muscle (vastus lateralis) were taken before, as well as repeatedly during, the training period. The muscle biopsies were histochemically stained for fibre-types (myofibrillar ATPase) and capillaries (amylase-PAS method), and analysed biochemically for succinate dehydrogenase and cytochrome oxidase activities.

2. The training programme resulted in a 16% increase in $\dot{V}_{O_2\,max.}$, a 20% increase in capillary density, a 20% increase in mean fibre area, and an approximately 40% increase in the activities of succinate dehydrogenase and cytochrome oxidase.

3. The capillary supply to type I, IIA and IIB fibres, expressed as the mean number of capillaries in contact with each fibre-type, relative to fibre-type area, increased equally.

4. The present study shows that endurance training constitutes a powerful stimulus for capillary proliferation in human skeletal muscle.

INTRODUCTION

Endurance training induces a marked increase in the activity levels of skeletal muscle oxidative enzymes, both in animals (see Holloszy & Booth, 1976) and in man (Varnauskas *et al.* 1970; Morgan *et al.* 1971; Gollnick *et al.* 1973; Henriksson & Reitman, 1977). In rat and guinea-pig muscle, capillary supply has also been reported to increase with endurance training (Petrén, Sjöstrand & Sylvén, 1937; Carrow, Brown & van Huss, 1967; Mai, Edgerton & Barnard, 1970), but in man the published data are somewhat conflicting. In a study by Saltin *et al.* (1968) no significant changes in capillary density were observed with inactivity (bed rest) and endurance

training. In agreement with this, Hermansen & Wachtlova (1971) found no difference in capillary density between untrained and endurance trained men. However, recent studies have shown both a higher capillary density in trained (compared to untrained) subjects and an increase in capillary density with endurance training (Andersen, 1975; Brodal, Ingjer & Hermansen, 1976).

In rabbit fast muscles, prolonged low frequency electrical stimulation has been shown to induce an increase in capillarization, preceding an increase in oxidative enzyme activities (Cotter *et al.* 1973). If this dissociation in the time course of changes also holds true in response to more physiological forms of training of human muscles, this could provide an opportunity to distinguish between the metabolic significance of these changes.

The purpose of the present investigation was to compare changes in human skeletal muscle capillarization, oxidative enzyme activities, and total body maximal oxygen uptake ($\dot{V}_{O_2}$ max.), not only before and after, but also during an 8-week period of endurance training. The five subjects studied were a part of a larger group studied for changes in $\dot{V}_{O_2}$ max., oxidative enzyme activities, and fibre-type distribution (Andersen & Henriksson, 1977; Henriksson & Reitman, 1977). The present paper focuses on changes in capillarization, but to permit a comparison of the time courses of the different adaptive changes, data on $\dot{V}_{O_2}$ max., oxidative enzyme activities and fibre-type distribution separately for this group of five subjects are also given.

METHODS

Five healthy men, aged 20 to 23 years (average 21), height 1·60 to 1·87 m (average 1·7) and wt. 57–80 kg (average 67·3), participated in the study. Body wt. hardly changed after the 8 weeks of training, being 58 to 81 kg (average 67·9). The subjects were all fully informed of the risks and discomfort associated with the experiments before they volunteered to participate. All had sedentary occupations and none had been engaged in any regular physical training during the preceding year.

The subjects trained for 8 weeks by pedalling a bicycle ergometer for an average of 40 min/day, 4 times per week. The work intensity was progressively increased during the training period to maintain a constant relative work load corresponding to 80 % of $\dot{V}_{O_2\,max.}$ All training sessions were supervised. Before, as well as repeatedly during the training period, $\dot{V}_{O_2\,max.}$ was determined and muscle biopsies (20–40 mg each) were taken from the quadriceps femoris muscle (vastus lateralis), 12–16 cm above the patella (Bergström, 1962). On testing days the subjects always came to the laboratory in the morning, after a light breakfast. All tests and biopsy sampling were performed at least 72 h after any exhaustive exercise.

Maximal oxygen uptake determinations. The $\dot{V}_{O_2\,max.}$ value was determined with the subjects pedalling a bicycle ergometer at 60 rev/min. Expired air was collected in Douglas bags and subsequently analysed using an I.R. CO_2-analyser (Beckman LB-1) and a paramagnetic O_2-analyser (Servomex OA 184). The reliability of these analyses was verified with the Scholander micro-technique.

Before training, at least three different $\dot{V}_{O_2\,max.}$ determinations were made on each subject, using work loads demanding an oxygen uptake slightly under, at, and slightly above the expected $\dot{V}_{O_2\,max.}$ value. When tested during the training period, the subjects always started to work at the highest load that had elicited $\dot{V}_{O_2\,max.}$ in the preceding test. If the subject was able to continue for more than 5 min at this load, the work load was increased to ensure that the actual $\dot{V}_{O_2\,max.}$ was reached.

Biopsy sampling. On each testing day two muscle biopsies were taken, one for biochemical and one for histochemical analysis. Before the training period biopsies were obtained before as well as after the 3–4 days of $\dot{V}_{O_2\,max.}$ determinations. As there were only small and unsystematic differences in enzyme activities and capillarization on these two occasions, the average values were used as pre-training values.

Histochemical methods. The samples were mounted in an embedding medium (Ames™ O.C.T. Compound), frozen in isopentane, cooled in liquid nitrogen, and stored at −80° C until analysis was performed. Serial transverse sections (10 μm) were cut with a microtome at −20° C and stained for myofibrillar ATPase activity (Gomori, 1941; Padykula & Herman, 1955) and with the amylase-PAS method to visualize capillaries (Andersen, 1975).

Sections to be stained for ATPase were incubated in a glycine buffer (pH 9·4) after acid (acetate buffer) or alkaline (glycine buffer) pre-incubation. To classify fibres into two main groups, type I and type II, and to identify type IIC fibres (Dubowitz & Brooke, 1973), pre-incubations at pH 4·4 (5 min, 21° C) and at pH 10·3 (8 min, 37° C) were used (Brooke & Kaiser, 1969). For classification into the subgroups IIA and IIB (Brooke & Kaiser, 1970) pre-incubations at both pH 4·6 (50 sec, 21° C) and pH 4·8 (5 min, 21° C) were used, to ensure the best possible differentiation between the subgroups.

In spite of this precaution a small number of type II fibres had an intermediate staining intensity and could not with certainty be categorized as either type IIA or IIB. On the average, these fibres made up 7 % (range 0–21) of the total number of type II fibres (4 % (range 0–12) of total number of fibres), with no systematic variation during the course of the training period. Half of these fibres in each section were considered as type IIA and half as type IIB.

The sections stained with the amylase-PAS method were photographed and copied (magnification × 150). On the photograph, one or several areas (A), without artifacts produced by the sectioning and staining procedure and without connective tissue structures splitting up the section in different parts, were framed by following the cell borders. The area of A (Table 1) was measured by planimetry, and capillaries were identified by direct microscopy of the sections (magnification × 200–400). In case a capillary was cut longitudinally, it was counted as one at each cell junction.

Fibre areas. Fibre-type areas were measured on the photographs of sections of biopsies taken before, and at the end of the 8-week training programme, using a grid method (Edström & Torlegård, 1968–9). Each square of the grid was 3×3 mm^2. On average, 52 type I fibres (range 45–71), 51 type IIA fibres (range 50–56) and 35 type IIB fibres (range 14–50) evenly distributed in A were measured. The total area of A, calculated from mean area and number of each fibre-type, agreed within ± 4 % to the planimetrically determined area.

Mean fibre area for all sections was calculated as the area of A divided by the number of fibres in A.

The area measurements on sections stained with the amylase-PAS method give an approximately 5 % larger area than measurements on sections stained for ATPase or NADH-dehydrogenase (Andersen, Kroese & Bonde-Petersen, preliminary results unpublished).

TABLE 1. Changes in different variables during the training period. Mean and S.E. of mean quoted for five subjects. Significant differences from pre-training values are indicated (a: $P < 0.05$; b: $P < 0.01$)

Week no. ...		0	1	2	3	5	8
$\dot{V}_{O_2\,max.}$ (ml.kg^{-1}.min^{-1})	$\bar{x}$	49·0	48·8	51·3	53·7^b	53·6^b	56·6^b
	S.E.M.	1·11	0·86	1·39	0·96	1·13	1·66
Fibre-type distribution (general) (%)							
Type I	$\bar{x}$	39	37	44	40	43	42
	S.E.M.	2·1	1·9	1·2	1·3	4·4	2·2
Type IIA	$\bar{x}$	36	37	35	39	40	42^a
	S.E.M.	2·9	3·1	1·1	0·4	1·9	2·4
Type IIB	$\bar{x}$	20	23	18	18	14^a	13^a
	S.E.M.	1·6	1·3	2·0	3·0	1·2	1·5
No. of fibres counted	$\bar{x}$	1035	617	421	436	398	937
	S.E.M.	126	87	71	50	121	270
Area of A (mm^2)	$\bar{x}$	1·695	1·027	0·676	0·706	0·699	1·603
	S.E.M.	0·410	0·264	0·138	0·149	0·189	0·345
Fibre-type distribution (in A) (%)							
Type I	$\bar{x}$	42	41	43	41	41	45
	S.E.M.	2·9	0·9	1·0	2·9	4·0	2·3
Type IIA	$\bar{x}$	37	35	36	37	45	40
	S.E.M.	3·3	2·9	1·5	5·0	5·8	2·2
Type IIB	$\bar{x}$	18	20	15	18	11	12
	S.E.M.	2·0	2·2	3·1	4·3	0·4	1·4
No. of fibres in A	$\bar{x}$	421	235	152	155	153	328
	S.E.M.	114	49	37	34	44	78
Mean fibre area (μm^2)	$\bar{x}$	4150	4230	4720^a	4640	4870^b	5020^a
	S.E.M.	246	245	365	314	359	254
Cap.mm^{-2}	$\bar{x}$	329	343	355	346	376	395^b
	S.E.M.	11	22	19	25	26	16
Succinate dehydrogenase activity (μmol.g^{-1}.min^{-1})	$\bar{x}$	8·6	8·4	9·0	10·0	10·2	12·2^a
	S.E.M.	0·5	0·6	0·9	0·4	0·7	1·0
Cytochrome oxidase activity (μmol.g^{-1}.min^{-1})	$\bar{x}$	6·2	5·7	6·5	6·9	7·5	8·8^a
	S.E.M.	0·4	0·4	0·5	0·6	0·6	0·7
Cap.fibre^{-1}	$\bar{x}$	1·36	1·43	1·67	1·62	1·84^a	2·00^b
	S.E.M.	0·07	0·11	0·16	0·19	0·21	0·17

Calculations

Fibre-type distribution was expressed as the number of fibres of each type relative to the total number of fibres within the whole section (*general*) and within A. Fibre-type distribution in A was always representative of the general fibre-type distri-

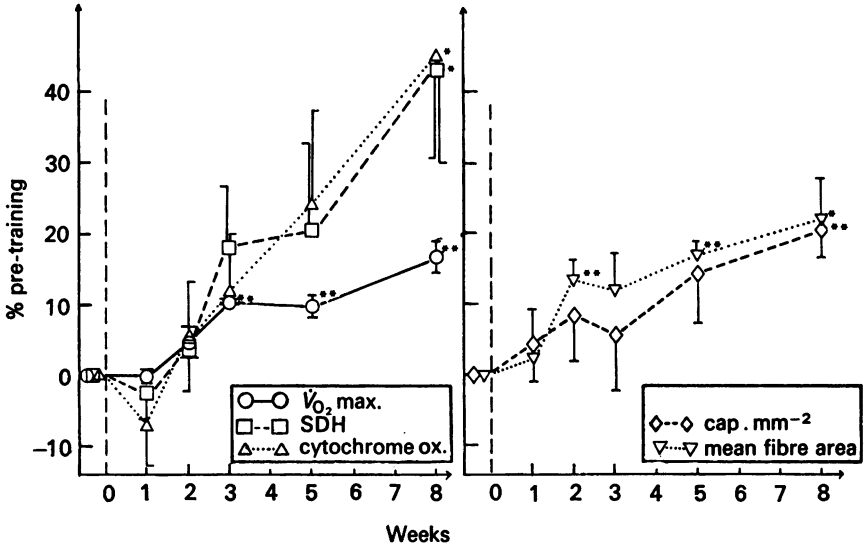
bution. The mean difference (*A*-general) in percentage of type I fibres was +1·1 % (range −7 to +7 %).

Capillary density (*cap.mm*$^{-2}$) was calculated as (the number of capillaries in *A*) × (the area of *A*)$^{-1}$. The number of capillaries in *A* included half the number of capillaries on the border line of *A*.

Capillaries per fibre (*cap.fibre*$^{-1}$) was calculated as (the number of capillaries in *A*) × (the number of fibres in *A*)$^{-1}$.

The mean number of capillaries in contact with fibres of each type (*CC*) was determined by counting all capillaries around each type I, IIA and IIB fibre in *A*. This was done only on biopsies taken before training and after 8 weeks of training. Furthermore, *CC relative to fibre-type area* was calculated for all three fibre-types.

Statistical methods. The significance of intra-individual differences was tested using the paired Student's *t*-test. Regression was calculated according to the method of the least squares.



Text-fig. 1. Time course of changes, relative to the pre-training values, for $\dot{V}_{O_2}$max., activities of succinate dehydrogenase and cytochrome oxidase, capillary density and mean fibre area during the training period. Mean and S.E. of mean is quoted for five subjects. Significant differences from pre-training values are indicated (* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$).

RESULTS

$\dot{V}_{O_2\ max.}$ The 8 weeks of training resulted in a 16 % increase in $\dot{V}_{O_2\ max.}$, from 49·0 ml.kg^{-1}.min^{-1} to 56·6 ml.kg^{-1}.min^{-1} ($P < 0{\cdot}01$; Table 1). The range for individual improvements was 10–27 %. The $\dot{V}_{O_2 max.}$ value was significantly different from the pre-training value after 3 weeks of training. The time course of the changes relative to the pre-training values is given in Text-fig. 1.

Fibre-type distribution. Before training, the fibre-type distribution was

39 % type I, 36 % type IIA and 20 % type IIB. The percentage of type I fibres was unchanged with training. However, the percentage of type IIA fibres increased, and was accompanied by a corresponding fall in the percentage of type IIB fibres ($P < 0.05$; Table 1). The percentage of type IIC fibres did not change significantly with training (4·7 % before *vs.* 2·1 % after). Because of the small number of these fibres, no further reference to them will be made.

TABLE 2. Fibre areas, no. of capillaries in contact with each fibre (CC) and CC relative to fibre area before and after the training period. Mean and S.E. of mean is quoted for five subjects. Significant differences, 0 *vs.* 8 weeks, and between fibre-types are indicated (*a*: $P < 0.05$; *b*: $P < 0.01$; *c*: $P < 0.001$)

Week no. ...		0	8
Fibre area (μm^2)			
Type I	$\bar{x}$	3880	4640
	S.E.M.	357	202
Type IIA	$\bar{x}$	4950	5780[a]
	S.E.M.	255	428
Type IIB	$\bar{x}$	3590	4590[b]
	S.E.M.	314	436
		IIA > I[a] IIA > IIB[b]	IIA > IIB[b]
No. of capillaries in contact with each fibre (CC)			
Type I	$\bar{x}$	3·9	5·4[a]
	S.E.M.	0·18	0·32
Type IIA	$\bar{x}$	4·2	5·5[a]
	S.E.M.	0·20	0·45
Type IIB	$\bar{x}$	3·0	4·2[a]
	S.E.M.	0·22	0·50
		I > IIB[a] IIA > IIB[c]	I > IIB[b] IIA > IIB[c]
CC relative to fibre area ($\mu m^{-2}.10^{-3}$)			
Type I	$\bar{x}$	1·03	1·16[a]
	S.E.M.	0·06	0·06
Type IIA	$\bar{x}$	0·86	0·95[a]
	S.E.M.	0·04	0·02
Type IIB	$\bar{x}$	0·84	0·92
	S.E.M.	0·02	0·03
		I > IIA[a] I > IIB[a]	I > IIA[a] I > IIB[a]

Fibre areas. Mean area of fibres was 4150 μm^2 before and 5020 μm^2 after training ($P < 0.05$; Table 1). This increase was due to an increase in the area of all three fibre-types (Table 2). The fibre areas (μm^2) before

and after training, respectively, were for type I 3880 and 4640 (+20% N.S.), type IIA 4950 and 5780 (+17%, $P < 0.05$), and type IIB 3590 and 4590 (+28%, $P < 0.01$). Mean fibre area was significantly increased after 2 weeks of training (Table 1; Text-fig. 1). The time course of the changes, expressed relative to the pre-training values, is illustrated in Text-fig. 1. Before training, the area of type IIA fibres was 1·28 times that of type I ($P < 0.05$) and 1·38 times that of type IIB fibres ($P < 0.01$). After training, the corresponding factors were 1·25 (N.S.) and 1·26 ($P < 0.01$).

Capillaries per fibre increased from 1·36 to 2·00 ($P < 0.01$) during the 8 weeks of training and was significantly different from the pre-training value after 5 weeks of training (Table 1).

Capillary density was 329 cap.mm^{-2} before training, and increased gradually during the training period to be significantly different from the pre-training value after 8 weeks of training (395 cap.mm^{-2}; $P < 0.01$) (Table 1; Text-fig. 1; Pl. 1).

The mean number of capillaries in contact with fibres of each type (*CC*) increased with training for all three fibre-types (Table 2), from 3·9 to 5·4 (type I), 4·2 to 5·5 (type IIA), and 3·0 to 4·2 (type IIB) ($P < 0.05$). Before training, CC for type IIB was 0·77 times that of type I ($P < 0.05$) and 0·71 times that of type IIA ($P < 0.001$). After training, the corresponding factors were 0·78 ($P < 0.01$) and 0·76 ($P < 0.001$), respectively. There was no difference between CC for type I and type IIA fibres.

CC relative to fibre-type area ($\mu m^{-2}.10^{-3}$) increased by approximately 10% for all three fibre-types as a result of the training. The values before and after training were 1·03 and 1·16 for type I ($P < 0.05$), 0·86 and 0·95 for type IIA ($P < 0.05$) and 0·84 and 0·92 for type IIB (N.S.) (Table 2). CC relative to fibre-type area for type I was always greater than the corresponding values for type IIA and IIB fibres ($P < 0.05$).

Oxidative enzyme activities. Both succinate dehydrogenase (E.C. 1.3.99.1) and cytochrome oxidase (E.C. 1.9.3.1) activities for the five subjects increased gradually during the training period and were approximately 40% above the pre-training levels after 8 weeks of training ($P < 0.05$) (Table 1 and Text-fig. 1).

DISCUSSION

How to express capillary supply

A complete description of capillary supply to a tissue can only be given in a three-dimensional system. However, since the capillary arrangement in mammalian muscle is, in general, quite regular, with capillaries running parallel to the muscle fibres (Krogh, 1918–19), it is possible to quantify the capillary supply from counts made on transverse sections (Spalteholz,

1888; Krogh, 1918–19; Schmidt-Nielsen & Pennycuik, 1961; Hermansen & Wachtlova, 1971; Andersen, 1975). For this purpose, several variables have been used: cap.mm^{-2} (Krogh, 1918–19; Petrén *et al.* 1937), cap.fibre^{-1} (Martin, Wooley & Miller, 1932; Smith & Giovacchini, 1956) and number of capillaries around fibres (Valdivia, 1958; Romanul, 1965). As discussed below, none of these variables alone will provide sufficient information with regard to capacities of diffusion and heat transport.

The variable *cap.mm*$^{-2}$ will vary with artifactual changes in area produced by the preparative technique used. Thus, the absolute values do not necessarily reflect the *in vivo* conditions. Different methods have given markedly different results, even if the same species and muscle group has been studied (for human quadriceps muscle, see Saltin *et al.* 1968; Hermansen & Wachtlova, 1971; Andersen, 1975). By use of the same method, however, cap.mm^{-2} tends to give a good indication of inter- and intra-individual differences in the average distance of diffusion, provided the muscle groups examined have a similar capillary arrangement, and not too great differences in fibre area.

With the variable *cap.fibre*$^{-1}$, variations in areas due to the preparation technique are eliminated, and therefore it has been used extensively in the literature to compare results from different studies (see Plyley & Groom, 1975). An increase in cap.fibre^{-1} reflects formation of new capillaries. The disadvantage of the variable is, however, that it provides no information as to the diffusion conditions. Therefore, for comparative purposes, mean fibre area must also be taken into consideration.

The two variables mentioned above only describe the muscle as a whole, and do not take into account differences in capillary supply to fibres of different types. For muscles with a mixed distribution of fibre-types, such as most human muscles, counts of the number of capillaries in contact with fibres of different types (CC) can be used to describe capillary supply to different fibre-types.

In counts of CC, capillaries are counted more than once, as belonging equally to the surrounding fibres, in case of differences in type and size of these fibres. CC can be subjected to the same criticism as cap.fibre^{-1} since it does not take into account the size of the different fibre-types. This can be achieved by calculation of *CC relative to the fibre-type area.* However, when using this variable alone in the evaluation of diffusion capacities for the different fibre-types, possible differences in the distribution of the metabolic processes within the fibres are not taken into consideration. Another problem may be a slight over-estimate of the diffusion capacities of large fibres, since a proportionally high number of capillaries around a fibre cannot compensate completely for the longer diffusion distance to the central part of the fibre. Moreover, this variable

has the general limitations of an area-dependent variable (see cap.mm^{-2}), which, however, can be disregarded if relative differences between fibre-types are studied.

In summary, no single variable adequately describes diffusion and heat transport capacities of the capillary network, since it will inherently be influenced by changes in area due to the preparative technique used. Furthermore, no variable characterizes capillary supply both to the muscle as a whole and to the different fibre-types. Consequently, it is recommended to use both cap.mm^{-2} and CC relative to fibre-type area, together with mean fibre area and the area of each fibre-type to permit correction for artifactitious area changes.

Capillarization and training

The training programme resulted in a considerable proliferation of the capillary network, as revealed by the pronounced increase (50 %) in cap.fibre^{-1}. This formation of new capillaries exceeded the 20 % increment in mean fibre area, and consequently capillary density, indicative of capillary supply to muscle as a whole, increased with the endurance training. It is noticeable that the enhanced capillarization was not restricted to a specific fibre-type, since CC relative to fibre-type area increased equally (10–13 %) for both type I, IIA and IIB fibres. Here it should, however, be pointed out that an increased number of capillaries around one fibre-type will inherently result in an increase in the number of capillaries also around the other fibre-types, as the fibre-types are distributed in a mixed fashion within the muscle.

Stimuli for the capillary proliferation

At present, a discussion of the factors initiating the formation of new capillaries can only be speculative (see Myrhage & Hudlická, 1977). It is conceivable that an interplay between a high capillary blood flow and a recurrent local mechanical compression of capillaries second to muscle contraction brings about a high local strain on the capillary walls, which either through an injury, or merely a distension of the capillary wall, might induce proliferative processes. Other possible triggering factors may be blood-borne substances, such as hormones, or metabolites produced by muscle cells during contraction.

Metabolic significance of increased capillary density

Maximal exercise. The time course of the increase in capillary density resembled that of $\dot{V}_{O_2}$max., whereas succinate dehydrogenase and cytochrome oxidase activities, in the latter part of the training period, appeared to increase more rapidly (Text-fig. 1). A causal relationship between

capillary density and $\dot{V}_{O_2 max.}$ relative to body weight could therefore be assumed, although this interpretation at first sight seems contradicted by a correlation coefficient as low as 0·44 between the two variables calculated from all paired values of the present study. This low correlation coefficient may, however, be explained partly by the methodological error in determining capillary density, and partly by different ratios for the different subjects between maximal oxygen uptake per kg body weight during bicycling and the oxygen uptake per weight unit of the lateral part of the quadriceps femoris muscle during these conditions. The latter is due mainly to different body compositions of the subjects, with corresponding differences in the percentage of the body weight constituted by the quadriceps muscle. The low correlation coefficient may, on the other hand, also indicate that the maximal oxygen uptake of this muscle is, in fact, not reached during these circumstances.

Submaximal exercise. During work at submaximal intensities the increased capillary density could be of importance by enabling a better distribution of blood flow to a local demand. This mechanism could, for example, enhance transport of free fatty acids by shortening the diffusion distance, and thus, together with an increased enzymatic oxidative capacity, contribute to the increased fat (relative to carbohydrate) metabolism observed in endurance trained subjects (Christensen & Hansen, 1939; Hermansen, Hultman & Saltin, 1967), and in the trained leg after one-legged endurance training (Henriksson, 1977).

Capillary supply of different fibre-types

The reported difference in oxidative capacity between the fibre-types (Dubowitz & Brooke, 1973; Essén *et al.* 1975) was not found to be accompanied by a similar difference in the number of capillaries around the fibres (CC), in contrast to what has been reported by Romanul (1965). However, the difference in oxidative capacity should rather be expected to be matched by differences in CC relative to fibre-type area. Accordingly, the 50 % higher oxidative potential of type I (compared to type II) fibres (Essén *et al.* 1975; Henriksson & Reitman, 1976) is accompanied by a 20 % difference in CC relative to fibre-type area. On the other hand, the higher oxidative potential in type IIA relative to IIB fibres (Essén *et al.* 1975) is not paralleled by a similar difference in capillary supply.

Fibre hypertrophy

Evidently, characterization of subjects as untrained with respect to aerobic work capacity does not necessarily imply a corresponding untrained state with respect to strength. Therefore no consistent pattern regarding the effect of endurance training at submaximal intensities on

fibre areas can be expected. In cross-sectional studies of endurance-trained male athletes, Hermansen & Wachtlova (1971), Gollnick *et al.* (1972), Andersen (1975) and Prince, Hikida & Hagerman (1976) reported *increased*, Edström & Ekblom (1972) and Brodal *et al.* (1976) *unchanged* and Jansson & Kaijser (1977) *decreased* fibre areas in the lateral part of the quadriceps femoris muscle, when compared to controls. Costill *et al.* (1976) found increased fibre areas in the lateral head of the gastrocnemius muscle in five distance runners. In these studies, both type I and II fibres appeared to vary in parallel. In a longitudinal study by Gollnick *et al.* (1973), 5 months of bicycle endurance training at a similar intensity as that used in the present study resulted in a 20 % increase in type I area, whereas no change was observed in type II area. In the present study, a marked hypertrophy of all three fibre-types occurred.

If active tension development is the critical event in initiating growth of fibres, this implies that fibres of all three types have been activated during the training sessions. Although it is the general view that there is a primary reliance upon type I fibres during submaximal exercise (Gollnick, Piehl & Saltin, 1974), a recruitment also of type IIA and IIB fibres has been observed at a work intensity demanding 85 % of $\dot{V}_{O_2 max.}$ (Andersen & Sjøgaard, 1975). Even if the relative workload was slightly lower during the training sessions in the present study, the observed conversion of type IIB into IIA fibres supports the idea of a recruitment of all three fibre-types. As type I, IIA and IIB fibres, at these work intensities, are not activated to the same extent, the similar degree of hypertrophy of all three fibre-types therefore indicate a different sensitivity to activation. This might be explained by initial differences in the 'training state' of the individual fibre-types, combined with different sensitivity due to qualitative differences between the fibre-types. An alternative explanation for the hypertrophy of all fibre-types could be that, in addition, passive stretch of the fibres during the bicycling might act as a stimulus for fibre hypertrophy (see Goldberg, Etlinger, Goldspink & Jablecki, 1975).

In conclusion, capillarization of the human quadriceps femoris muscle has been shown to increase with training at a heavy submaximal work intensity. The time course of this change was similar to that of $\dot{V}_{O_2 max.}$, whereas the activity of oxidative enzymes appeared to increase more rapidly. The capillary supply increased to the same extent for all three fibre-types. Furthermore, the training programme induced hypertrophy of both type I, IIA and IIB fibres.

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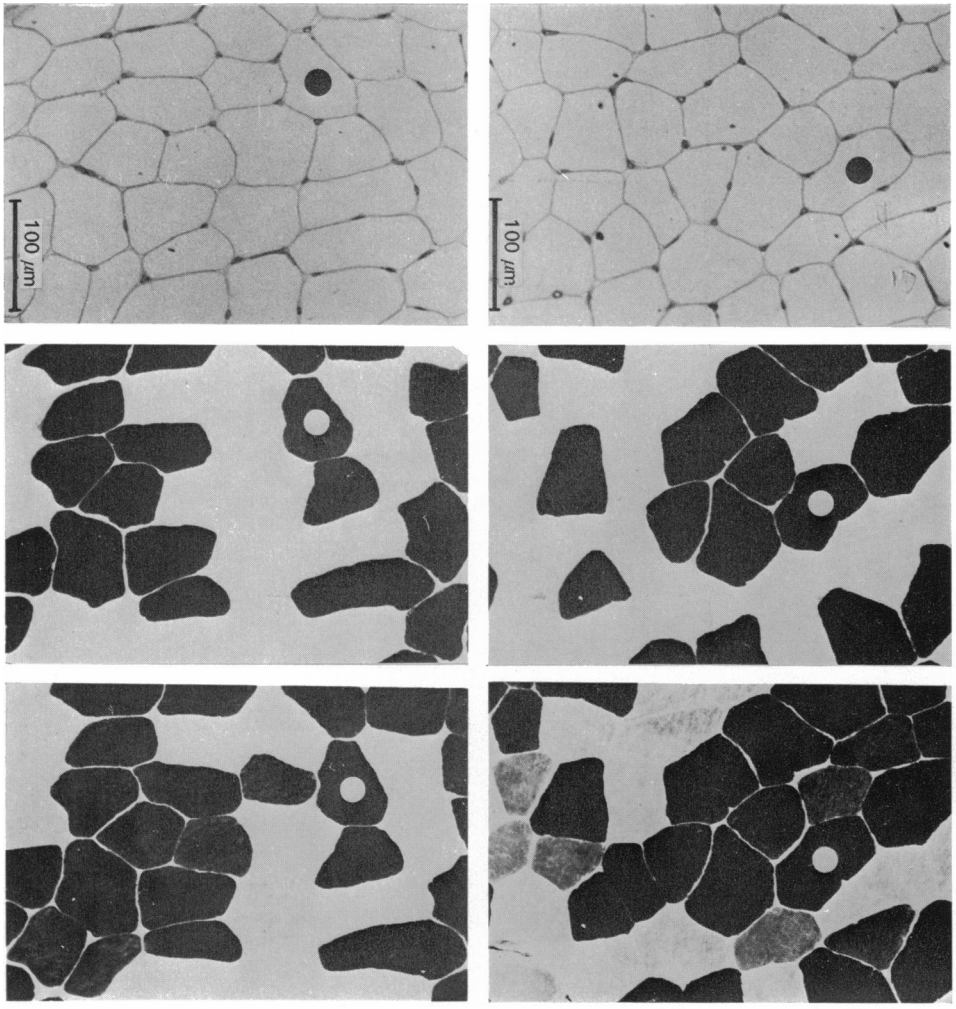
EXPLANATION OF PLATE

Photomicrographs of transverse sections of muscle biopsies taken before (left panel) and after 8 weeks of endurance training (right panel) from the same subject. From the top to the bottom: sections stained for capillaries (amylase-PAS method), for myofibrillar ATPase after pre-incubation at pH 4·4 (type I fibres darkly stained) and for myofibrillar ATPase after preincubation at pH 4·8 (type I and IIB fibres darkly stained). Spot for identification of the same fibre in the three sections, before and after training, respectively.



Before training After training