J. Physiol. (1979), **294**, *pp.* 419–432
Printed in Great Britain

EFFECTS OF ENDURANCE TRAINING ON MUSCLE FIBRE ATP-ASE ACTIVITY, CAPILLARY SUPPLY AND MITOCHONDRIAL CONTENT IN MAN

BY FRANK INGJER

From the Laboratory of Physiology, Norwegian College of Physical Education and Sport, Oslo, and Anatomical Institute, University of Oslo, Norway

(*Received 27 October 1978*)

SUMMARY

Seven young females were subjected to 24 weeks of intensive endurance training. Adaptive changes in myofibrillary ATP-ase activity, capillary supply and mitochondrial content were investigated with light- and electron microscopy in needle biopsies from the quadriceps femoris.

1. The average value for the maximal oxygen uptake increased from 45·7 to 57·2 (ml. kg^{-1} min^{-1}) (25·2%, $P < 0·005$).

2. The average number of capillaries per muscle fibre increased from 1·39 to 1·79 (28·8%, $P < 0·005$). Since no significant change in fibre area was found, this suggests that a considerable number of new capillaries have been formed during the training period.

3. An increased capillary supply of all fibre types was found, being greatest for type I and smallest for type IIB.

4. The relative amount of type I fibres before and after the training period was 57·9 and 56·5% respectively (n.s.), for type IIA fibres 26·4 and 31·5% ($P < 0·005$), for type IIB fibres 9·2 and 3·4% ($P < 0·005$) and for type IIC fibres 0·4 and 2·2% ($P < 0·005$). Thus, in the type II group, significant changes in subtypes take place during the endurance training. The data suggest that type IIAB may represent a transitional state between type IIA and IIB.

5. Correlation of capillary supply, myofibrillar ATP-ase activity and mitochondrial content (determined semiquantitatively) of individual muscle fibres indicates that the capillary supply to a given fibre is more closely related to its mitochondrial content than to the fibre type as determined on the basis of myofibrillar ATP-ase activity.

INTRODUCTION

The effects of endurance training on myofibrillar ATP-ase activity, mitochondrial content and capillary supply of human muscle fibres have been investigated in cross-sectional studies comparing data from trained athletes and untrained subjects (e.g. Edström & Ekblom, 1972; Hoppeler, Lüthi, Claassen, Weibel & Howard, 1973; Howald, 1975; Costill, Daniels, Evans, Fink, Krahenbuhl & Saltin, 1976; Brodal, Ingjer & Hermansen, 1977; Ingjer, 1978*a*). Although these studies indicate that training leads to an increase in the factors mentioned, it is impossible to decide with

0022-3751/79/4900-0807 $01.50

certainty whether the observed differences depend on the effect of training or on genetic differences. This problem can only be solved by longitudinal studies. Unfortunately, differences between data obtained before and after training in the same subjects are usually small, and the extreme values often found in elite athletes are seldom or never obtained during a relatively short training period.

In the few published longitudinal studies investigating the effects of endurance training on the capillary supply and muscle fibre type distribution in human muscle, most of the training programs have consisted of pedalling a bicycle ergometer 3–4 times a week for not more than 7–8 weeks (e.g. Andersen, 1975; Andersen & Henriksson, 1977*a*, *b*). The resulting changes have therefore often been relatively small. Increase in mitochondrial content in human muscle fibres during endurance training has been reported earlier (Morgan, Cobb, Short, Ross & Gunn, 1971; Kiessling, Piehl & Lundquist, 1971), but much less is known about this increase in relation to the different muscle fibre types (Bylund, Bjurö, Cederblad, Holm, Lundholm, Sjöström, Angquist & Schersten, 1977). Therefore, further data from longitudinal studies in this field are needed and especially from studies with longer training periods and other types of endurance training programs than bicycling.

The present investigation was undertaken to study the effects of 24 weeks of intense cross-country running on the myofibrillar ATP-ase activity, capillary supply and mitochondrial content of individual muscle fibres in seven previously untrained female volunteers.

In order to ensure as exact quantitative data as possible, the capillary supply and mitochondrial content of the muscle fibres was determined with the electron microscope (Brodal *et al.* 1977).

METHODS

The methods used have been published elsewhere (Brodal *et al.* 1977; Grønnerød, Dahl & Vaage, 1977; Ingjer, 1977; Ingjer, 1978*a*) and these studies shoud be consulted for particulars and discussion of their validity.

The subjects were fifteen healthy females 20–23 years of age. They had not been engaged in any regular endurance training during the last 5 years (Table 1). Some of the subjects started very early to complain about the training intensity, and eight of them dropped out of the training programme after 7–9 weeks (Ingjer & Dahl, 1978). At the time the eight subjects discontinued the training, no data other than the maximal oxygen uptake taken before the training started were known for any of the fifteen subjects. There was no significant difference in the pre-training oxygen uptake between the drop-outs as a group and the seven subjects that continued the whole training programme. This study concerns only the seven subjects who continued the whole training programme.

The subjects trained for 24 weeks by cross-country running for an average of 45 min a day, 3 times per week. The training consisted of 1 day a week with continuous submaximal work for the whole 45 min period, the work load ranging from 50 to 90% of maximal oxygen uptake depending on the terrain at the time. The two other days consisted of 15–20 min continuous running (warm-up) followed by intermittent training; 1 day with 3 work periods of 3–4 min continuous running, each separated by 4 min rest intervals, the other day with 2 periods of 6–9 min work consisting of 15 sec heavy work and 15 sec rest intervals. Both these intermittent training regimes were taxing the whole maximal oxygen uptake during the last part of each working period. The training sessions were supervised. The heart rate was taken frequently during the training in order to estimate and control the working intensity. The work load was progressively increased during the training period to maintain a constant intensity.

After being thoroughly familiarized with the procedures, all subjects gave their consent for the determination of maximal oxygen uptake and taking of muscle biopsies.

TABLE 1. Age, weight, maximal oxygen uptake, area of muscle fibres and number of capillaries per fibre and per mm² in the seven subjects before and after the training period

	Number of fibres	Age (yr)	Wt. (kg)	Maximal oxygen uptake (ml.kg^{-1} min^{-1})	Muscle fibre area (μm²)	Capillaries per fibre	Capillaries per mm²	Fibres per mm²
Before training [7]	1228	22 (20–23)	63·4 (55–76)	45·7 (41·4–50·6)	3561 ± 234	1·39 ± 0·06	348 ± 29	253 ± 32
After training [7]	1081	22 (21–24)	60·5 (51–71)	57·2 (53·7–62·4)	3755 ± 226	1·79 ± 0·08	438 ± 31	243 ± 23

Values are the mean for each state of training ± 1 S.E.
[ ], number of subjects.
( ), range.

TABLE 2. General muscle fibre type distribution in the seven subjects before and after the training programme

	Number of fibres		Type I		Type IIA		Type IIB		Type IIAB		Type IIC		Type IIAC	
Subjects	Before training	After training	B	A	B	A	A	B	A	A	B	A	B	A
1	786	830	66·9	67·5	17·8	19·7	9·4	2·3	5·3	9·0	0·6	1·5	0·0	0·0
2	828	818	58·9	56·1	33·1	38·3	3·5	0·0	4·5	3·2	0·0	2·1	0·0	0·4
3	810	800	50·9	46·9	38·0	43·4	5·4	4·6	4·0	2·6	1·8	2·4	0·0	0·0
4	844	467	63·7	60·5	14·7	23·3	15·3	5·3	6·2	9·4	0·1	1·5	0·0	0·0
5	799	855	50·8	52·3	26·0	34·4	12·0	0·1	11·1	13	0·0	0·1	0·1	0·0
6	827	952	63·2	61·2	28·1	32·2	3·5	0·0	5·3	2·7	0·0	3·6	0·0	0·3
7	801	847	51·3	51·1	27·6	29·2	15·5	11·4	5·5	4·5	0·0	3·9	0·1	0·0
Mean values	814	796	57·9	56·5	26·4	31·5	9·2	3·4	6·0	6·3	0·4	2·2	0·03	0·1

B, before training; A, after training.

Maximal oxygen uptake was measured before and within 3 days after the training period during running on a motor-driven treadmill at 3° uphill inclination according to the procedure of Åstrand & Rodahl (1970). Expired air was collected in Douglas bags and subsequently analysed using a direct reading paramagnetic oxygen analyzer (Beckman E2) and an indirect reading infra-red carbon dioxide analyzer (Beckman IR 215A). The accuracy of the analysis was verified with the Scholander technique (Scholander, 1957).

Needle biopsies (Bergström, 1962) were obtained from the lateral part of the quadriceps muscle (biopsies were taken from both legs, 10–15 cm above the proximal part of the patella) before and whtin 3 day after the training period, and immediately frozen in Freon 22 (Virginia Chemicals) cooled to the freezing point by liquid nitrogen. Transverse sections (10 μm) were cut in a cryostat at $\div$ 20 °C, and stained for myofibrillar ATP-ase after sequential preincubation in alkaline and acid buffers for fibre type classification (Grønnerød *et al.* 1977). Serial sections were subjected to different buffer combinations. Most often a buffer pH 10·3 containing 50 mM-$CaCl_2$ followed by fixation and buffer pH 4·6, and buffer pH 10·1 containing 20 mM-$CaCl_2$ followed by fixation and buffer pH 4·2 were used. An identical population of muscle fibres (e.g. 200–600) from two serial sections subjected to different combinations of alkaline and acid buffers was then photometrically evaluated to get a numerical estimate of the reaction intensity. Absorbance values for each fibre are plotted on a diagram giving rise to clusters of fibres with nearly similar reaction to the treatment. The fibre types as determined with this method conform with those of Brooke & Kaiser (1969, 1970) with somewhat better resolution. In addition to types I, IIA, IIB and IIC, some fibres show staining characteristics inbetween types IIA and IIB, and types IIA and IIC. These are called type IIAB and IIAC, respectively. All the biopsies from each subject were sectioned and stained on the same day and on the same slide in order to eliminate possible risks of day to day differences in the quality of the staining. The general muscle fibre type distribution was determine with the light microscope using on the average 814 muscle fibres before and 796 after the training period for each subject (Table 2). As a rule, sections from two or more biopsy samples from one person were examined in order to obtain a large enough number of fibres. Individual muscle fibre areas were measured using planimetry on drawings ($\times$ 315) of the sections stained for myofibrillar ATP-ase.

Determination of number of capillaries and fibres per mm^2 was performed in rectangular areas of the ultrathin sections (see below) closely packed with fibres (Brodal *et al.* 1977). To avoid uncertainties introduced by the large and variable shrinkage of the tissue processed for electron microscopy, the size of each area was measured in the neighbour cryostat section (mentioned above).

When an adequate histochemical classification of the muscle fibres had been obtained, the remaining part of each tissue sample was immersed in a cooled (+ 1 °C) alhehyde fixative and further processed for electron microscopy (Ingjer, 1977). Ultrathin sections were cut parallel to the previous transverse section surface using an LKB ultrotome, and a representative area (average 175 muscle fibres before and 154 after the training period, see Table 3) from each subject was examined for capillaries and amount of mitochondria in a Siemens Elmiskop as described by Brodal *et al.* (1977). The identity of the individual muscle fibres in the electron microscope was secured in comparison of semithin plastic sections, serial to the ultrathin section and the previous cryostat section (Ingjer, 1977). The determination of mitochondrial content was done semiquantitatively by classifying the fibres on the basis of number of subsarcolemmal mitochondrial aggregates into one of the following categories: M_1, no subsarcolemmal mitochondrial aggregates present; M_2, one or two aggregates; and M_3, three or more present. Within the area examined fibres an capillaries were counted in the electron microscope and after determining the size of the area light microscopically, the number of fibres and capillaries per mm^2 could be calculated. The Wilcoxon tests for non-parametric samples were used for testing the significance for differences in parameters both before and after, and from before to after the training.

TABLE 3. Muscle fibre distribution, fibre area, capillaries around each fibre (*CA*) and *CA* relative to fibre area in the seven subjects before and after the training period

	Before training (1228)				After training (1081)			
Fibre types	Fibre type distribution (%)	Fibre area (μm^2)	*CA*	*CA* relative to fibre area ($\mu m^{-2} \times 10^{-3}$)	Fibre type distribution (%)	Fibre area (μm^2)	*CA*	*CA* relative to fibre area ($\mu m^{-2} \times 10^{-3}$)
I	58·2	3827	4·11	1·07	57·7	3954	5·04	1·27
	± 2·8	± 179	± 0·15		± 2·9	± 250	± 0·21	
IIA	24·9	3427	3·40	0·99	31·6	3670	4·15	1·13
	± 2·6	± 365	± 0·16		± 2·7	± 190	± 0·21	
IIB	11·8	2764	2·33	0·84	2·7	2911	2·68	0·92
	± 2·7	± 277	± 0·19		± 2·5	± 299	± 0·14	
IIAB	4·4	2811	2·41	0·86	6·1	2707	2·63	0·97
	± 1·7	± 251	± 0·27		± 1·8	± 300	± 0·31	
IIC	0·8	3806	4·5	1·18	1·8	3888	5·33	1·37
	± 1·3	± 563	± 0·50		± 0·6	± 263	± 0·23	
IIAC	0				0·1			
M_3	4·5	3884	4·81	1·24	29·1	4011	5·31	1·32
	± 1·6	± 100	± 0·23		± 1·7	± 189	± 0·15	
M_2	36·6	3806	4·16	1·09	54·0	3822	4·51	1·18
	± 2·6	± 220	± 0·09		± 2·0	± 263	± 0·15	
M_1	58·9	3383	3·23	0·95	16·9	3103	3·34	1·08
	± 4·2	± 283	± 0·16		± 2·0	± 155	± 0·15	
Mean values	100	3561	3·64	1·02	100	3755	4·55	1·21
		± 234	± 0·13			± 226	± 0·17	

Values are the mean for each state of training ± 1 S.E.
( ), total number of fibres in each group.
CA, number of capillaries around each fibre.

RESULTS

Maximal oxygen uptake, muscle fibre area, capillaries per fibre and capillaries and fibres per mm²

The mean maximal oxygen uptake expressed in ml. kg^{-1} min^{-1} increased from 45·8 to 57·2 (25·2 %, $P < 0.005$), the mean number of capillaries per fibre from 1·39 to 1·79 (28·8 %, $P < 0.005$) and the mean number of capillaries per mm^2 from 348 to 438 (25·9 %, $P < 0.005$) during the 24 weeks of training (Table 1). The muscle fibre area and the number of fibres per mm^2 did not change significantly during the training period (Table 1).

Fibre type distribution

The average percentage of the type I fibres was 57·9 % before and 56·5 % after the training (n.s., Table 2). Neither did the percentage of type IIAB fibres change significantly during the training period. However, the percentage of type IIA and IIC increased significantly (from 26·4 and 0·4 % to 31·5 and 2·2 %, respectively, $P < 0.005$), while the percentage of type IIB decreased from 9·2 to 3·4 % ($P < 0.005$, Table 2). The type IIAC will not be discussed further in this study because of the small number of fibres belonging to this type.

Fibre area, capillaries around each fibre (CA) and CA relative to fibre area for the different fibre types

The data given in Table 3 is based upon the study of 168–265 fibres before and of 137–197 muscle fibres after the training for each subject. No significant increase in fibre area was found for any of the fibre types (Table 3), but both before and after the training period the type I fibres were larger ($P < 0.005$) than the type IIB and IIAB fibres. Although the type IIA fibres seem to be smaller than the type I fibres, the difference is not significant, either before or after the training.

The values for the mean number of capillaries around each fibre (*CA*) and the *CA* relative to the fibre area were significantly higher ($P < 0.01$) after the training than before for all fibre types. *CA* and *CA* relative to fibre area were highest both with regard to absolute values and in terms of increase during the training period for type I fibres (4·11–5·04 and 1·07–1·27) and type IIC fibres (4·50–5·33 and 1·18–1·37) (Table 3). There was a significant difference between type I, IIA and IIB fibres ($P < 0.005$) in *CA* and *CA* relative to fibre area both before and after training.

Table 3 shows that also when the nuscle fibres are classified into M_1, M_2 and M_3 groups the *CA* and *CA* relative to the fibre area are larger after than before training for all the three groups ($P < 0.01$). The highest capillarization values are found in the M_3 groups (4·81 and 1·24 before –5·31 and 1·32 after training, while the lowest capillarization values are found in the M_1 groups (3·23 and 0·95 before –3·34 and 1·08 after training). The difference in capillarization values between the M_1, M_2 and M_3 groups are significant ($P < 0.005$) both before and after the training. The average cross sectional fibre area was highest in the M_3 fibres and smallest in the M_1 fibres, both before and after training, but the difference was only significant ($P < 0.01$) between the M_1 and each of the two other groups (Table 3).

TABLE 4. Correlation of histochemical classification and classification based on mitochondrial content of identical muscle fibres in the seven subjects before and after the training period

	Fibre types (ATP-ase)*				
	I	IIA	IIB	IIAB	IIC
	Before training				
Mitochondrial content					
M_3	53 (96·4)	2 (3·6)	—	—	—
	[7·4]	[0·7]			
M_2	372 (82·7)	63 (14·0)	6 (1·3)	2 (0·4)	7 (1·6)
	[52·0]	[20·7]	[4·2]	[3·7]	[66·7]
M_1	290 (40·1)	240 (33·2)	138 (19·1)	52 (7·2)	3 (0·4)
	[40·6]	[78·7]	[95·8]	[96·3]	[33·3]
	After training				
Mitochondrial content					
M_3	258 (81·9)	50 (15·9)	—	1 (0·3)	6 (1·9)
	[41·4]	[14·6]		[1·5]	[30·0]
M_2	336 (57·3)	213 (36·5)	4 (0·7)	17 (2·9)	14 (2·4)
	[53·9]	[62·3]	[13·8]	[25·8]	[60·0]
M_1	30 (16·5)	79 (43·4)	25 (13·7)	48 (26·4)	—
	[4·8]	[23·1]	[86·2]	[72·7]	

( ), horizontal percentage distribution.

[ ], vertical percentage distribution.

* the fibres are classified on the basis of histochemical analyses (incubated for myosin adenosine triphosphatase) stained for myofibrillar ATP-ase.

† Fibres classified on the basis of number of subsarcolemmal mitochondrial aggregates.

Fibre types and mitochondrial content

There was a significant increase ($P < 0.005$) in the number of mitochondria-rich fibres during the training period (M_3, from 4·5 to 29·1 %). The percentage of M_2 fibres also increased during the training period (from 36·6 to 54 %, $P < 0.01$), while the percentage of fibres with very few mitochondria (M_1) decreased (from 58·9 % to 16·9 %, $P < 0.005$) (Table 3).

Table 4 shows the percentage distribution of the M_1, M_2 and M_3 fibres in the different fibre types (I, IIA, IIB, IIC and IIAB). On the average muscle fibre type I has the highest percentage of mitochondria-rich fibres (M_3+M_2), while type IIB has the lowest content of the M_3+M_2 fibres both before and after the training period. The vertical percentage distribution shows that for all fibre types a transition towards more mitochondria-rich fibres takes place during the training period. This increase in mitochondrial content is highest for type I and smallest for type IIB

DISCUSSION

Training and capillary supply in man

The present study clearly demonstrates a marked increase in capillary supply to skeletal muscle fibres during endurance training, in accordance with earlier longitudinal studies (Andersen, 1975; Andersen & Henriksson, 1977*a*). Thus, both the capillary per fibre ratio and the capillary density (per mm^2) were significantly increased (Table 1). A marked proliferation of capillaries therefore seems to be the most reasonable explanation for this, although it cannot be excluded that altered lengths and patterns of the capillaries may play a role (Appell & Hammersen, 1978).

In untrained females the mean number of capillaries around each fibre (*CA*) is significantly higher for type I fibres than for type IIA (Table 3), in contrast to in untrained males (Andersen & Henriksson, 1977*a*; Ingjer, 1978*a*), where the *CAs* for type I and IIA fibres are very similar. This sex difference may probably be explained by the fact that in untrained females type I fibres have the largest cross-sectional areas (Brooke & Engel, 1969; Nygaard, Bentzen, Houston, Larsen, Nielsen & Saltin, 1977; Table 3), while in untrained males the type IIA are the largest fibres (e.g. Ingjer, 1978*a*). The lowest values for *CA* and *CA* relative to fibre area were found in type IIB (Table 3). Nygaard *et al.* (1977), however, report in a short abstract much smaller differences in *CA* values between the fibre types in untrained females. They also found substantially larger *CA* and *CA* relative to fibre area for the IIB fibres (3·7 and 1·68, respectively) than those found here (2·33 and 0·84, Table 3). No reasonable explanations can be found for these large differences in results between the two studies.

The increase in *CA* and *CA* relative to fibre area during the training period was largest in type I and smallest in type IIB fibres, most probably reflecting the manner in which the muscles were used during the training.

An interesting observation in the present study is that the increase in number of capillaries per fibre and per mm^2 (28·8 and 25·9 %, respectively, Table 1) are of the same order of magnitude as the increase in maximal oxygen uptake (25·2 %) during

the training period. This finding is in accordance with results from some cross-sectional studies (Brodal *et al.* 1977; Ingjer, 1978*b*). On the other hand, Andersen (1975) and Andersen & Henricksson (1977*a*) in their 8 weeks longitudinal studies of bicycle training obtained only 13 and 16% increases in maximal oxygen uptake, while the number of capillaries increased by 40 and 47% (capillaries per fibre) and by 23 and 20% (capillaries per mm^2), respectively. Increased muscle fibre area found by Andersen & Henriksson (1977*a*) can presumably account for part of the differences between their results and the present ones. However, the large difference reported by Andersen (1975) between increase in maximal oxygen uptake and capillary supply cannot be explained in this way, since he found no increase of fibre area during training, but a more intensive and selective use of the quadriceps muscle during cycling than during running may be of importance.

In accordance with previous cross-sectional studies (e.g. Brodal *et al.* 1977; Ingjer & Brodal, 1978), the largest values for capillary supply were found in the mitochondria-rich (M_3) fibres and smallest in the M_1 fibres both before and after training (Table 3).

Fibre type distribution

The percentage of type I fibres (about 57%, Table 2) in the present group is higher than usually reported for normal populations of untrained female subjects (Hedberg & Jansson, 1976; Komi & Karlsson, 1978). Since athletes engaged in endurance sports usually have a higher proportion of type I fibres than untrained subjects (e.g. Jansson & Kaijser, 1977) it seems reasonable to assume that those volunteering in a training programme like the present find endurance training fairly attractive e.g. because their high percentage of type I fibres makes them more fit for this type of activity. This is supported by the fact that the seven subjects who completed the training programme had a higher average percentage of type I fibres than the drop-outs (58 and 47%, respectively) (Ingjer & Dahl, 1978). The average maximal oxygen uptake of the present subjects before the training period started is also higher than usually reported in untrained females (e.g. Costill *et al.* 1976; Komi & Karlsson, 1978), further indicating that they are more fit for endurance work than an average of females of the same age.

It is generally accepted that endurance training does not change the percentage distribution of the muscle fibre types when classified with the ATP-ase method only into two groups (I and II), (Barnard, Edgerton & Peter, 1970; Gollnick, Armstrong, Saltin, Saubert, Sembrowich & Shepherd, 1973). However, using a more refined ATP-ase method, subdividing the group II fibres into IIA, IIB, IIAB and IIC, the present study shows (Table 2) in agreement with Andersen & Henriksson (1977 *b*), that the distribution of type II fibres in subgroups may change within the same subject during a period of endurance training. There is a clear increase in the percentage of type IIA fibres (26·4–31·5%, Table 2) and a corresponding decrease in the percentage of type IIB fibres (9·2–3·4%). Although the manner in which this change takes place so far remains unknown, it is of interest that the type IIAB fibres (Grønnerød *et al.* 1977) have a size, capillary supply and mitochondrial content in-between the type IIA and IIB (Ingjer, 1978*a*; present study, Table 3), and this may

represent a transitional state between type IIA and IIB. The change in amount of type IIA and IIB fibres (Table 2) therefore seems to be due to a conversion of type IIB via IIAB to IIA fibres during the training period.

Although a few cross-sectional studies have reported relatively high numbers of type IIC muscle fibres in endurance trained subjects (e.g. Jansson & Kaijser, 1977), no previous longitudinal studies have reported any significant change in the number of IIC fibres with endurance training. The present results show, however, a significant increase in the percentage of IIC fibres (0·4 and 2·2 %, Table 2), although their absolute numbers are small before as well as after the training. Longer training period (24 weeks) and probably also higher intensity (taxing the whole aerobic power during the intervals) used in the present study as compared with earlier ones may explain this difference. At present it is not possible to decide where the new type IIC muscle fibres may come from, but they resemble type I fibres both in capillary supply, muscle fibre size, oxidative enzyme activity and mitochondrial content (e.g. Essén, Jansson, Henriksson, Taylor & Saltin, 1975; Ingjer, 1978*a*). This suggests that they are heavily engaged in endurance work rather than being undifferentiated fibres, as proposed by Dubowitz & Brooke (1973), or fibres related to increased rate of degenerative–regenerative processes in heavily used muscle fibres (Jansson & Kaijser, 1977) or fibres subjected to a sudden loss of functional contact with their motor nerve (Saltin, Henriksson, Nygaard, Andersen & Jansson, 1977). On the whole, the changes in the muscle types IIA, IIB and IIC shown to take place during intense endurance training may be regarded as meaningful adaptations, since they all lead to higher aerobic power, and therefore would contribute to improved performance in endurance activities.

Fibre types and mitochondrial content

Earlier longitudinal studies have shown that the mitochondrial content in the muscle fibres increases with endurance training (e.g. Morgan *et al.* 1971; Kiessling *et al.* 1971; Bylund *et al.* 1977). This is confirmed in the present study (Table 3). In addition by use of correlated light microscopic histochemistry and electron microscopy (Ingjer, 1977) it has been possible to determine mitochondrial content semi-quantitatively in individual fibres with known ATP-ase activity (Table 4). *All* fibre types are found to increase their mitochondrial content, but the type I fibres which from the beginning have the highest percentage of mitochondrion-rich fibres also increase this percentage more than the other fibre types during the training period (Table 4). Among type II fibres the percentage of mitochondria-rich fibres increased most for type IIA and least for type IIB. The increase in mitochondrial content and amount of mitochondria-rich fibres represent another part of the adaptive response leading to higher oxidative capacity in the trained muscles.

Factors related to capillary supply of muscle fibres

Previous studies (e.g. Andersen & Henriksson, 1977*a*; Ingjer, 1978*a*) as well as the present have shown large differences between the capillary supplies to the various muscle fibre types, both when classified on the basis of myofibrillar ATP-ase activity and on the basis of mitochondrial content (Table 3). Since both ATP-ase activity and

TABLE 5. Fibre area, number of capillaries around each fibre (*CA*) and *CA* relative to fibre area in the type I, IIA and IIB fibres belonging to the M_1 and M_3 fibres before and after training (♀) and in groups of untrained and endurance trained men

Fibre type combinations	Number of fibres	Fibre area (μm^2)	*CA*	*CA* relative to fibre area ($\mu m^{-2} \times 10^{-3}$)	Number of fibres	Fibre area (μm^2)	*CA*	*CA* relative fibre area ($\mu m^{-2} \times 10^{-3}$)
		Before training				After training		
I/M_3	53	3907 ± 154	4·85 ± 0·18	1·24	258	4043 ± 247	5·33 ± 0·16	1·32
I/M_1	290	3606 ± 256	3·33 ± 0·17	0·92	30	3527 ± 228	3·46 ± 0·27	0·98
IIA/M_3	2	—	—	—	50	3913 ± 114	5·07 ± 0·19	1·30
IIA/M_1	240	3411 ± 301	3·13 ± 0·19	0·92	79	3432 ± 189	3·31 ± 0·46	0·96
IIB/M_3	0	—	—	—	0	—	—	—
IIB/M_1	138	2767 ± 273	2·46 ± 0·21	0·89	25	2854 ± 202	2·65 ± 0·27	0·93
		Untrained men*				Endurance trained men*		
I/M_3	104	4400 ± 304	5·46 ± 0·36	1·24	280	4928 ± 552	8·32 ± 0·32	1·69
I/M_1	25	3879 ± 176	3·46 ± 0·39	0·89	10	4520 ± 912	4·72 ± 0·29	1·04
IIA/M_3	55	5270 ± 294	6·02 ± 0·30	1·14	17	5491 ± 545	8·61 ± 0·36	1·57
IIA/M_1	73	4223 ± 347	3·67 ± 0·16	0·86	37	5000 ± 708	5·34 ± 0·20	1·07
IIB/M_3	2	—	—	—	—	—	—	—
IIB/M_1	76	3923 ± 349	3·05 ± 0·31	0·78	27	4576 ± 380	4·68 ± 0·35	1·02

Values are the mean for each group ± 1 S.E.

* The values for untreated and endurance trained men are calculated from a previous study (Ingjer, 1978).

mitochondrial content as well as capillary supply are known for the individual muscle fibres in the present study, a correlation between these three factors can be made to elucidate the relative importance of the ATP-ase activity and the mitochondrial content for the capillary supply. This is shown in Table 5 where *CA* and *CA* relative to fibre area have been calculated for the muscle fibres in the M_1 and M_3 groups that belong to the type I, IIA and IIB. The same calculations have been performed on data from two male groups (Ingjer, 1978*a*). If the ATP-ase activity is the dominant factor for the determination of capillary supply, one would expect to find a large difference between capillary supply to fibres of types I, IIA and IIB, all belonging to the same M group (M_1 or M_3) and a very small difference between M_1 and M_3 fibres belonging to the same ATP-ase determined group. On the other hand, if the mitochondrial content is the dominant factor, one would expect to find exactly the reverse pattern of differences in capillary supply. Table 5, particularly the data from the females, clearly shows a strong relation between mitochondrial content and capillary supply, while the capillary supply shows no such clear relation to ATP-ase activity. Thus ATP-ase fibre type groups as defined by myofibrillar ATP-ase are not homogeneous with regard to mitochondrial content and capillary supply. Since the ultrastructural features of the muscle fibres have been studied in single ultrathin sections, it cannot be excluded that mitochdrial content and capillary supply may vary from place to place along a single fibre. However, the finding of subgroups of type I fibres by incubation for oxidative enzymes (Askanas & Engel, 1975) points toward metabolic heterogeneity in fibres with equal myofibrillar ATP-ase properties as a more probable explanation.

The mechanisms involved in controlling the capillary supply are not known, but the present findings further support the assumption (Ingjer, 1978*a*) that factors controlling the increase in mitochondrial content also take part in the control of the capillary supply to the various muscle fibres.

This study was supported by the Norwegian Sport Federation. The author is indebted to Dr S. Mæhlum and Dr Å. Ekroll for taking the biopsies, and to Dr P. Brodal and Dr H. A. Dahl for advice and help throughout this work.

REFERENCES

ANDERSEN, P. (1975). Capillary density in skeletal muscle of man. *Acta physiol. scand.* **95**, 203–205.

ANDERSEN, P. & HENRIKSSON, J. (1977*a*). Capillary supply of the qudriceps femoris muscle of man Adaptive response to exercise. *J. Physiol.* **270**. 677–690.

ANDERSEN, P. & HENRIKSSON, J. (1977*b*). Training induced changes in the subgroups of human type II skeletal muscle fibers. *Acta physiol. scand.* **99**, 123–125.

APPELL, H.-J. & HAMMERSEN, F. (1978). Capillary density and patterns in skeletal muscle II. The effect of the capillary patterns on capillary counts. *Pflügers Arch.* suppl. R60.

ASKANAS, V. & ENGEL, W. K. (1975). Distinct subtypes of type I fibres of human skeletal muscle. *Neurology* **25**, 879–887.

ÅSTRAND, P. O. & RODAHL, K. (1970). *Textbook of Work Physiology*, pp. 281–286 and 613–616. New York: McGraw-Hill.

BERGSTRÖM, J. (1962). Muscle electrolytes in man. *Scand. J. clin. Lab. Invest.* suppl. 68.

BARNARD, R. J., EDGERTON, V. R. & PETER, J. B. (1970). Effect of exercise on skeletal muscle. 1. Biochemical and histochemical properties. *J. appl. Physiol.* **28**, 762–766.

BRODAL, P., INGJER, F. & HERMANSEN, L. (1977). Capillary supply of skeletal muscle fibers in untrained and endurance trained men. *Am. J. Physiol.* **232**, H705–H712.

BROOKE, M. H. & ENGEL, W. K. (1969). The histographic analysis of human muscle biopsies with regard to fiber types. *Neurology* **19**, 221–233.

BROOKE, R. E. & KAISER, K. K. (1969). Some comments on the histochemical characterization of muscle adenosine triphorphatase. *J. Histochem. Cytochem.* **17** 431–432.

BROOKE, R. E. & KAISER, K. K. (1970). Three 'myosin ATPase' systems: The nature of their pH lability and sulfhydryl dependence. *J. Histochem. Cytochem.* **18**, 670–672.

BYLUND, A. C., BJURÖ, T., CEDERBLAD, G., HOLM, J., LUNDHOLM, K., SJÖSTRÖM, M., ÅNGQUIST, K. A. & SCHERSTEN, T. (1977). Physical training in man. Skeletal muscle metabolism in relation to muscle morphology and running ability. *Eur. J. appl. Physiol.* **36**, 151–169.

COSTILL, D. L., DANIELS, J., EVANS, W., FINK, W., KRAHENBUHL, G. & SALTIN, B. (1976). Skeletal muscle enzymes and fiber composition in male and female track ahtletes. *J. appl. Physiol.* **40**, 149–154.

DUBOWITZ, V. & BROOKE, M. H., (1973). Muscle biopsy: a modern approach. In *Major Problems in Neurology*, vol. 2. London: W. B. Saunders Co., Ltd.

EDSTRÖM, L. & EKBLOM, B. (1972). Differences in sizes of red and white fibers in vastus lateralis of musculus quadriceps femoris of normal individuals and athletes. Relation to physical performance. *Scand. J. clin. Lab. Invest.* **30**, 175–181.

ESSÉN, B., JANSSON, E., HENRIKSSON, J., TAYLOR, A. W. & SALTIN, B. (1975). Metabolic characteristics of fibre types in human skeletal muscles. *Acta physiol. scand.* **95**, 153–165.

GOLLNICK, P. D., ARMSTRONG, R. B., SALTIN, B., SAUBERT, C. W. IV, SEMBROWICH, L. & SHEPHERD, R. E. (1973). Effect of training on enzyme activity and fiber composition of human skeletal muscle. *J. appl. Physiol.* **34**, 107–111.

GRØNNERØD, O., DAHL, H. A. & VAAGE, O. (1977). Easy typing of human muscle fibers in sequentially preincubated myofibrillar ATP-ase sections. *Proc. Int. Union. Physiol. Sci.* **13**, 285.

HEDBERG, G. & JANSSON, E. (1976). Skelettmuskelfiberkomposition. Kapacitet och intresse för olika fysiska aktiviteter bland elever i gymnasieskolen. *Raport* **54** Pedagogiska Inst., Umeå: .

HOPPELER, H., LÜTHI, P., CLAASSEN, H., WEIBEL, E. R. & HOWARD, J. (1973). The ultrastructure of the normal human skeletal muscle. A morphometric analysis on untrained men, women and well-trained orienteers. *Plügers Arch*, **344**, 217–232.

HOWALD, H. (1975). Ultrastructural adaptation of skeletal muscle to prolonged physical exercise. In *Metabolic Adaptation to Prolonged Physical Exercise*, vol. 8, ed. HOWALD, H. & POOTMANS, J. R. Basel, Switzerland: Birkhäuser Verlag.

INGJER, F. (1977). A method for correlating ultrastructural and histochemical data from individual muscle fibers. *Histochemistry* **54**, 169–172.

INGJER, F. (1978*a*). Capillary supply and mitochondrial content of different skeletal muscle fiber types in untrained and endurance trained men. A histochemical and ultrastructural study. *Eur. J. appl. Physiol.* (in the Press).

INGJER, F. (1978*b*). Maximal aerobic power related to the capillary supply of the quadriceps femoris muscle in man. *Acta physiol. scand.* **104**, 238–240.

INGJER, F. & BRODAL, P. (1978). Capillary supply of skeletal muscle fibers in untrained and endurance trained women. *Eur. J. appl. Physiol.* **38**, 291–299.

INGJER, F. & DAHL, H. A. (1978). Drop-outs during an endurance training programme. Some histochemical and physiological aspects. *Scand. J. Sport and Sciences* (in the Press).

JANSSON, E. & KAIJSER, L. (1977). Muscle adaptation to extreme training in man. *Acta physiol. scand.* **100**, 315–324.

KIESSLING, K. H., PIEHL, K. & LUNDQUIST, C. G. (1971). Effect of physical training on ultrastructural features in human skeletal muscle. In *Muscle Metabolism During Exercise*, vol. 11, ed. PERNOW, B. & SALTIN, B. New York: Plenum.

KOMI, P. V. & KARLSSON, J. (1978). Skeletal muscle fibre types, enzyme activities and physical performance in young males and females. *Acta physiol. scand.* **103**, 210–218.

MORGAN, T. E., COBB, L. A., SHORT, F. A., ROSS, R. & GUNN, D. R. (1971). Effects of long-term exercise on human muscle mitochondria. In *Muscle Metabolism During Exercise*, vol. 11, ed. PERNOW, B. & SALTIN, B. New York: Plenum.

NYGAARD, E., BENTZEN, H., HOUSTON, M., LARSEN, H., NIELSEN, E. & SALTIN, B. (1977). Capillary supply and morphology of trained human skeletal muscle. *Proc. Int. Union. Physiol. Sci.* **13**, 557.

SALTIN, B., HENRIKSSON, J., NYGAARD, E., ANDERSEN, P. & JANSSON, E. (1977). Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners. *Ann. N.Y. Acad. Sci.* **301**, 3–29.

SCHOLANDER, P. F. (1947). Analyses for accurate estimation of respiratory gases in one-half cubic centimeters samples. *J. biol. Chem.* **167**, 235–250.