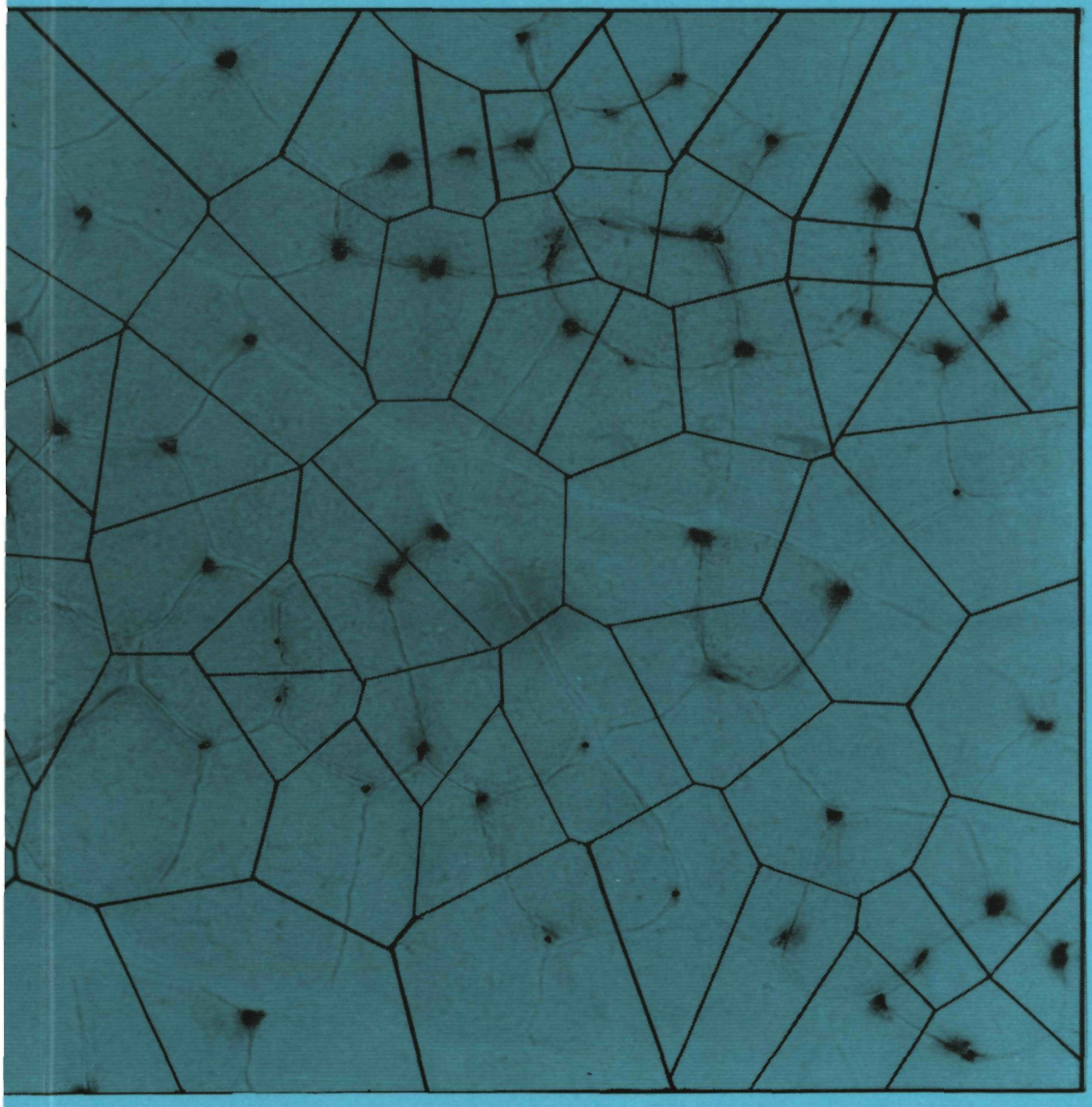
HYPERTROPHY IN THE M. PLANTARIS OF THE AGEING RAT

FUNCTIONING AND CAPILLARISATION RELATED TO FIBRE TYPES



H. DEGENS

HYPERTROPHY IN THE M. PLANTARIS OF THE AGEING RAT

FUNCTIONING AND CAPILLARISATION RELATED TO FIBRE TYPES

een wetenschappelijke proeve op het gebied van
de Medische Wetenschappen

Proefschrift

ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op dinsdag 9 maart 1993
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CONTENTS

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List of abbreviations

ANOVA: Analysis of Variance

AP: Alkaline Phosphatase

ATP: Adenosine 5'-TriPhosphate

BW: Body Weight

C: Control

CD: Capillary Density

CFD: Capillary Fibre Density

CK: Creatine Kinase

CK-NAC: Creatine Kinase N-acetyl-L-cysteine

CNT: Control Not Trained

CS: Citrate Synthase

CT: Control Trained

DPP-IV: Dipeptidyl Peptidase IV

C/F: Capillary-to-Fibre ratio

EDL: Extensor Digitorum Longus

EDTA: Ethylene Diamine Tetra Acetic Acid

ELISA: Enzyme Linked Immuno Sorbance Assay

FABP: Fatty Acid-Binding Protein

FAT: Fatigue Index

FCSA: Fibre Cross-sectional Area

FTET: Tetanic Force

FTW: Twitch Force

HADH: 3-Hydroxyacyl-CoA Dehydrogenase

HK: Hexokinase

LCFR: Local Capillary to Fibre Ratio

LogSD: Logarithmic standard deviation of the Domain areas i.e., SD of log-transformed variates, $\sigma \log_{10} x$ gives an indication of the heterogeneity of the capillary spacing

MW: Muscle Weight

NADH: Nicotinamide Adenine Dinucleotide

NADPH:	Nicotinamide Adenine Dinucleotide Phosphate
O:	Operated
ONT:	Operated Not Trained
OT:	Operated Trained
PHOS:	Phosphorylase
SD:	Standard Deviation
SDH:	Succinate DeHydrogenase
SEM:	Standard Error of the Mean
SR:	Sarcoplasmic Reticulum
Tris:	Tris hydroxyl ethyl amino methane
TPT:	Twitch Time to Peak Tension

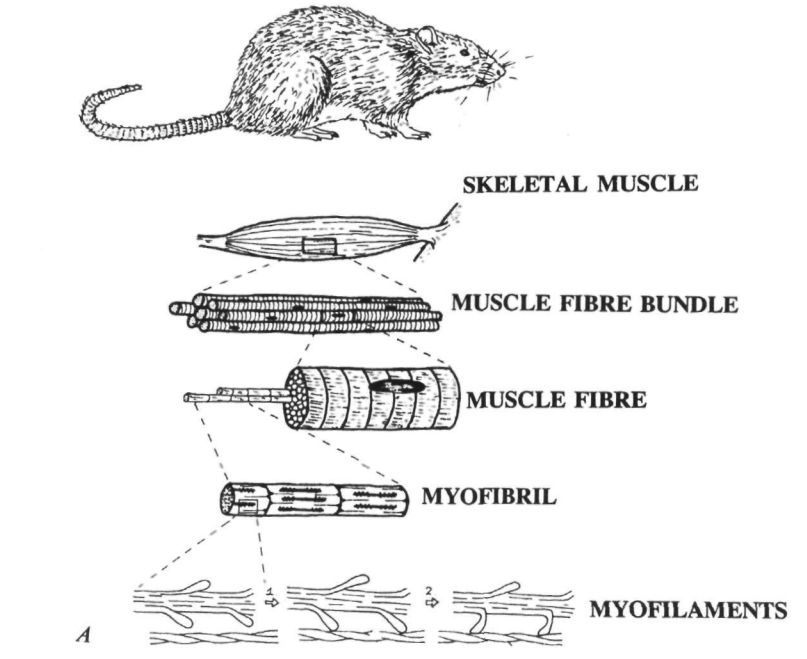
1. INTRODUCTION

1.1. The architecture of skeletal muscle

The principal functions of skeletal muscle are to move the body (locomotion) or body parts and to maintain body posture. Therefore the muscle has to be capable of shortening and producing force. The architecture is such that the muscle is able to optimally perform its function. In figure 1.1 it can be seen that a muscle is built up of muscle fibre bundles orientated in a longitudinal direction. Each muscle fibre bundle contains several muscle fibres and they in turn consist of myofibrils. The myofibril is composed of series sarcomeres and each sarcomere contains the myofilaments: actin and myosin. These filaments are the structures which cause the sarcomere to shorten by sliding along each other. This shortening is accompanied by force production and also gives the muscle the ability to move the body or body parts. Besides contractile structures, the muscle also contains non-contractile structures, such as connective tissue, blood vessels, etc.

1.2. How the muscle works

In the previous section, it is stated that force production takes place in the sarcomeres, when the filaments slide along each other. To gain a better understanding, we will describe the sarcomeres in more detail. In the myosin filament, a light and a heavy-meromyosin can be distinguished. The heavy meromyosin is the head of the myosin molecule and it is this part of the myosin molecule which can bind with the actin filament. The present concept is that after the myosin heads have bounded with the actin filaments, rotation of the myosin heads takes place. In this way the myosin heads pull the actin into the centre of the sarcomere. When the heads have finished their rotation, they might detach and bind to another place on the actin filament and pull it further by rotation towards the centre of the sarcomere. Tropomyosin and troponin on the actin filament, the enzyme myosin ATP-ase, as well as free Ca^{2+} play an important role in this process. The Ca^{2+} changes the configuration of tropomyosin by binding to troponin and thereby initiates the binding of the myosin heads to the actin filament. Myosin ATP-ase provides the energy necessary for contraction by splitting ATP into ADP and Pi.
The muscle will be stimulated by nerve pulses. When an appropriate pulse arrives at the synapse on the fibre, the membrane and the T-tubuli depolarise as a reaction. This causes delivery of Ca^{2+} from the sarcoplasmic reticulum (SR), which diffuses into the actin filaments. When the stimulation is finished the Ca^{2+} is actively pumped back into the SR.



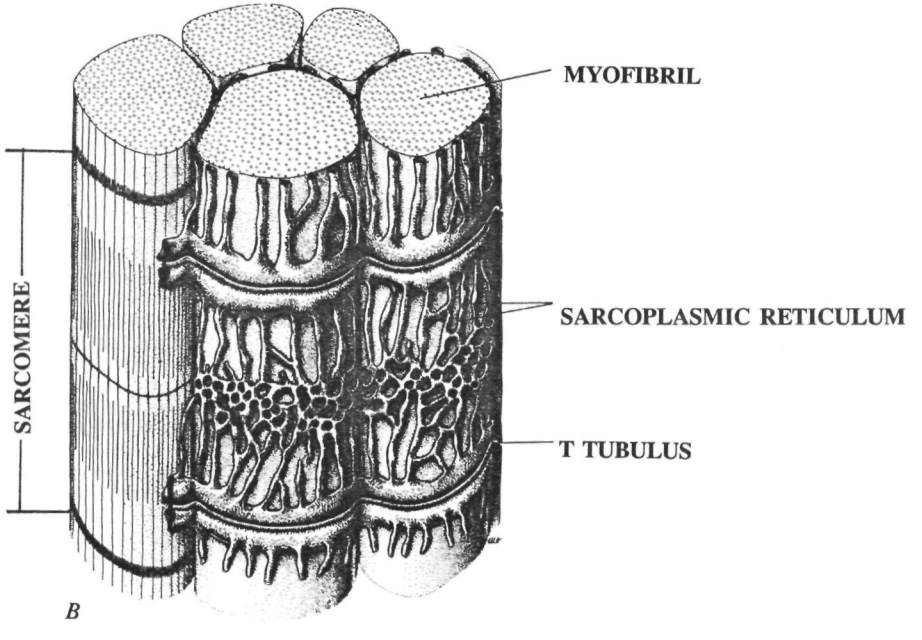


Fig. 1.1: The skeletal muscle. A. the architecture of a muscle (Peeze Binkhorst, 1989). B. Myofibrils with sarcoplasmic reticulum and T-tubuli (Ham and Cormack, 1979).

1.3. Some modes of muscle contraction

The sliding of the actin and myosin filaments along each other results in shortening of the sarcomere. Because the sarcomeres in the myofibril are arranged in series, this shortening results in contraction of the muscle. There are several types of contraction, for example isometric and isotonic contractions. If the tension remains constant during a contraction, the muscle is said to contract isotonically. In this thesis the contractile properties of the m. plantaris were studied by isometric contractions. During these contractions the length of the muscle remains constant.

1.4. Motor unit types

Every muscle contains several motor units. A motor unit is a group of muscle fibres innervated by a single motoneuron. Every fibre of the motor unit has the same mechanical and metabolic properties as the other fibres of the unit. All the fibres of a motor unit contract simultaneously when an appropriate stimulus arrives from the innervating motoneuron. A motor unit is therefore a functional unit within a muscle (Saltin and Gollnick, 1983).

Functional characteristics

The motor units can roughly be divided into fast and slow units based on their contraction characteristics. The fast units have a short twitch contraction and relaxation time and a high fusion frequency in comparison with the slow units. As fast units contain more fibres than slow units the absolute maximal force production is higher in fast units than in slow units. The fast units can be subdivided, based on their resistance against fatigue, into fatigue resistant and fatigue sensitive units. In general, slow units (S units) are fatigue resistant, whereas fast units can be either fatigue resistant (FR units) or fast fatiguable (FF units) (Burke et al., 1973; Saltin and Gollnick, 1983).

Histochemical and Biochemical characteristics

Roughly three fibre types can be distinguished based on histochemistry. These are type I (S: Slow), type IIa (FOG: Fast Oxidative Glycolytic) and type IIb (FG: Fast Glycolytic) fibres. Differentiation between type I and type II fibres can be made by the differences in staining intensity for myosin-ATP-ase after preincubations at different pH. Type II fibres have a myosin ATP-ase which is alkaline-stable, whereas type I fibres have acid-stable myosin ATP-ase (Brooke and Kaiser, 1970). Fibres with a high staining intensity for SDH (Succinic Dehydrogenase) are oxidative and fibres with a weak staining intensity are glycolytic fibres. Type II fibres which are more oxidative are subclassified as IIa and those which are more

glycolytic are subclassified as IIb in this thesis. Lind and Kernell (1991) found that the mean intensity of SDH staining per fibre type, classified on the basis of the myosin ATPase stain, was ranked such that IIa > I > IIbd > IIbm. IIbd fibres are dark staining IIb fibres and IIbm fibres are moderately staining IIb fibres for fixed alkaline ATPase (Sections treated with 5% paraformaldehyde followed by preincubation at pH 10.4).

Relation between histochemical and functional motor unit types

Glycogen depletion experiments have made it possible to establish a relationship between the metabolic and contractile properties of motor units. It appeared that there is a relationship between the histochemical and functional motor unit typing. Both classification systems identify similar units as fast units and the resistance to fatigue is related to the staining intensity of the units for oxidative enzymes. So S, FR and FF units in the functional classification represent type I, IIa and IIb units respectively (See table 1.1; Close, 1972; Burke et al., 1973; Saltin and Gollnick, 1983). However, this comparison has limitations i.c. the variation in the twitch contraction time of motor units was larger than was expected from the differences in the staining intensity for ATPase, and the oxidative enzyme activity was not the only factor which determined the fatigue resistance (Burke et al., 1973; Burke and Edgerton, 1975; Saltin and Gollnick, 1983), which might also be due to, for example, impaired excitation contraction coupling.

Table 1.1. *Comparison of histochemical and functional motor unit classification. For abbreviations see text.*

Histochemical	I	IIa	IIb
	SO	FOG	FG
pH 4.3 ATP-ase	+	-	-
SDH	+	+	-
Functional	S	FR	FF
Twitch Contr Time	long	short	short
Fatigue Resistance	high	medium	low

+: indicates high staining intensity; -: indicates low staining intensity.

1.5. Capillarisation of skeletal muscle

Architecture of the skeletal muscle vascular bed

The artery is the blood supplying vessel of the muscle. It feeds a meshwork of interconnected arterioles, designated as arcade arterioles. From the arcading systems vessels branch off at regular intervals; the transverse arterioles (Engelson et al., 1985; Oude

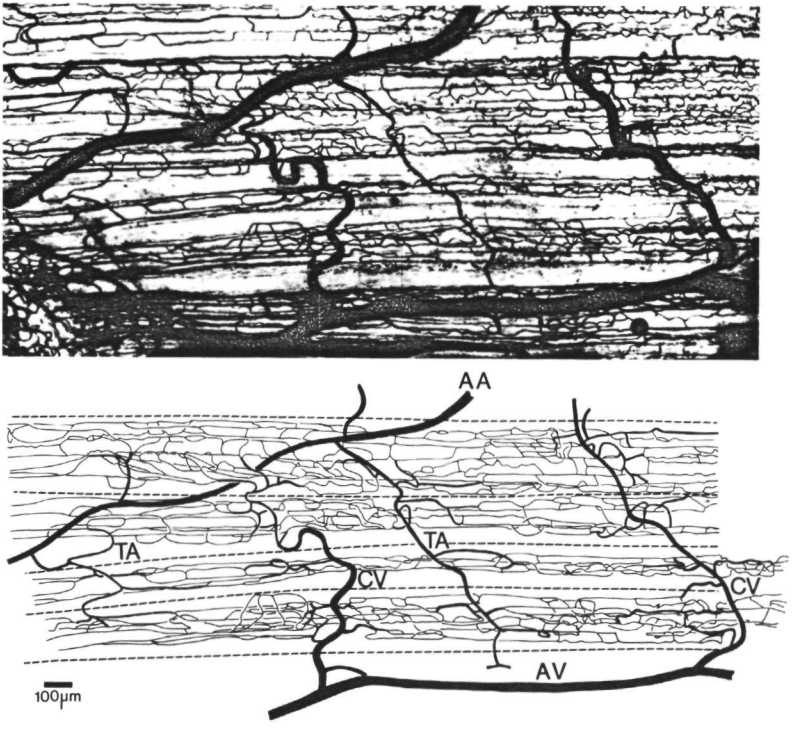


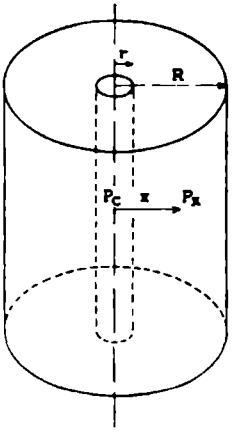
Fig. 1.2: Microphoto montage and line drawing of a capillary network in rat m. spinotrapezius. A segment of the arcade arterioles (AA), arcade venules (AV), transverse arterioles (AT), and collecting venule (CV) are displayed. Four capillary bundles are shown; their division are enhanced by the dashed lines. (Skalak and Schmid-Schönbein,1986).

Vrielink, 1988). The terminal branches of the transverse arterioles are the terminal arterioles, which empty directly into capillaries (Skalak and Schmid-Schönbein, 1986; Oude Vrielink, 1988).

The capillaries run essentially parallel with the muscle fibres (Wiedeman, 1984; Skalak and Schmid-Schönbein, 1986) and are arranged in capillary bundles (fig. 1.2). A capillary bundle consists of a capillary network, its blood supplying transverse arteriole and a collecting venule (Skalak and Schmid-Schönbein, 1986). Within a bundle, the capillaries interconnect in dichotomies; higher order branch points are not seen. There are no connections with capillaries from other bundles. Connections between bundles are provided by the transverse arterioles or collecting venules (Skalak and Schmid-Schönbein, 1986). The capillary bed ends in the collecting venules which converge into transverse venules. Finally, the blood is drained off by the vein (Oude Vrielink, 1988). In addition Kreuzer et al. (1991)

mention the possibly existence of preferential channels across large capillaries.
A pre-capillary sphincter is considered to be the final smooth muscle cell of an arteriole or terminal arteriole guarding the entrance to a capillary network; therefore it is suggested to be a control site for blood flow into the exchange area (Wiedeman, 1984).

The oxygenation of the muscle



$$P_x = P_c - \frac{M}{K}\left(\frac{R^2}{2}\ln\frac{x}{r} - \frac{x^2-r^2}{4}\right)$$

Fig. 1.3: Krogh cylinder with formula of Krogh-Erlang. P_x: tissue oxygen pressure at any distance x from the capillary; P_c: capillary oxygen pressure; R: radius of tissue cylinder; r: radius of capillary; M: oxygen consumption; K: Krogh's diffusion coefficient (Kreuzer, 1986).

The exchange of oxygen and carbon dioxide between the blood and muscle fibres takes place at the level of the capillaries. Therefore, the capillarisation of a muscle gives a rough indication of the maximal capacity of oxygen supply. A simplified way of estimating this capacity is the Krogh model (Kreuzer, 1982). The idea is that a central capillary supplies a surrounding cylinder of tissue with oxygen by means of diffusion (fig. 1.3). An increase in the R (radius) of the cylinder causes a decrease in tissue P_{O_2}, according to this model. The capillaries are distributed irregularly in the muscle. This heterogeneity in capillary spacing results in heterogeneity in R and might affect tissue oxygenation (Turek et al., 1991).
Most muscles contain several fibre types with different oxygen demands (Gray and Renkin, 1978; Kreuzer et al., 1991). Oxidative fibres have a richer capillary supply than glycolytic fibres, both in terms of capillaries around a fibre (Saltin and Gollnick, 1983) and the number of capillaries per cross-sectional fibre area (Gray and Renkin, 1978; Egginton and Ross, 1989).

1.6 Training of skeletal muscle

Several types of training can be distinguished, including endurance and strength training. Strength training increases the capacity to perform high-intensity, high-resistance exercise of a single or few repetitions, such as weight lifting. Endurance training, such as running or cycling training, increases the capacity to sustain repetitive high-intensity, low resistance exercise (Hickson et al., 1988; Klitgaard et al., 1989[a]).

Hypertrophy

Skeletal muscle responds to strength training by increasing its size. Even more rapid hypertrophy develops in muscles following elimination of their synergists by tenotomy, denervation or extirpation (Binkhorst, 1969; Roy et al., 1982; Michel et al., 1989). In the m. plantaris of the rat, this increase in size is accompanied by an increase in maximum tetanic tension, whereas the maximal force per cross-sectional muscle area remains the same (Binkhorst, 1969) or decreases (Roy et al., 1982; Olha et al., 1988).

Riedy and colleagues (1985) found no difference in the activities of oxidative enzymes between normal and hypertrophied rat plantaris muscles. Baldwin et al. (1982) reported reduced ATPase activity and an increased amount of slow myosin heavy chain components in hypertrophied rat plantaris muscles. In accordance with this, an increased proportion of type I fibres was found in these muscles (Ianuzzo and Chen, 1979; Baldwin et al., 1982; Roy et al., 1982; Riedy et al., 1985; Michel et al., 1989), which is probably reflected in the observed increase in twitch duration and fatigue resistance (Roy et al., 1982; Michel et al., 1989).

The increase in size with hypertrophy of rat muscles is characterised by hypertrophy of existing fibres (Gollnick et al., 1981; Riedy et al., 1985; Michel et al., 1989). This increase in fibre size might result in increased diffusion distances from capillary to fibre, which impedes the delivery of oxygen to the interior of a fibre and thus might ultimately result in anoxic tissue areas (Kreuzer et al., 1991). However, the blood flow per unit muscle mass both at rest and during exercise (Armstrong et al., 1986) and glycogen depletion during treadmill exercise (Riedy et al., 1985) was the same for hypertrophied and normal rat plantaris muscles. These observations and the even increased fatigue resistance (Roy et al., 1982; Michel et al., 1989) argue against impaired oxygen delivery in hypertrophied rat muscles.

Endurance training

Endurance training can be given in the form of swimming or running training on a motor driven treadmill. With endurance training, there is no change in the oxygen cost for the same tension development. Therefore increased performance is reached by an increased

maximal O_2 uptake (McAllister and Terjung, 1991), which can be achieved by an increase in the oxidative capacity of the trained muscle (Riedy et al., 1985; Booth and Thomason, 1991). Based on histochemical data, Kovanen (1989) described fibre transformation in several rat muscles towards more fatigue-resistant muscle fibres with slower contractile speeds by live-long endurance training on a treadmill. This can also explain the increased twitch duration as described by Fitts et al. (1984).
It has also been reported that endurance training results in an increased capillary density (Hudlicka, 1985; Booth and Thomason, 1991) which may be related to an increase in the maximal oxygen uptake. However, others did not observe any change in capillarisation with endurance training (Banchero et al., 1979).

1.7 Ageing of skeletal muscle

Morphology
The age-related decrease in muscle mass is accompanied by a decrease in the number of muscle fibres (Grimby and Saltin, 1983) and muscle fibre atrophy (Brooks and Faulkner, 1988). The loss of contractile material is more pronounced in fast units than in slow units (Larsson and Salviati, 1989). At the same time, the amounts of fat and connective tissue show at least a relative increase (Kovanen, 1989).
In the soleus muscle of the rat, which contains mainly type I fibres, the proportion of type I fibres increases, whereas the proportion of type II fibres decreases (Eddinger et al., 1985; Klitgaard et al., 1989[b]; Kovanen, 1989; Larsson and Salviati, 1989). In the Tibialis Anterior and the Extensor Digitorum Longus (EDL), which contain mainly type II fibres no change was seen in the fibre type composition (Eddinger et al., 1985; Larsson and Salviati, 1989). Larsson reviewed that the change in fibre type composition takes place at very old age, the so-called pre-mortal stage (Larsson, 1982). This might explain the absence of changes in fibre type composition in the Tibialis Anterior and the Extensor Digitorum Longus as reported by Larsson and Salviati (1989).

Contraction characteristics
In various studies on human, mouse and rat muscles, the decrease in muscle mass with ageing shows a concomitant decrease in the maximum tetanic tension (Grimby and Saltin, 1983; Fitts et al., 1984; Brooks and Faulkner, 1988; Campbell et al., 1991). In addition, a decrease in specific tension, i.e. the maximum tetanic force per muscle weight (N/g), might occur with age (Campbell et al., 1991 (rats); Phillips et al., 1991 (mice)). Others did not find any change in specific tension (Fitts et al., 1984 (rats); Grimby and Saltin, 1983 (human)).
Ageing is also associated with an increase in the isometric twitch contraction time (Larsson

and Salviati, 1989) and the half relaxation time (Larsson and Salviati, 1989; Fitts et al., 1984) of both fast and slow rat muscles. Phillips et al. (1991) did not find any significant increase in the tetanic contraction time in mice soleus muscle with increasing age.
Endurance, as assessed by intermittent isometric contractions, was found to be maintained or even enhanced in the soleus muscle of rats (Fitts et al., 1984). The rat plantar flexor muscles on the other hand, showed reduced resistance against fatigue at older age (Irion et al., 1987).

Aerobic power
A decline in maximal O_2 consumption generally occurs with increasing age which can partly be explained by the loss of muscle mass with advancing age (Larsson, 1982; Grimby and Saltin, 1983). With ageing, the metabolic differences between muscle types become less distinct. There is a shift towards a decrease in the ratios of glycolytic to aerobic-oxidative enzymes in the fast EDL, whereas no shift occurs towards a more aerobic-oxidative type of metabolism in the slow soleus muscle (Larsson, 1982; Klitgaard et al., 1989[b]). Campbell et al. (1991) reported a decreased anaerobic energy production in FG fibres.

Capillary supply
Senescent rats displayed a lower specific and total muscle blood flow and a lower potential to increase the specific and total blood flow than younger rats (Irion et al., 1987). In elderly humans, the capillary density is well maintained (Larsson, 1982), but little is known about the capillarisation of aged muscles in rats.

1.8 Aim of the study

As described above, compensatory hypertrophy is characterised by enlargement of existing fibres. It has therefore been suggested that hypertrophy of a muscle will result in impaired oxygenation, because the anatomical maximal diffusion distances increase (Kreuzer et al., 1991) and the heterogeneity of the capillary spacing increases (Turek and Rakusan, 1981). This might also affect muscle functioning. However, little is known about the effects of compensatory hypertrophy at different ages. In addition we investigated training effects on compensatorily hypertrophied muscles of different age.
Therefore, in this thesis the functioning of whole m. plantaris was studied. Furthermore, the capillarisation in relation to fibre types was investigated, by using the Domain method developed by Hoofd et al. (1985), that allows the estimation of the average radius of the Kroghian cylinders and the heterogeneity in capillary spacing. With an extension of this method it is possible to assess capillarisation as related to fibre types.
Muscles were enlarged by compensatory hypertrophy which was induced by denervation of

the synergists. Also endurance training was given. This was done with young, middle aged and old rats. Studied were the effects of hypertrophy, training and age on capillarisation, fibre type composition and function.

In chapter 2 the effects of training and hypertrophy on isometric contraction characteristics and fibre type composition in the muscles of young rats were studied. In chapter 3, the effects on capillarisation of these muscles is described. Chapter 4 deals with age effects on the isometric contraction characteristics of normal, hypertrophied, trained and hypertrophied trained m. plantaris. In chapter 5 the fibre type composition and capillarisation in hypertrophied muscles at several different ages were studied. In chapter 6 changes in fatigue resistance due to hypertrophy and age are related to the activities of several enzymes, the fibre type composition and capillarisation.

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2. HISTOCHEMISTRY AND FUNCTIONING OF THE TRAINED HYPERTROPHIED M. PLANTARIS OF THE RAT

SUMMARY

1. Hypertrophy of the m. plantaris, amounting to 137% when expressed as muscle weight/body weight, was obtained 6 weeks after denervation of its synergists. A proportion of the rats were trained on a treadmill.
2. Isometric twitch time to peak tension ($P < 0.001$) and half relaxation time ($P < 0.05$) were prolonged in the hypertrophied muscles.
3. The tetanic force/muscle weight ratio and the twitch/tetanus ratio were not significantly changed, indicating that there was a similar relative increase in the contractile and non-contractile material of the muscle with hypertrophy.
4. Fatigue resistance tended to be higher ($P = 0.08$) in the hypertrophied muscles.
5. The applied training had no significant effect on the isometric contraction characteristics and fatigue resistance of the control and hypertrophied muscles.
6. All the muscles contained mainly IIa and IIb fibres and a smaller amount type I fibres. Type I fibre proportion was increased in hypertrophied muscles ($P < 0.02$) as assessed with ATP-ase and SDH histochemistry.
7. Type I fibres in the deep region of the muscle had smaller cross-sectional areas than in the superficial region, while for IIb fibres the opposite was found ($P < 0.001$). The most significant hypertrophy was observed in type I and IIa fibres in the Operated Trained group ($P < 0.05$).
8. There was no significant correlation between the fatigue resistance and percentage area occupied by a fibre type.

INTRODUCTION

Compensatory hypertrophy of a muscle can be induced by denervating its synergists (Binkhorst and van 't Hof, 1973). Compensatory hypertrophy is characterised by enlargement of the existing fibres (Gollnick et al., 1981; Riedy et al., 1985; Michel et al., 1989). Hypertrophy is accompanied by an increase in the maximum tension generating capacity (Binkhorst and van 't Hof, 1973; Michel and Gardiner 1989; Michel et al., 1989), twitch time to peak tension and fatigue resistance (Roy et al., 1982; Michel et al., 1989). There is also an increase in the percentage of type I fibres in the cross-sectional segments of hypertrophied muscle (Ianuzzo and Chen, 1979; Baldwin et al., 1982; Riedy et al., 1985;

Michel et al., 1989). These functional and histochemical characteristics reflect an increase in their slow motor unit complement (Olha et al., 1988).

Only a few studies have dealt with the influence of endurance training on compensatorily hypertrophied m. plantaris. It was found that the isometric contraction characteristics of both normal and hypertrophied m. plantaris were not affected by endurance training (Binkhorst and van 't Hof, 1973). Riedy and colleagues (1985) found that the rates of oxidative enzymes and glycogen depletion of hypertrophied muscle responded to training and exercise in a manner similar to that of normal muscle. This suggests that hypertrophied muscle possesses the capacity to adjust to endurance training in the same manner as normal muscle. This might be reflected in an increased fatigue resistance in the trained muscle compared to untrained hypertrophied muscle. This aspect was not included, however, by Riedy et al. (1985).

Therefore, the main aim of this investigation was to study, whether the fatigue resistance of compensatorily hypertrophied muscle can be increased by additional moderate activity. This was done by isometric contractions and the fibre type composition of the muscle was established by histochemistry.

MATERIALS AND METHODS

Animals

Female Wistar rats weighing 160-264 g and aged 15-18 wks at the start of the training period were used. The rats were kept, one to a cage, in a room maintained at 22°C with 12 h light and 12 h darkness per 24 h period. Food and water were provided ad libitum. The rats were assigned at random to four groups: Control Not Trained (CNT), Control Trained (CT), Operated Not Trained (ONT) and Operated Trained (OT). Before the operation the rats were anaesthetised with pentobarbital sodium (70 mg/kg i.p.). The operation to produce hypertrophy of the left m. plantaris consisted of denervation of the m. soleus and m. gastrocnemius by cutting the nerves as far distally as possible and transplanting the distal tendon of the m. plantaris to the tendon of the m. gastrocnemius. Regeneration of the nerves was prevented by sewing them into the m. semimembranosus or the m. biceps femoris using ligatures (Binkhorst, 1969). At the time of the contraction measurements no reinnervation was found. The day after the operation the CT and OT rats were placed on the treadmill, set at an inclination of 30^0 upwards and zero speed for 15 min to make them familiar with the treadmill. Over the next four days, the time of running at a speed of 320 m/h and an inclination of 30^0 was gradually increased from 20 min on the second day after the operation to 2 h. This training programme was carried out 5 days per week for the next 5 weeks. To prevent muscle fibre degeneration, the training periods were not extended. The

objective of this training programme was to put additional stress on the muscle to obtain further hypertrophy and an increased endurance capacity.

Contraction measurements

The rats (21 - 24 wks) were anaesthetised with pentobarbital sodium (70 mg/kg i.p.). The body temperature and muscle temperature were maintained at 35 $\pm$ 1°C. The m. plantaris was prepared free, keeping the blood supply intact. The left leg was fixed at the point of the condyli of the femur to ensure isometric contractions. The distal tendon of the muscle was connected to a force transducer (Statham Transducing cell UC2, load cell accessory UL4-5). The isometric contractions were induced by stimulating the muscle with double pulses via the n. ischiadicus. Each pulse was a square pulse of 0.2 ms with an amplitude of 3 V. There was a 0.05 ms time lapse between the double pulses to prevent a back-response (Brown and Matthews, 1960). The muscle was set at its optimal length (L_0), defined as the length at which the muscle produced its maximal twitch force with a minimal twitch duration. A twitch was elicited every 30 s during the experiments to keep the preparation in a steady state. The first five twitch contractions were analysed by measuring the twitch force (FTW), twitch time to peak tension (TPT) and twitch half relaxation time (½RT). After this, two tetani were elicited with a 5 min interval between them, by a 185 Hz pulse train of 330 ms duration. The maximal tetanic force (FTET) of each tetanus was determined. The last tetanus was followed by a 5-min rest period. Then the fatigue sensitivity of the muscle was measured. This was done by stimulating the muscle with 330 ms bursts of 40 Hz every second for 4 min (Burke et al., 1973). A fatigue index (FAT) was calculated as the ratio (%) between the peak force of the contraction encountered 2 min after the strongest contraction, and the peak force of the strongest contraction during the test. This FAT was the same as the fatigue index A of Kernell et al. (1983).

Histochemistry

Directly following the contraction measurements, the muscles were excised, mounted at their L_0 on cork and frozen in isopentane cooled in liquid nitrogen. The muscles were stored at -135°C until processing. Cross-sections (12 μm) were cut in a cryostat at -25°C and stored at -135°C until staining. Sections were stained at room temperature for myofibrillar ATP-ase activity at pH 9.4 after 5 min preincubation at pH 4.35. Adjacent sections were stained for succinic dehydrogenase activity (SDH) at 37°C. Fibres were classified as type I or II based on the ATP-ase staining with type II fibres subclassified into oxidative (IIa) and glycolytic (IIb) based on SDH staining.

Fibre type distribution and fibre areas were determined on photomicrographs of two regions of the muscle: a deep region close to the tendon and a superficial region close to a large blood vessel. On one photomicrograph there were 40 - 115 fibres. From each region of the

muscle, one photomicrograph was analysed. FCSAs (fibre cross-sectional areas) were derived from complete fibre contours on these photographs, using a computer programme similar to the one used by Egginton et al. (1988). It was found that IIb fibres had the largest FCSA and type I fibres the smallest (Degens et al., 1992[b]). Consequently, the contribution of IIb fibres was underestimated and that of type I fibres overestimated, both in terms of their contribution to the total tension generated by a muscle and its oxidative potential; the latter is related to fatigue resistance (Burke et al., 1973). Therefore we calculated the percentage of area occupied by a fibre type in a muscle region as:

$$\frac{(\% * FCSA)_x}{(\% * FCSA)_I + (\% * FCSA)_{IIa} + (\% * FCSA)_{IIb}} * 100\%$$

where % = numerical percentage and *FCSA* = mean FCSA for that fibre type. *I, IIa, IIb*: fibre types; *x*: fibre type of interest.
The % area occupied by a fibre type for whole muscle was then calculated as:
(% area occupied by a fibre type deep + % area occupied by a fibre type superficial)/2.
These values are plotted against the fatigue index.

Statistics
A 2-way ANOVA was used to test for main effects of hypertrophy and training. Differences in fibre type distribution and fibre areas in the deep and superficial region were determined by using a two-tailed paired *t*-test, because they were dependent variables. Pearson's correlation coefficients were calculated between the fatigue index as a dependent variable and the % area occupied by a fibre type. Differences were considered to be significant if $P < 0.05$. Unless stated otherwise, mean values are given $\pm$ SEM with n in parentheses.

RESULTS

Animals
The body weights of the rats in the different groups were similar at the start of the training period (203 $\pm$ 3 (38); mean $\pm$ SEM (n)) and did not differ significantly after the training period (220 $\pm$ 3 (38); mean $\pm$ SEM (n)). The muscle wet weight was increased in the hypertrophied muscles ($P < 0.001$; table 2.1). The degree of hypertrophy, expressed as muscle weight/body weight, was 137% ($P < 0.001$) for the operated groups compared to the control groups (table 2.1).

Table 2.1. *Weight, isometric contraction characteristics and fatiguability of the m. plantaris.*

	CNT	CT	ONT	OT
MW (mg)	329 ± 18 (10)	330 ± 16 (9)	422 ± 22 (8)	446 ± 13 (8)
MW/BW (g/g*100%)	0.142 ± 0.009 (10)	0.155 ± 0.008 (9)	0.198 ± 0.013 (8)	0.208 ± 0.007 (8)
TPT (ms)	16.5 ± 0.7 (8)	16.9 ± 0.4 (9)	19.6 ± 0.9 (8)	19.5 ± 0.5 (8)
FTW (N)	0.79 ± 0.08 (8)	1.04 ± 0.07 (9)	1.30 ± 0.14 (8)	1.34 ± 0.12 (8)
FTET (N)	5.65 ± 0.27 (8)	5.95 ± 0.34 (9)	7.19 ± 0.26 (8)	7.57 ± 0.28 (8)
FTW/FTET	0.14 ± 0.01 (8)	0.18 ± 0.002 (9)	0.18 ± 0.02 (8)	0.18 ± 0.01 (8)
FAT	0.29 ± 0.04 (8)	0.35 ± 0.03 (9)	0.42 ± 0.02 (8)	0.38 ± 0.08 (7)

CNT: Control Not Trained; CT: Control Trained; ONT: Operated Not Trained; OT: Operated Trained. Values are mean ± SEM; number of animals is given in parentheses. MW: muscle wet weight; MW/BW: muscle wet weight/body weight; TPT: twitch time to peak tension; FTW: twitch force; FTET: tetanic force; FTW/FTET: twitch/tetanus ratio; FAT: fatigue index.

Contraction measurements

The twitch time to peak tension was increased ($P < 0.001$) as a result of hypertrophy. This was also true ($P < 0.05$) for the twitch half relaxation time. The twitch force of the hypertrophied muscles was significantly higher ($P < 0.001$) than that of the control muscles. The tetanic force of the hypertrophied muscles was significantly higher ($P < 0.001$) than that of the control muscles, whereas the tetanic force/muscle weight ratio was not significantly changed. The twitch/tetanus ratio tended to be higher for the hypertrophied muscles ($P < 0.10$). The fatigue index was not significantly affected by either training or hypertrophy, although it tended to be higher in the hypertrophied muscles ($P = 0.08$).

Histochemistry

The plantaris muscle contained mainly type II fibres. In the deep region of the muscle, more type I and IIa fibres and fewer IIb fibres were found than in the superficial region ($P < 0.001$). A moderate amount of type I fibres was found in the deep region. A higher proportion of type I fibres appeared in both regions of the m. plantaris ($P < 0.02$

superficial; $P < 0.001$ deep) in the ONT and the OT group, compared to the CNT and CT group. The percentage of type IIb fibres was significantly decreased with hypertrophy in the deep region of the muscle ($P < 0.005$). The percentage of type IIa fibres was not significantly changed in either region (table 2.2).

Table 2.2. *Fibre type composition as a percentage of incidence.*

A:	CNT	CT	ONT	OT
I	16.0 ± 2.0 (7)	14.8 ± 1.6 (8)	29.9 ± 3.5 (8)	33.9 ± 2.6 (7)
IIa	51.6 ± 3.0 (7)	51.7 ± 1.2 (8)	49.0 ± 3.3 (8)	49.3 ± 5.4 (7)
IIb	32.5 ± 4.3 (7)	33.6 ± 1.3 (8)	21.1 ± 2.9 (8)	16.8 ± 5.2 (7)
B:	**CNT**	**CT**	**ONT**	**OT**
I	4.9 ± 0.9 (6)	1.2 ± 0.6 (8)	7.4 ± 2.6 (7)	10.7 ± 4.2 (6)
IIa	41.6 ± 4.3 (6)	39.8 ± 2.9 (8)	42.8 ± 3.5 (7)	36.6 ±.3.6 (6)
IIb	53.5 ± 4.1 (6)	59.8 ± 2.9 (8)	49.8 ± 3.9 (7)	52.7 ± 4.2 (6)

A: deep region; B: superficial region. CNT: Control Not Trained; CT: Control Trained; ONT: Operated Not Trained; OT: Operated Trained. Values are mean ± SEM; no. of animals is given in parentheses. Deep region contained more type I and IIa and fewer IIb fibres than the superficial region ($P < 0.001$); Increased % type I as a result of hypertrophy ($P < 0.002$).

Table 2.3. *Fibre cross-sectional areas in* μm^2.

A:	CNT	CT	ONT	OT
I	1375 ± 93 (7)	1194 ± 44 (8)	1964 ± 233 (8)	2575 ± 144 (7)
IIa	1657 ± 119 (7)	1574 ± 76 (8)	2369 ± 152 (8)	3170 ± 280 (7)
IIb	2481 ± 109 (7)	2249 ± 118 (8)	3121 ± 208 (8)	3511 ± 253 (7)
B:	**CNT**	**CT**	**ONT**	**OT**
I	925 ± 76 (5)	815 ± 27 (2)	1459 ± 114 (5)	1951 ± 140 (5)
IIa	1614 ± 145 (5)	1642 ± 174 (6)	2408 ± 167 (7)	2618 ± 226 (6)
IIb	3652 ± 374 (5)	3326 ± 282 (6)	4815 ± 368 (7)	4634 ± 437 (6)

See table 2 for abbreviations. Values are mean ± SEM; no. of animals is given in parentheses. Type IIb > IIa > I ($P < 0.05$); IIb fibre area superficial > deep ($P < 0.001$); I fibre area deep > superficial ($P < 0.001$); All fibre areas increased with hypertrophy ($P < 0.002$).

Type IIb fibres had larger cross-sectional areas than type IIa fibres, and they again had larger areas than type I fibres ($P < 0.001$). The area of IIb fibres was larger in the superficial region of the muscle than in the deep region ($P < 0.001$), whereas the area of type I fibres was larger in the deep than in the superficial region ($P < 0.001$). In hypertrophied muscles, a significant increase was found in the cross-sectional areas of fibres of all types ($P < 0.002$). The cross-sectional areas of type I fibres in both of the regions of the muscle, and of type IIa fibres in the deep region, were even more increased as a result of an interaction between hypertrophy and training (OT), than with hypertrophy alone ($P < 0.05$) (ONT) (table 2.3).

Fatigue and fibre types

In figure 2.1 the fatigue index is plotted against the % area occupied by the fibre types. There were no significant correlations between the area occupied by a fibre type and the fatigue resistance of a muscle.

DISCUSSION

The degree of hypertrophy expressed as muscle weight/body weight was 137% as a result of the operation. This was also found in a previous report (Binkhorst, 1969). Other authors observed a more pronounced increase (Michel and Gardiner, 1989) and even a doubling in size of the hypertrophied muscles (Ianuzzo and Chen, 1979; Roy et al., 1982; Armstrong et al., 1986). In these experiments, hypertrophy was induced by extirpation of the synergists of the m. plantaris, whereas we denervated its synergists. Denervated muscles may not completely lose the ability to bear tension during standing and locomotion, which might reduce the stimulus for hypertrophy on the innervated plantaris muscle. In line with this, Roy et al. (1982) and Armstrong et al. (1986), who found the most pronounced hypertrophy, operated both legs.

Both the twitch and tetanic force were significantly higher for the hypertrophied muscles than for normal muscles. Yet, the specific force expressed as tetanic force/muscle weight and the twitch/tetanus ratio were unchanged. This was also found in a previous study (Binkhorst, 1969). These results indicate that the increase in muscle mass can be attributed to a similar relative increase in contractile proteins and non-contractile material. Others found, however, a decreased specific force, expressed as tetanic force per unit cross-sectional muscle area, and a decreased twitch/tetanus ratio (Roy et al., 1982; Michel et al., 1989). The difference in normalisation might be an explanation for the discrepancies regarding the specific force. Furthermore, an increased twitch time to peak tension and twitch half relaxation time was found for hypertrophied m. plantaris, consistent with the

findings of others (Roy et al., 1982; Michel et al., 1989). This is, however, in contrast with Binkhorst (1969) who used the same model. This difference can most probably be explained by the method used to determine the twitch time to peak tension and the twitch half relaxation time. We used a computer analysis with a sampling frequency of 2500 Hz, while Binkhorst (1969) determined the twitch time to peak tension and twitch half relaxation time by measuring it on the screen of an oscilloscope.

Hypertrophy tended to increase the fatigue resistance ($P = 0.08$) in our experiments, whereas others found a significant increase (Roy et al., 1982; Michel et al., 1989). These authors used a fatigue index (fatigue index according to Michel and Roy) defined as the peak tension developed within the 120th second, divided by the peak tension developed within the strongest contraction of the total protocol, whereas in our study the fatigue index was defined as the peak tension developed 120 sec after the strongest contraction divided by the peak tension in this strongest contraction. In figure 2.2, the fatigue index we used was plotted against their index. Both indices gave numerically almost similar results, as was also found by Kernell and co-workers (1983). This shows that the difference between their results and ours is probably not related to differences in the fatigue index used. Roy et al. (1982) and also Michel et al. (1989) however, induced compensatory hypertrophy in both hindlimbs. The animals were thereby forced to use the overloaded m. plantaris in both limbs. In our experiments, the rats were in principle able to spare the operated limb by putting an additional load on the normal leg. Furthermore, denervation might not completely remove the ability of the denervated muscles to bear some tension (Kandarian and White, 1990). This might be an explanation for the discrepancy in the fatigue index between their and our results. However, after one week we did not see an abnormal walking pattern in the operated rats.

Training did not have any significant effect on the contraction characteristics of the control or hypertrophied muscles, as was also found by Binkhorst (1969). The same applied to the fatigue resistance. It is thought that the training programme was too mild to induce effects on the contractile properties. We did not want to apply a more intensive training programme, because we wanted to avoid the chance of muscle damage in the operated rats.

The histological analyses showed that in each group, the cross-sectional areas of type IIb fibres were smaller and those of the type I fibres were larger in the deep region of the muscle than in the superficial region. The deep region also contained more type I and IIa and fewer type IIb fibres than the superficial region. This might be explained by the use of the deep part of the muscle for posture maintenance, whereas the superficial part is recruited during more forceful activities, such as walking. The overload resulted in an increase in the cross-sectional areas of fibres of each type. Training on its own did not have such an effect. A combination of overload and endurance training, however, resulted in a more pronounced increase in the cross-sectional areas of type I fibres in both regions of the

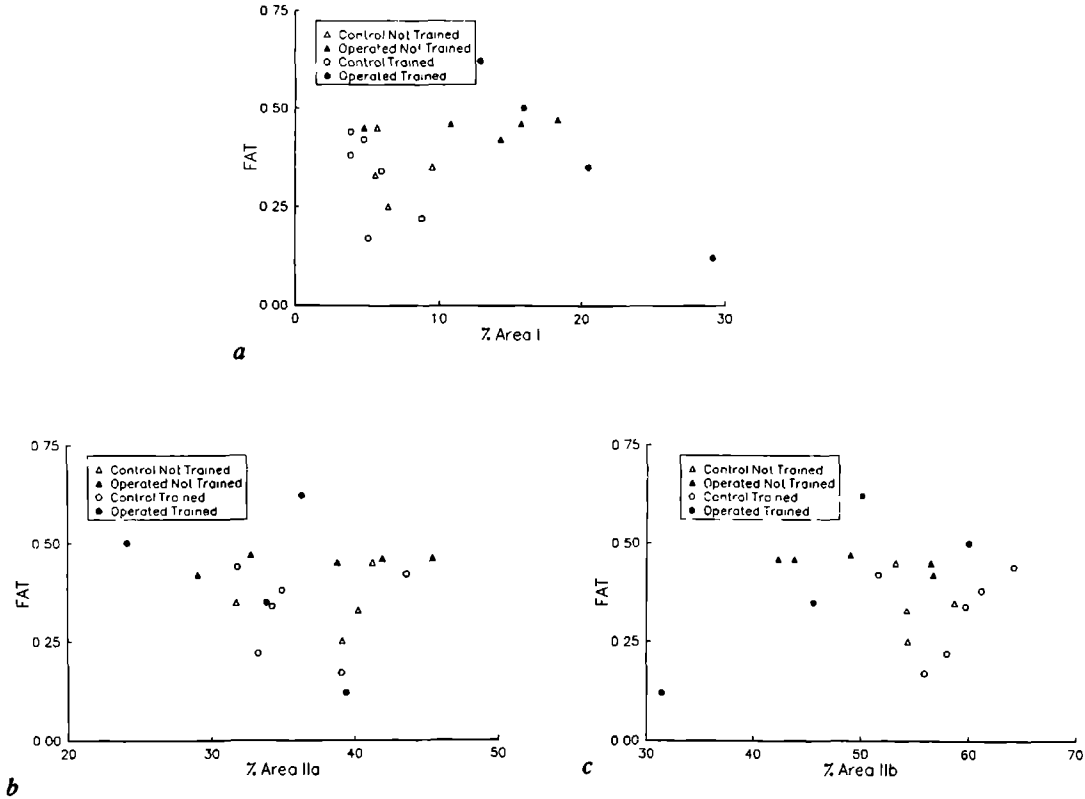

Fig. 2.1: Fatigue Index plotted against percentage area occupied by fibre types. a: FAT against % type I ($r = -0.11$; $P = 0.66$; $n = 19$; pooled values). b: FAT against % type IIa ($r = -0.13$; $P = 0.61$; $n = 19$; pooled values). c: FAT against % type IIb ($r = 0.17$; $P = 0.49$; $n = 19$; pooled values).

muscle and type IIa fibres in the deep region, than overload alone, as was also reported earlier (Riedy et al., 1985). It is possible that our training programme required an activity, which lays within the reserves of the normal muscle, without it being necessary to increase the fibre cross-sectional areas. However, the hypertrophied muscle might have a smaller reserve for performing the training, due to the activity required as a result of the overload. This extra stress on the overloaded muscle might be revealed in further increases in the cross-sectional areas of type I and IIa fibres, as compared to muscles overloaded alone, as was also discussed by Riedy et al. (1985). It is feasible that the training demands an activity of mainly type I and IIa fibres, because all of the rats were able to complete the training sessions in the last four weeks.

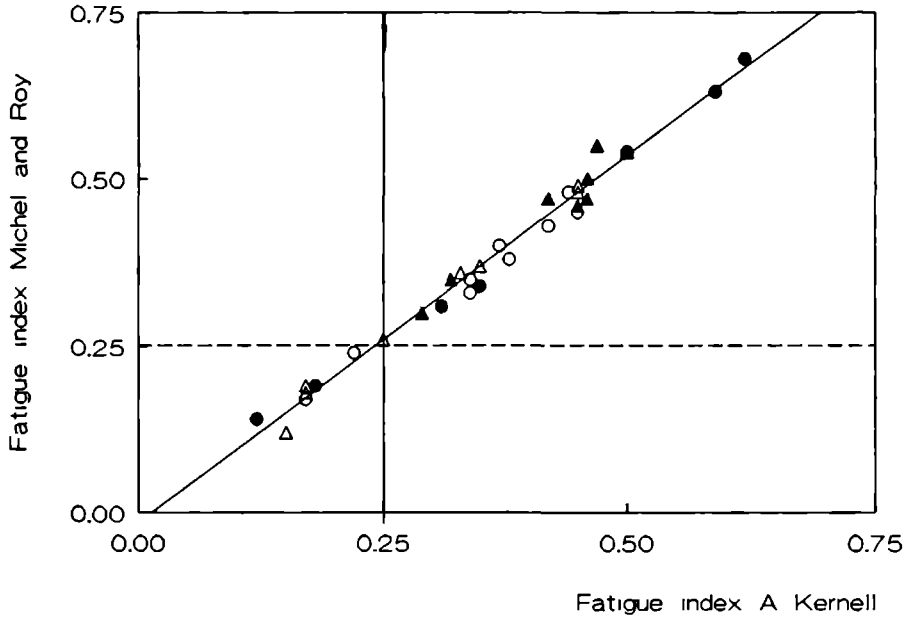

Fig. 2.2: Plot of fatigue Indices. Fatigue index according to Roy et al. (1982) and Michel et al. (1989) versus fatigue index A according to Kernell et al. (1983). Δ: Control Not Trained; O: Control Trained; ▲ Operated Not Trained; ●: Operated Trained. Horizontal and vertical interrupted lines drawn for a fatigue index value of 0.25. The correlation coefficient was 0.99 (n = 32).

It was found that the muscles of all the groups contained mainly type IIa and IIb fibres and a smaller amount of type I fibres. There was an increased proportion of type I fibres in the hypertrophied muscles. This was also observed by others (Ianuzzo and Chen, 1979; Baldwin et al., 1982; Roy et al., 1982; Riedy et al., 1985; Michel et al., 1989). Although the absolute cross-sectional area of type II fibres changed more than that of type I fibres, the proportion of cross-sectional muscle area occupied by type I was also increased, as can be calculated from table 2.1 and 2.2. Thus the increase in the percentage of type I fibres can be an explanation for the tendency towards higher fatigue resistance, as these fibres have a high fatigue resistance (Burke et al., 1973). In addition, a significant decrease was found in the percentage of type IIb, fatigue sensitive (Burke et al., 1973), fibres in the deep region. However, in figure 2.1 it is shown that there was no significant correlation between the percentage area occupied by a fibre type and the fatigue index. This indicates that the fatigue resistance for whole muscle, assessed by applying the 'Burke test', was not closely related to its oxidative capacity, in contrast to what has generally been found for motor units (Burke et al., 1973). Others reported that in normal plantaris muscle, the whole muscle response was less fatigue resistant than a composite of its individual motor unit

responses (Gardiner and Olha, 1987). They suggested that this might be due to metabolic changes which were more severe during whole muscle activity than during single motor unit activity and by changing the ionic composition affecting neuromuscular propagation of excitation (Gardiner and Olha, 1987). Therefore only global estimations of the motor unit composition can be made based on this test.

The type IIa distribution was not significantly affected by the operation or training. This suggests that the proportion of type I fibres increased to the expense of the type IIb fibres. Nevertheless, in a previous study, transitional fibre types were found in compensatorily hypertrophied m. plantaris, especially during the period between 14 and 28 days after the operation. According to their ATP-ase and SDH histochemistry, these fibres were between the type I and IIa fibres (unpublished results of Meessen, Wirtz and Binkhorst). In conformity with this, others found transitional motor units in overloaded plantaris muscle (Pettigrew and Noble, 1989). This study and the present results suggest that with compensatory hypertrophy there is a shift from IIb to IIa followed by a shift from IIa to I.

The histochemical data correspond with biochemical data in the literature, which indicate reduced ATP-ase activity and an increased amount of slow myosin light chain components in the hypertrophied m. plantaris (Baldwin et al., 1982). Furthermore an increased proportion of S (Slow) units and a lower proportion of FI (fast, intermediate fatigue resistant) and FR (fast, fatigue resistant) units was found in hypertrophied m. plantaris (Olha et al., 1988). This shift is probably reflected in the increased twitch time to peak tension and twitch half relaxation time with compensatory hypertrophy.

This investigation was carried out to study the effects of compensatory hypertrophy, obtained by denervation of its synergists and training on the m. plantaris of the rat. Histochemistry showed that the cross-sectional areas of type I fibres were larger in the deep than in the superficial region of the muscle, while for type IIb fibres the opposite was found. The number of type I fibres in the cross-sections was larger in the hypertrophied than the normal muscles. Hypertrophied muscles had increased twitch contraction times. However, both contraction measurements and histochemistry demonstrated that hypertrophied muscles remained fast. The fatigue resistance, the maximal tetanic force/muscle weight as well as the twitch/tetanus ratio were not significantly changed. These results indicate that the increase in muscle mass can be attributed to a similar relative increase in contractile proteins and non-contractile material. Training, however, did not have any significant effect on either the control or hypertrophied muscles, except for more pronounced hypertrophy of type I and IIa fibres in hypertrophied muscles.

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3. THE RELATIONSHIP BETWEEN CAPILLARISATION AND FIBRE TYPES DURING COMPENSATORY HYPERTROPHY OF THE PLANTARIS MUSCLE IN THE RAT

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SUMMARY

Compensatory hypertrophy of the plantaris muscle was obtained by denervation of its synergists. This hypertrophy is characterised by a 32% increase in muscle mass. The muscle consists of type I and IIa (oxidative), and IIb (glycolytic) fibres. Fibres of all types were enlarged in hypertrophied muscles and the proportion of type I fibres was increased. We investigated the capillarisation after hypertrophy as related to fibre types. In order to obtain this information a new technique was used, capable of estimating not only the traditional overall capillary density (CD) but also an index of heterogeneity in capillary spacing (LogSD), the 'local capillary-to-fibre ratio' (LCFR), obtained separately for each muscle fibre type, and finally a capillary density for each respective fibre type, the 'capillary fibre density' (CFD). It was found in both control and hypertrophied muscles that CD was higher in the deep (few IIb fibres) than in the superficial part of the muscle (considerable number of IIb fibres). The LogSD was lower, indicating less heterogeneity, in the deep than in the superficial part of the muscle. The LCFR and CFD of each fibre type were greater in the deep than in the superficial region of both control and hypertrophied muscles. Furthermore the CFD and LCFR were larger in type I and IIa fibres than in IIb fibres in each region of control and hypertrophied muscles. In hypertrophied muscles the CD was not significantly different from that of control muscles. However, LCFR of all fibre types was increased significantly in hypertrophied muscles as compared with controls, demonstrating capillary proliferation. The decreased CFD of type I and IIa fibres in the deep region of hypertrophied muscles as compared with controls suggests that here the capillary proliferation lags behind the increase in muscle mass. Endurance training had no significant effects for any region in any of the indices that were used.

INTRODUCTION

Compensatory hypertrophy of a muscle can be induced by denervating its synergists (Binkhorst, 1969). This hypertrophy is characterised by enlargement of existing fibres (Riedy et al., 1985; Michel et al., 1989). In the absence of accompanying capillary proliferation, this hypertrophy of muscle fibres would result in increased diffusion distances from capillary to the interior of a fibre, thus impeding delivery of oxygen to the interior of the fibre. This ultimately may result in anoxic tissue areas (Kreuzer *et al.*, 1991). Hypertrophy can also result in an increased heterogeneity in capillary spacing which might impair oxygen transport to tissue (Turek et al., 1991).

Little is known about capillary proliferation in compensatorily hypertrophied muscle.

Reitsma (1973) described capillary neoformation in compensatorily hypertrophied soleus muscle based on the observation of capillaries at sites where they are never seen normally. However, the capillarisation was not studied quantitatively and thus no conclusion can be drawn as to capillary proliferation from this report.
Endurance training can increase the number of capillaries in skeletal muscle (Hudlicka, 1985). A primary decrease in capillarisation in hypertrophied muscle might thus be compensated for by endurance training. A moderate degree of endurance training was therefore included in the present study. So far, studies on capillarisation of skeletal muscle have mainly been performed by using the traditional indices of capillary density (CD) and capillary to fibre ratio (C/F). The CD and C/F give global estimates of the capillary supply to a muscle but no information about the capillary supply to different fibre types. We have developed a computerised method based on the method of capillary domains (Hoofd et al., 1985) that enables us to analyse the capillarisation as related to fibre types (I, IIa, IIb). It runs automatically, with no subjective bias, and it also yields values of capillary spacing and heterogeneity (Egginton et al., 1988). This method may therefore be helpful in modelling tissue oxygenation. It has been found in skeletal muscle that there is a lower heterogeneity in capillary distribution in slow (oxidative) muscle than in the extensor digitorum longus (EDL), a mixed fast muscle (Egginton et al., 1988). This possibly has a positive effect on oxygen supply to the muscle fibres (Kreuzer et al., 1991).
The plantaris muscle of the rat is a mixed fast muscle containing type I (SO or β), IIa (FOG or αR) and IIb (FG or αW) fibres (Armstrong & Phelps, 1984; Klitgaard et al., 1989; Torgan et al., 1989). This muscle is therefore useful for studying the effects of compensatory hypertrophy on the capillarisation as related to fibre types and was chosen for the present study. We have also investigated the effect of additional endurance training.

MATERIALS AND METHODS

Animals

Female Wistar rats weighing 160 - 264 g and aged 15 - 18 wk at the start of the experiment were used. The rats were kept, one to a cage, in a room maintained at 22°C with 12 h light and 12 h darkness per 24 h period. Food and water were provided ad libitum. The rats were assigned at random to a Control Not Trained (CNT), Control Trained (CT), Operated Not Trained (ONT) and an Operated Trained (OT) group. Before the operation the rats were anaesthetised with pentobarbital sodium (70 mg/kg i.p.). The operation to produce hypertrophy of the left plantaris muscle consisted of denervation of the soleus and gastrocnemius muscles by cutting the nerves as far distally as possible and transplanting the distal tendon of the plantaris to the tendon of the gastrocnemius. Regeneration of the nerves was prevented by sewing them into the biceps femoris (Binkhorst, 1969). At the time of the termination of the experiments no reinnervation was found. The day after the operation both CT and OT rats were placed for 15 min on the treadmill, set at an inclination of 30^0 and zero speed to make them familiar with the treadmill. Over the next four days the time of running at a speed of 320 m/h and an inclination of 30^0 was gradually increased from 20 min on the second day after the operation to 2 h. This training programme was carried out

5 d per wk for the next 5 wk. After this training period the rats were anaesthetised with pentobarbital sodium (70 mg/kg i.p.) and in situ isometric contractions were elicited. It was shown that the ratio tetanic force/muscle weight was not significantly changed, either with training or with the operation, indicating that all muscles were in a good condition (Degens et al., 1990). At that time, the rats were aged 21 - 24 wk and had weights of 178 - 287 g.

Muscle preparation

Directly following the contraction measurements, the muscles were excised, mounted at their L_0 length on cork and frozen in isopentane cooled in liquid nitrogen. L_0 was defined as the length at which the muscle produces its maximal twitch force with a minimal twitch duration. The muscles were stored at -135°C until processed. Cross-sections (12 μm) were cut in a cryostat at -25°C and stored at -135°C until staining.

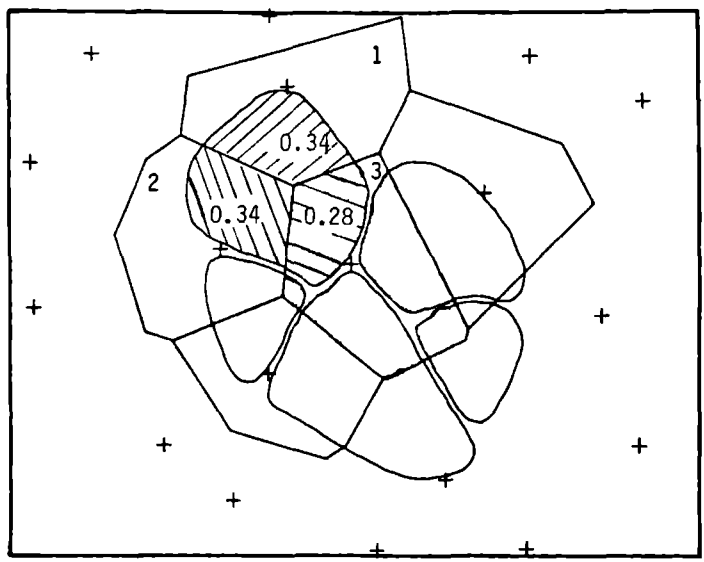

Fig. 3.1: Illustration of the interaction between domains and fibres. For each capillary (crosses) only that fraction of the domain that actually overlaps the fibre (dashed) is counted. All such fractions together for a fibre provide the LCFR. For the dashed fibre the LCFR is 0.96 (0.34 of domain 1 + 0.34 of domain 2 + 0.28 of domain 3 = 0.96). The CFD is the LCFR of this fibre divided by its area i.e. 0.96/fibre area. (After Egginton et al., 1988).

Histochemistry

Sections were stained at room temperature for myofibrillar ATP-ase activity at pH 9.4 after 5 min preincubation at pH 4.35. Adjacent sections were stained for SDH activity at 37°C. Fibres were classified as type I or II based on the m-ATPase staining with type II fibres subclassified into oxidative (IIa) and glycolytic (IIb) based on SDH staining.

In another consecutive section the capillaries were stained by a modification of the combined staining method for AP (alkaline phosphatase) and DPP-IV (dipeptidyl peptidase

IV). This method demonstrates both the arterial and venular portions of a capillary, whereas after AP staining alone, the venular portion of a capillary remains unstained (Mrazkova et al., 1986; Batra et al., 1989). The sections were therefore incubated for 90 min at room temperature in the dark in a DPP-IV reaction medium containing 64 mg glycyl-l-proline-4-methoxy-β-naphthylamide (Bachem Feinchemikalien AG, Bubendorf, Switzerland), dissolved in 8 ml N,N-dimethylformamide mixed with 160 mg fast blue B salt (Sigma, St. Louis, USA) dissolved in 160 ml 0.1 M phosphate buffer pH 7.4. After rinsing the sections 3 times in distilled water, they were transferred to the AP incubation medium with the following composition (Ziada et al., 1984): nitroblue tetrazolium chloride (Analytical Grade, Serva, Feinbiochemica, Heidelberg, Germany) 160 mg and 5-bromo-4-chloro-3-indolylphosphate-p-toluidine salt (Sigma, St. Louis, USA) 32 mg dissolved in 160 ml of a buffer containing 6.9 mM $MgSO_4$ and 27.5 mM $Na_2B_4O_7$, adjusted to pH 9.2 with NaOH, for 60 min at room temperature. Then the sections were rinsed 3 times in distilled water and postfixed in 4% formalin for 2 h. Finally, the sections were washed three times with distilled water and mounted in glycerine-gelatine.

Analysis

As stated in the Introduction, the capillarisation of a muscle has traditionally been described by the overall indices capillary density (CD) and capillary to fibre ratio (C/F). These indices do not take into account the presence of different fibre types in a muscle and therefore give little information about capillarisation as related to fibre types. The heterogeneity in capillary spacing in a muscle also is not indicated by these parameters, although this heterogeneity may influence the oxygenation of a muscle markedly. The method of capillary domains, developed in our Department (Hoofd et al., 1985), estimates CD and heterogeneity of capillary spacing. This method was expanded in order to relate these indices to fibre types. The analysis was performed on photomicrographs of the deep region, near the head of the tendon, and the superficial region, near a large blood vessel in the core. Each photomicrograph contained 40-115 fibres and from each region of each muscle 1 photomicrograph was taken. For each photograph the fibre type (I, IIa and IIb) composition was assessed. Using a digitising tablet, fibre outlines of complete fibres were read into the computer as contour coordinates and capillary locations as coordinates of the capillary centres. The overall capillary density (CD) was defined as the number of capillaries per square millimetre of tissue (muscle fibres plus intercellular space). Capillary domains were constructed (fig. 3.1), defined as the area surrounding a capillary delineated by equidistant boundaries from adjacent ones (Voronoi tesselation) and their surface area calculated (DOM). Domain areas have a lognormal distribution (Hoofd et al., 1985; Egginton et al., 1988). Therefore the logarithmic standard deviation of the DOM areas (i.e. the SD of log-transformed variates, $\sigma \log_{10} x$, denoted by the abbreviation LogSD), gives an indication of the heterogeneity of the capillary spacing. The LogSD of the DOM areas is numerically equal to twice the LogSD of the Krogh tissue cylinder radii used in some other publications (Egginton et al., 1988). Fibre areas were derived from complete fibre contours on these photomicrographs. From overlapping of domains and muscle fibres 2 indices were derived. First, for each fibre the local capillary-to-fibre ratio (LCFR) was determined. It was defined as the sum of the fractions of each domain area overlapping the fibre (fig. 3.1).

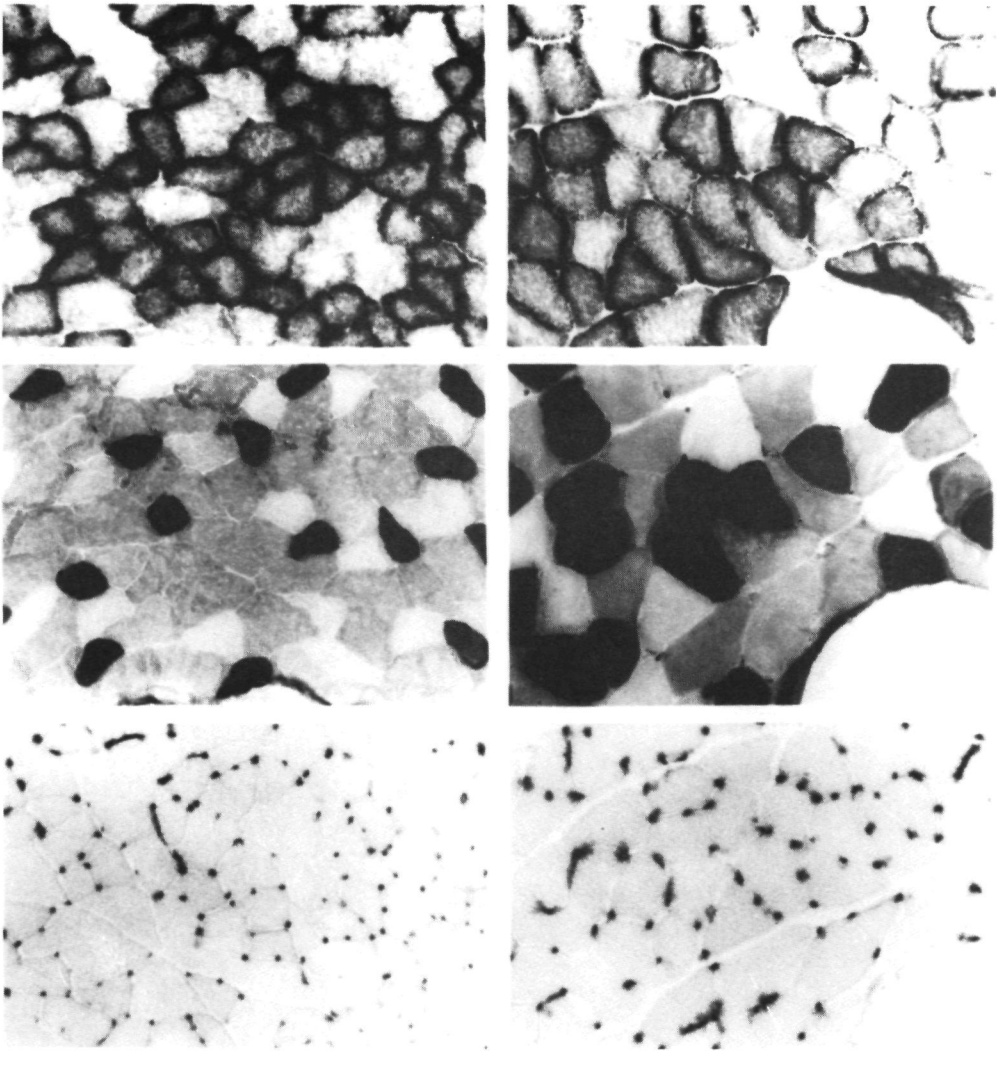

Fig. 3.2: The effect of denervation of synergists on morphology of the plantaris muscle. On the left, sections from the Control (a, c, e) and on the right from the Operated (b, d, f) groups. Sections stained for SDH (a, b), ATP-ase at pH 4.35 (c, d) and with combined staining for alkaline phosphatase and dipeptidyl peptidase IV to demonstrate capillaries (e, f). Scale bar, 50 μm. The pooled LCFR in f was higher than in e (2.091 $\pm$ 0.104 (13) vs. 1.764 $\pm$ 0.097 (31); mean $\pm$ SEM (n)).

Whereas a capillary count can only have discrete values, the LCFR has a continuous distribution of values. Furthermore, it also takes account of remote capillaries. Hence it

may provide a good estimate of the capillary supply to a fibre in glycolytic muscle areas, where muscle fibres can lack direct capillary contact and thus depend on these remote capillaries (Egginton et al., 1988). As one domain is the region geometrically supplied by one capillary, the LCFR can be interpreted as a number of capillaries geometrically supplying a muscle fibre. Secondly, the LCFR divided by the cross-sectional area of that fibre will provide the capillary density for that particular fibre, the capillary fibre density (CFD) (fig. 3.1). Thus these indices give the possibility to estimate capillarisation as related to separate fibre types, type I, IIa and IIb fibres. The same indices were used in the reports of Egginton et al. (1988) and Egginton and Ross (1989) and can be seen as a refinement of the indices introduced by Gray and Renkin (1978).

Statistics

First, an ANOVA was used to test for training and operation effects per region and fibre type. The ANOVA was applied assuming no interaction. For the indices calculated for type I fibres in the superficial region a Wilcoxon test was used and testing was for fibre type distribution, fibre area, LCFR and CFD. CFD was calculated as the mean LCFR divided by the mean corresponding area for each rat. Differences between the deep and superficial regions and between the 3 fibre types were determined by using a 2-tailed paired *t*-test. The CFD for each fibre type in each region of each group was plotted against the percentage area occupied by IIb fibres. The correlation coefficient was calculated and tested for significance by the Pearson correlation test. Differences were considered significant if $P < 0.05$. Unless stated otherwise, mean values are given $\pm$ SEM.

Table 3.1. *Fibre type composition (percentage incidence).*

A:	CNT	CT	ONT	OT
I	16.0 ± 2.0 (7)	14.8 ± 1.6 (8)	29.9 ± 3.5 (8)	33.9 ± 2.6 (7)
IIa	51.6 ± 3.0 (7)	51.7 ± 1.2 (8)	49.0 ± 3.3 (8)	49.3 ± 5.4 (7)
IIb	32.5 ± 4.3 (7)	33.6 ± 1.3 (8)	21.1 ± 2.9 (8)	16.8 ± 5.2 (7)
B:	**CNT**	**CT**	**ONT**	**OT**
I	4.9 ± 0.9 (6)	1.2 ± 0.6 (8)	7.4 ± 2.6 (7)	10.7 ± 4.2 (6)
IIa	41.6 ± 4.3 (6)	39.8 ± 2.9 (8)	42.8 ± 3.5 (7)	36.6 ± 3.6 (6)
IIb	53.5 ± 4.1 (6)	59.8 ± 2.9 (8)	49.8 ± 3.9 (7)	52.7 ± 4.2 (6)

A: deep region; B: superficial region. CNT: Control Not Trained; CT: Control Trained; ONT: Operated Not Trained; OT: Operated Trained. Values are mean ± SEM; number of animals given in parentheses.

RESULTS

From the ANOVA, there appeared to be no significant training effects. The effects of

operation and fibre and region differences are described below.
The amount of muscle hypertrophy obtained by the operation was about 32% (muscle mass in mg: CNT 329 ± 18; CT 330 ± 16; ONT 422 ± 22; OT 446 ± 13) ($P < 0.001$). Figure 3.2 gives typical examples of photomicrographs of the deep region of the muscles in both Control and Operated rats in which the effects described below are illustrated.
Table 3.1 shows that the plantaris muscle contains mainly type II fibres. A moderate number of type I fibres were found in the deep region and a smaller number in the superficial region. The proportion of type I fibres was increased in the Operated group as compared with the Control group ($P < 0.05$). In the deep region of the muscles of the Operated group the proportion of type IIb fibres was decreased ($P < 0.001$).
Table 3.2 shows that the cross-sectional area of type I fibres was smallest and that of type IIb fibres the largest, with type IIa fibres being intermediate (all $P < 0.001$). The cross-sectional areas of type I fibres were larger in the deep than in the superficial region ($P < 0.05$). For type IIb fibres the opposite was true ($P < 0.001$). This was found for both the Control and Operated groups. The cross-sectional areas of all fibre types were increased by the operation ($P < 0.01$).
Table 3.3 shows that the CD was higher in the deep part of the muscle than in the superficial part ($P < 0.001$). This corresponds to larger DOM areas in the superficial than in the deep region ($P < 0.001$). Heterogeneity, given by LogSD, was less in the deep region than in the superficial region ($P < 0.001$). This was found in both the Control and Operated groups. The DOM areas in the deep region of the muscle were larger in the Operated as compared with the Control rats ($P < 0.05$). There was, however, no significant effect of the operation on the CD ($P = 0.122$).
Table 3.4 shows that the LCFR was higher in the deep than in the superficial region of the muscle for all fibre types in both the Control and Operated groups ($P < 0.02$). In both regions of the muscles of the Control group the LCFR for type I fibres was lowest and type

Table 3.2. *Fibre cross-sectional areas (μm^2).*

A:	CNT	CT	ONT	OT
I	1375 ± 93 (7)	1194 ± 44 (8)	1964 ± 233 (8)	2575 ± 144 (7)
IIa	1657 ± 119 (7)	1574 ± 76 (8)	2369 ± 152 (8)	3170 ± 280 (7)
IIb	2481 ± 109 (7)	2249 ± 118 (8)	3121 ± 208 (8)	3511 ± 253 (7)
B:	**CNT**	**CT**	**ONT**	**OT**
I	925 ± 76 (5)	815 ± 27 (2)	1459 ± 114 (5)	1951 ± 140 (5)
IIa	1614 ± 145 (5)	1642 ± 174 (6)	2408 ± 167 (7)	2618 ± 226 (6)
IIb	3652 ± 374 (5)	3326 ± 282 (6)	4815 ± 368 (7)	4634 ± 437 (6)

A: deep region; B: superficial region. CNT: Control Not Trained; CT: Control Trained; ONT: Operated Not Trained; OT: Operated Trained. Values are mean ± SEM; number of animals given in parentheses.

IIb fibres highest with IIa fibres in between (all $P < 0.05$). The same was found in the superficial region of the muscles of the Operated group ($P < 0.01$). In the deep region of these muscles the LCFR of type I fibres remained lower than that of IIa and IIb fibres ($P < 0.01$), but there was no significant difference between IIa and IIb fibres. The operation resulted in an increased LCFR for all fibre types both in the deep (I, IIa: $P < 0.001$; IIb: $P < 0.025$) and the superficial region (I: $P < 0.02$; IIa: $P < 0.001$; IIb: $P < 0.004$) of the muscle as compared with Controls.

Table 3.3. *Overall indices of capillarisation.*

A:	CNT	CT	ONT	OT
n	7	8	8	7
CD	865 ± 35	897 ± 54	870 ± 67	724 ± 28
DOM	1157 ± 47	1127 ± 60	1207 ± 100	1386 ± 59
LogSD	0.150 ± 0.010	0.165 ± 0.010	0.161 ± 0.007	0.168 ± 0.005
B:	**CNT**	**CT**	**ONT**	**OT**
n	7	8	7	6
CD	465 ± 57	422 ± 53	422 ± 39	457 ± 52
DOM	2241 ± 289	2545 ± 297	2409 ± 208	2136 ± 220
LogSD	0.195 ± 0.016	0.199 ± 0.006	0.197 ± 0.019	0.215 ± 0.007

A: deep region; B: superficial region. CNT: Control Not Trained; CT: Control Trained; ONT: Operated Not Trained; OT: Operated Trained. n: number of animals; CD: capillary density, in mm^{-2}; DOM. domain area, in μm^2; LogSD: logarithmic standard deviation of DOM, as index of heterogeneity. Values are mean ± SEM.

Table 3.4 also shows that the CFD for all fibre types in both the Control and Operated groups was higher in the deep region of the muscle than in the superficial region ($P < 0.05$). In the deep region the CFD for type I fibres was highest and that for IIb fibres the lowest with IIa fibres in between (all $P < 0.05$). In the superficial region of the Control and Operated groups the CFD for type I and IIa fibres was higher than that for type IIb fibres (all $P < 0.01$). The CFD of type I and IIa fibres, however, was not significantly different. Operation resulted in a lower CFD for type I ($P < 0.05$) and IIa fibres ($P < 0.02$) as compared with the Control group, but only in the deep region.

In figure 3.3 the CFD for each fibre type is plotted against the percentage of the cross-sectional area occupied by IIb fibres. There was a significant negative correlation in the superficial region of control muscles between the CFD for any fibre type and the area occupied by IIb fibres (type I: $r = 0.73$, $P < 0.05$; IIa: $r = 0.66$, $P < 0.01$; IIb: $r = 0.76$, $P < 0.01$). For the other cases, however, there was no significant correlation between the CFD of a fibre type and the percentage area occupied by IIb fibres. Accepting that the data for the superficial and deep region can be pooled, the resulting correlations are

found for each fibre type in both Control (I: $r = 0.79$, $P < 0.001$; IIa: $r = 0.79$, $P < 0.001$; IIb: $r = 0.89$, $P < 0.001$) and hypertrophied muscles (I: $r = 0.68$, $P < 0.01$; IIa: $r = 0.63$, $P < 0.001$; IIb: $r = 0.81$, $P < 0.001$).

Table 3.4. *Capillarisation with respect to fibre types.*

A:	CNT	CT	ONT	OT
I: n	7	8	7	7
LCFR	1.324 ± 0.070	1.210 ± 0.067	1.670 ± 0.063	2.009 ± 0.125
CFD	972 ± 50	1035 ± 63	980 ± 64	785 ± 32
IIa: n	7	8	8	7
LCFR	1.514 ± 0.093	1.531 ± 0.086	2.118 ± 0.147	2.242 ± 0.196
CFD	927 ± 40	988 ± 59	894 ± 69	719 ± 31
IIb: n	7	8	8	6
LCFR	2.011 ± 0.097	1.667 ± 0.105	2.211 ± 0.194	2.253 ± 0.195
CFD	808 ± 31	759 ± 59	727 ± 52	700 ± 54
B:	**CNT**	**CT**	**ONT**	**OT**
I: n	6	2	4	5
LCFR	0.579 ± 0.092	0.518 ± 0.069	0.794 ± 0.082	1.193 ± 0.195
CFD	639 ± 96	668 ± 135	526 ± 85	611 ± 93
IIa: n	6	8	7	5
LCFR	0.992 ± 0.142	0.789 ± 0.078	1.129 ± 0.082	1.671 ± 0.134
CFD	668 ± 107	546 ± 96	508 ± 46	621 ± 55
IIb: n	6	8	7	6
LCFR	1.518 ± 0.089	1.268 ± 0.065	1.661 ± 0.088	1.752 ± 0.143
CFD	440 ± 55	403 ± 41	368 ± 36	417 ± 35

A: deep region; B: superficial region. CNT: Control Not Trained; CT: Control Trained; ONT: Operated Not Trained; OT: Operated Trained. n: number of animals; LCFR: local capillary-to-fibre ratio; CFD: capillary fibre density in mm^2. Values are mean ± SEM.

DISCUSSION

Hypertrophy

The degree of hypertrophy of the plantaris muscle obtained by denervation of its synergists, as expressed by the increase of muscle mass, was about 32%. In the hypertrophied muscles the cross-sectional areas of all fibre types were increased. The muscle remained a fast muscle (Degens et al., 1990) even though there was an increased proportion of type I fibres, as has also been covered by others (Riedy et al., 1985; Michel et al., 1989).

Training

The applied endurance training did not result in significant changes in our experiments. Binkhorst (1969), applying the same training programme, did not find significant changes in the isometric contraction characteristics with training in hypertrophied muscles. Thus this mode and intensity of training induces changes neither in contraction characteristics nor in fibre type composition and capillarisation. We did not apply heavier training in order to avoid possible muscle damage in operated rats.

Capillarisation

In the present study the capillarisation of the muscles was analysed with a computerised method based on the method of capillary domains (Hoofd et al., 1985). With this method the capillarisation can also be related to fibre types. Glycolytic muscles have a lower CD than oxidative muscles (Ripoll et al., 1979; Gray et al., 1983; Egginton et al., 1988). In this presentation it was found that the overall CD was higher in the deep (more oxidative) region of the plantaris muscle than in the superficial (more glycolytic) region. The same was found by Romanul (1965) in plantaris and by Ziada et al. (1989) in tibialis anterior. Maxwell et al. (1980), however, found no correlation between oxidative capacity and capillarisation in various skeletal muscles from several species, but they did find significant correlations within a single species.

Heterogeneity in capillary spacing, expressed as logSD of the domain areas, was higher in the superficial region of the muscle than in the deep region. This agrees with the finding that heterogeneity in capillary spacing in the EDL (extensor digitorum longus) was higher than in the soleus of rats (Egginton et al., 1988). This was explained by the presence in the soleus of only oxidative fibres, whereas in the EDL there were both glycolytic and oxidative fibre types. Likewise the differing heterogeneity in both regions of the plantaris in our study might be explained by the predominance of oxidative fibres in the deep region and the more equal distribution of glycolytic and oxidative fibres in the superficial region of the muscle.

A higher CFD for oxidative than for glycolytic fibres was also found by Egginton and Ross (1989). This implies that the capillary supply per surface area is higher for oxidative fibres than for glycolytic fibres, in accordance with observations of other authors using different methods (Gray & Renkin, 1978; Green et al., 1989). Type II fibres, however, had a higher LCFR than type I fibres and in the superficial region the LCFR was higher for IIb than for IIa fibres. This indicates that the capillary supply expressed as the number of capillaries per fibre was higher for IIb (glycolytic) fibres than for type I and IIa (oxidative) fibres, IIb fibres having larger cross-sectional areas than type I and IIa fibres. These findings and the increased LCFR for all fibre types with hypertrophy indicate that the capillary supply to a fibre is also influenced by its cross-sectional area, as has been suggested by others (Gray & Renkin, 1978; Myrhage, 1978; Ripoll et al., 1979; Egginton et al., 1988). Egginton and Ross (1989) even stated that "capillarisation is primarily determined by fibre size, although this basic relationship may be modulated by the absolute level of oxidative metabolism".

Gray and Renkin (1978), using a parameter for capillary supply per fibre type comparable to our LCFR, found an inverse relation between fibre capillarisation and the percentage incidence of IIb fibres. We found a significant negative correlation in the superficial region of control muscles between the CFD of any fibre type and the cross-sectional area occupied

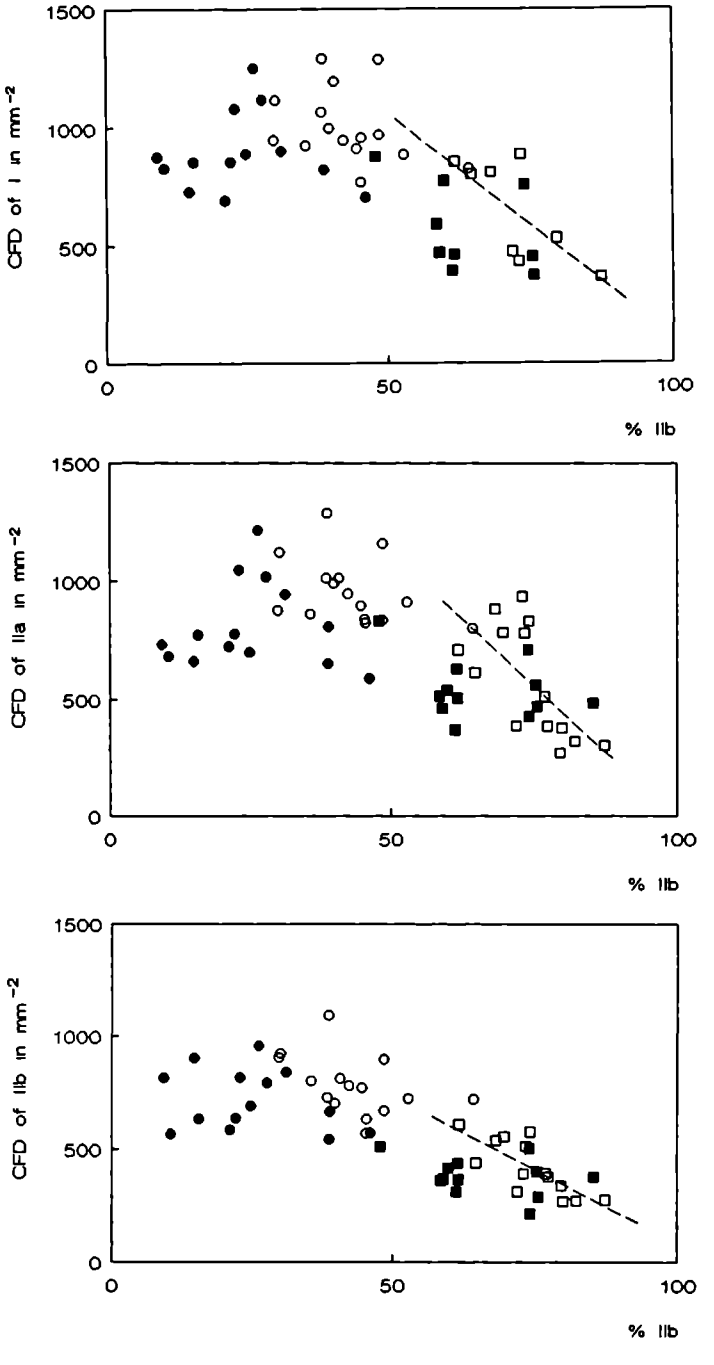

Fig. 3.3: Capillary fibre density (CFD) plotted against percentage of area occupied by IIb fibres for each fibre type. ○ Control deep region; □ Control superficial region, ● Operated deep region; ■ Operated superficial region. **A.** CFD of type I; Control: n = 23, Operated. n = 22. **B.** CFD of type IIa; Control· n = 29; Operated: n= 26. **C.** CFD of type IIb, Control. n = 29, Operated: n = 26. --:regression lines for Control superficial region.

by IIb fibres (fig. 3.3), in conformity with their findings. For the other instances, however, there was no significant correlation between the CFD for a fibre type and the percentage of area occupied by IIb fibres. Both from figure 3.3 and from table 3.4 it can be seen that the CFD was higher in the deep region of the muscle than in the superficial region. However, these regional differences for CFD might be related to the same negative correlation, as seen from figure 3.3. On the assumption that the data of the superficial and deep region can be pooled, significant negative correlations are found for each fibre type in both control and hypertrophied muscles. Thus this might indicate that the capillary supply to a fibre in terms of CFD also follows an inverse relation to the percentage area occupied by IIb fibres.

Reitsma (1973) concluded that there was capillary neoformation in the compensatorily hypertrophied soleus because of the occurrence of capillaries in clefts between fibres. He did not undertake quantitative analysis of the capillarisation, so no definite conclusion can be drawn as to the extent of capillary proliferation from his report. Capillary proliferation was found in stretch-induced hypertrophied muscles of the chicken as assessed by a capillary to fibre ratio (Holley et al., 1980). In the present study the LCFR was increased in all fibre types in both regions of the muscle with hypertrophy. This can only be explained by capillary proliferation with compensatory hypertrophy.

The CFD was not significantly altered with hypertrophy for all fibre types in the superficial region of the muscle. The CFD of type I and IIa fibres in the deep (more oxidative) region, however, was decreased. This is reflected in the increased DOM area in the deep region of the muscle, although the CD was not significantly changed. Thus in the superficial region the capillary proliferation matched the hypertrophy of the fibre cross-sectional areas, whereas in the deep region capillary proliferation lagged behind the increase in fibre cross-sectional areas, especially in type I and IIa fibres. Similar to our results, Holley et al. (1980) found with stretch-induced hypertrophy no significant change in CD in the patagialis, a twitch muscle and a decreased CD in the anterior latissimus dorsi, a slow-tonic muscle, indicating that the capillary proliferation matched hypertrophy in the former muscle but lagged behind in the latter.

The increased LCFR with compensatory hypertrophy in both glycolytic and oxidative fibres might indicate that oxidative capacity is increased in both fibre types. Riedy et al. (1985), however, suggested that the activity of several oxidative enzymes of compensatorily hypertrophied muscles was similar or decreased as compared with normal muscles.

With compensatory hypertrophy there was a concomitant increase in blood flow both at rest and during exercise. The maximum flow, however, expressed as $ml.min^{-1}.100g^{-1}$ was identical in both control and hypertrophied muscles. Thus the maximum absolute flow in ml/min is increased with hypertrophy (Armstrong et al., 1986). It has been suggested that increased blood flow is an important factor in the induction of capillary proliferation (Hudlicka & Price, 1990). This might have been the initiating process for the capillary proliferation observed in our study.

Although the capillary supply to type I and IIa fibres, in terms of CFD, in the deep region of hypertrophied muscles was decreased, no decreased fatiguability was observed in these muscles, compared with their control counterparts, as assessed by the Burke fatigue test (Degens et al., 1990). Both these results and the unchanged glycogen depletion as related to the oxidative capacity during prolonged treadmill exercise (Riedy et al., 1985) suggest that

the oxygen supply is sufficient. Our results indicate that this is at least partly accomplished by capillary proliferation.
It is interesting to note that our results show the opposite trend to that found with denervation atrophy in human skeletal muscle, where the number of capillaries per transverse muscle fibre area tended to increase and C/F to decrease, indicating loss of capillaries (Carpenter & Karpati, 1982).

Conclusion

In conclusion, we have found that in both normal and hypertrophied rat plantaris muscle capillary density was higher in the deep (oxidative) region than in the superficial (glycolytic) region. The opposite was found for the heterogeneity in capillary spacing. The capillary supply to muscle fibres was dependent on their metabolic character, cross-sectional area and the metabolic character of surrounding fibres. Training did not result in significant changes in fibre type composition, fibre cross-sectional areas and capillarisation. In hypertrophied muscles the relation between capillary supply and fibre types was not significantly changed when compared with normal muscles. Furthermore, with hypertrophy obtained by denervation of synergists, a concomitant capillary proliferation was found which lagged behind the increase in muscle mass, at least in the deep region of the muscle.

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4. COMPENSATORY HYPERTROPHY AND TRAINING EFFECTS ON THE FUNCTIONING OF AGEING RAT M. PLANTARIS

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SUMMARY

The influence of age, compensatory hypertrophy and training on isometric contraction characteristics of rat m. plantaris were investigated in 5, 13 and 25-month-old rats. Each age group was subdivided into Control Not Trained, Control Trained, Operated Not Trained and Operated Trained groups. Run training was applied. The Operation i.c. denervation of synergists induced a 30% compensatory hypertrophy ($P < 0.001$). Age and training did not significantly affect muscle weight. The tetanic force was highest in 13-month-old and lowest in 25-month-old muscles as was the tetanic force/muscle weight ($P < 0.001$). Trained and hypertrophied muscles had increased tetanic force ($P < 0.01$), but tetanic force/muscle weight was not significantly affected. Twitch contraction time was longer in hypertrophied muscles than in controls ($P < 0.001$). Half relaxation time decreased with training ($P < 0.05$). Resistance to fatigue was increased in hypertrophied muscles as compared to controls ($P < 0.04$) and was lower in 5-month-old than in 13-month-old muscles, but their fatigue resistance did not differ from the 25-month-old muscles. Each age group showed comparable effects of training and hypertrophy on the contraction characteristics. These results indicate that the force generating capacity of the m. plantaris was optimal in the 13-month-old animals compared to the other age groups. Furthermore, our data show that aged muscles react in a similar way to increased functional demand as muscles of younger age.

INTRODUCTION

Most studies on ageing described a decrease in muscle mass and a concomitant decrease in the maximum tetanic tension with increasing age (Campbell et al., 1991; Fitts et al., 1984; Grimby and Saltin, 1983; Klitgaard et al., 1989). In addition it was found in some investigations that maximum tetanic force/muscle weight ratio decreased with ageing (Campbell et al., 1991), whereas others reported no change in this ratio (Fitts et al., 1984; Grimby and Saltin, 1983). Also age-associated increases in the twitch contraction time (Larsson and Salviati, 1989) and half relaxation time (Fitts et al., 1984; Larsson and Salviati, 1989) were reported. As observed in plantar flexors it was found that the fatigue resistance decreased with increasing age (Irion et al., 1987) or remained the same as was

found for the m. soleus (Klitgaard et al., 1989).
In young adult rats compensatory hypertrophy of the m. plantaris could be obtained by denervation of its synergists. This hypertrophy was characterised by a concomitant increase in maximal tetanic force (Binkhorst, 1969; Degens et al., 1990; Michel et al., 1989; Roy et al., 1982). The same hypertrophy could be obtained in plantaris muscles of old rats (Pettigrew and Noble, 1991; Tomanek and Woo, 1970). It is not known, however, whether the tension generating capacity of old muscle would change with compensatory hypertrophy. Our investigation concerned the contraction characteristics and resistance to fatigue of aged compensatorily hypertrophied m. plantaris. Also the influence of an additionally applied moderate endurance training on the isometric contraction characteristics was investigated. These measurements were done in young adult, adult and old rats, to study the influence of ageing and whether muscles of old rats can adapt in a similar way as muscles of young adult and adult rats to increased functional demands. Three age groups were studied to obtain a better insight in the influence of ageing than when using two age groups as is often done.

MATERIALS AND METHODS

Animals

Female Wistar rats were used. These were kept 10 to a cage until use. During the experimental period the rats, including controls, were housed individually. The environment was maintained at 22°C with 12 h light and 12 h darkness. Food and water were provided ad libitum. The rats were randomly assigned to three age groups with ages of 5, 13 and 25 months respectively at the time of the contraction measurements. The rats of each age group were divided over a Control Not Trained (CNT), Control Trained (CT), Operated Not Trained (ONT) and Operated Trained (OT) group. Animals that had large tumours, were apparently sick, moribund or had extremely atrophied m. plantaris, i.e. muscle weight less than 50% of mean muscle weight in the corresponding group, were excluded from the experiments.

Operation

The rats were anaesthetised with pentobarbital sodium (60 mg/kg i.p.) The operation that resulted in compensatory hypertrophy of the left m. plantaris consisted of denervation of the m. soleus and m. gastrocnemius by cutting the nerves as distally as possible and transplantation of the distal tendon of the m. plantaris to the tendon of the m. gastrocnemius. To prevent reinnervation of the m. soleus and m. gastrocnemius their nerves were sewed into the m. semimembranosus or the m. biceps femoris (Binkhorst, 1969). At the

time of the contraction measurements no reinnervation was found.

Training

The day after the operation both CT and OT rats were placed for 15 min on a treadmill set at an inclination of 30° and at zero speed to make them familiar with the treadmill. During this period the rats were not forced to walk. So they had a recovery period of approximately 48 h. The next four days the time of running at a speed of 320 m/h and an inclination of 30° was gradually increased from 20 min, on the second day after the operation, to 2 h. Rats having to much problems with running uphill during these days were taken from the belt before the end of a session, but all rats were able to fulfill the 2 h run after this period. This training program was carried out 5 days a week for the next 5 weeks. The rats were forced to run by mechanical or electrical stimulation. The objective of this training was to put an additional stress on the muscle to obtain a further hypertrophy and an increased endurance capacity.

Measurements

The rats were anaesthetised with pentobarbital sodium (60 mg/kg i.p.). The body temperature and muscle temperature were maintained at 35 $\pm$ 1°C. The m. plantaris was prepared free, keeping the blood supply intact. The leg was fixed at the point of the condyli of the femur to ensure isometric contractions. The distal tendon of the muscle was connected to a force transducer (Statham Transducing cell UC2, load cell accessory UL4-5). Stimulation and on line data sampling were done with a computer. The isometric contractions were elicited by supramaximal stimulation of the muscle with double pulses via the n. ischiadicus. Each pulse was a square pulse of 0.2 ms duration and an amplitude of 3 V for the 5 and 13-month-old rats and 5 V for the 25-month-old rats. Five V stimulation was applied to the 25-month-old rats for 3 V stimulation was not always maximal for those rats. There was a time lapse of 0.05 ms between the double pulses to prevent a back-response (Brown and Matthews, 1960). The muscle was set at its optimal length (L_0), defined as the length at which the muscle produced its maximal twitch force with a minimal twitch duration. During the entire experiment each 30 s a twitch was elicited to keep the preparation in a steady state. The first five twitch contractions were analysed. Measured were the twitch force (FTW), twitch time to peak tension (TPT) and twitch half relaxation time (½RT). After this, two tetani 5 min apart from each other were elicited by a 185 Hz pulse train of 330 ms duration. The maximal tetanic force (FTET) was then determined. When the FTET of the second tetanus was decreased 5% or more when compared to the first tetanus, the rat was excluded from the contraction experiments. Each tetanus was followed by a 5-min rest period with only double pulses each 30 s. Then the fatigue sensitivity of the muscle was measured. This was done by stimulating the muscle by 330 ms

bursts of 40 Hz each second during 4 min (Burke et al., 1973). A fatigue index (FAT) was calculated as the ratio between the peak force of the contraction encountered 2 min after the strongest contraction and the peak force of the strongest contraction during the test. This FAT is the same as the fatigue-index A of Kernell et al. (1983). Then the muscle was rapidly excised and the wet weight was determined on an analytical balance. The rats were killed under anaesthesia by excision of the heart.

Statistics

ANOVA was applied to test for age, operation, and training effects. When an age effect was found a *t*-test was applied to test for differences between age groups and then a Bonferroni correction was done (Wallenstein et al., 1980). Differences were considered significant at $P < 0.05$. Unless stated otherwise, mean values are given $\pm$ SEM.

RESULTS

By applying an ANOVA it was possible to study the effects of ageing, operation and training separately. Only when interactions were present this indicated that a combination of the respective effectors gave a different outcome than these effectors on their own (Wallenstein et al., 1980).

Animals

Both body weight at the start of the experimental period (203 $\pm$ 3 (38); 311 $\pm$ 5 (39); 319 $\pm$ 6 (30); mean $\pm$ SEM (n) in g of 5, 13 and 25-month-old rats respectively) and at the time of the contraction measurements were lower for the 5-month-old rats than for the other age groups (table 4.1 and 4.3). The operated rats had lower body weights at the time of the experiments than the control counterparts in each age group (table 4.1 and 4.3).

Training

Table 4.1, 4.3 and fig. 4.1 show that the muscle weight/body weight ratio is increased with training. This is also found for the twitch force and tetanic force (table 4.2 and 4.3). Training resulted in a shortened twitch half relaxation time (16.0 $\pm$ 0.3 (48); 17.0 $\pm$ 0.4 (51); mean $\pm$ SEM (n) in ms; Trained vs. Control) (table 4.3), which may not be functionally meaningful. No changes in the Fatigue resistance were found as a result of the applied training.

Table 4.1. *Body and muscle weights.*

	5 MONTHS				13 MONTHS				25 MONTHS			
	CNT	CT	ONT	OT	CNT	CT	ONT	OT	CNT	CT	ONT	OT
MW (mg)	329 ± 18 (10)	330 ± 16 (9)	422 ± 22(8)	446 ± 13 (8)	313 ± 24 (10)	364 ± 15 (10)	431 ± 21 (9)	461 ± 26 (9)	332 ± 12 (10)	400 ± 17 (6)	461 ± 28 (9)	447 ± 24 (6)
BW2 (g)	233.2 ± 8.7 (10)	215.9 ± 5.9 (10)	215.5 ± 3.5 (9)	213.8 ± 4.4 (9)	300.1 ± 12.1 (10)	306.1 ± 6.4 (10)	294.5 ± 8.0 (9)	276.2 ± 9.1 (10)	311.7 ± 12.0 (10)	301.1 ± 10.1 (7)	294.1 ± 12.6 (9)	283.1 ± 9.3 (6)
MW/BW2 (g/g * 100%)	0.142 ± 0.009 (10)	0.155 ± 0 008 (9)	0.198 ± 0.013 (8)	0.208 ± 0.007 (8)	0.112 ± 0.008 (10)	0.119 ± 0.004 (10)	0.146 ± 0.005 (9)	0.166 ± 0.008 (9)	0.107 ± 0.003 (10)	0.133 ± 0.002 (6)	0.156 ± 0.005 (9)	0.152 ± 0.012 (6)

CNT: Control Not Trained; CT: Control trained; ONT: Operated Not Trained; OT: Operated trained. Values are mean ± SEM; number of animals is given in parentheses.
MW: Wet weight of m. plantaris; BW2: body weight at time of contraction measurements; MW/BW2: muscle weight/body weight.

Table 4.2. *Contraction characteristics of m. plantaris.*

	5 MONTHS				13 MONTHS				25 MONTHS			
	CNT	CT	ONT	OT	CNT	CT	ONT	OT	CNT	CT	ONT	OT
TPT (ms)	16.5 ± 0.7 (8)	16.9 ± 0.4 (9)	19.6 ± 0.9 (8)	19.5 ± 0.5 (8)	14.6 ± 0.4 (9)	14.1 ± 0.4 (8)	15.0 ± 0.5 (8)	15.7 ± 0.6 (10)	14.8 ± 0.4 (10)	15.2 ± 0.4 (7)	16.7 ± 0.8 (8)	16.8 ± 0.7 (6)
FTW (N)	0.79 ± 0.08 (8)	1.04 ± 0.07 (9)	1.30 ± 0.14 (8)	1.34 ± 0.12 (8)	1.20 ± 0.07 (9)	1.23 ± 0.05 (8)	1.14 ± 0.06 (8)	1.32 ± 0.08 (10)	0.97 ± 0.06 (10)	1.13 ± 0.12 (7)	1.24 ± 0.09 (8)	1.38 ± 0.16 (6)
FTET (N)	5.65 ± 0.27 (8)	5.95 ± 0.34 (9)	7.19 ± 0.26 (8)	7.57 ± 0.28 (8)	6.28 ± 0.38 (9)	6.98 ± 0.28 (8)	8.38 ± 0.37 (8)	9.15 ± 0.39 (10)	4.94 ± 0.36 (10)	5.77 ± 0.42 (7)	6.99 ± 0.53 (8)	7.28 ± 0.33 (6)
FTW/-FTET	0.14 ± 0.01 (8)	0.18 ± 0.002 (9)	0.18 ± 0.02 (8)	0.18 ± 0.01 (8)	0.19 ± 0.01 (9)	0.18 ± 0.004 (8)	0.14 ± 0.01 (8)	0.14 ± 0.01 (10)	0.20 ± 0.02 (10)	0.20 ± 0.01 (7)	0.18 ± 0.02 (8)	0.19 ± 0.02 (6)
FAT	0.29 ± 0.04 (8)	0.35 ± 0.03 (9)	0.42 ± 0.02 (8)	0.38 ± 0.08 (7)	0.40 ± 0.05 (7)	0.42 ± 0.05 (8)	0.51 ± 0.02 (8)	0.47 ± 0.03 (10)	0.30 ± 0.07 (10)	0.45 ± 0.08 (5)	0.32 ± 0.05 (7)	0.48 ± 0.08 (6)

CNT: Control Not Trained; CT: Control Trained; ONT: Operated Not Trained; OT: Operated Trained. Values are mean ± SEM; number of animals is given in parentheses.
TPT: twitch time to peak tension; FTW: twitch force; FTET: tetanic force; FTW/FTET: twitch/tetanus ratio; FAT: fatigue index.

Table 4.3. *The ANOVA results for each of the determined variables.*

	Train	Oper	Age	Interaction Oper Age
BW1 (g)			P < 0.001	
BW2 (g)		P < 0.03 ↓	P < 0.001	
MW (mg)	*	P < 0.001 ↑		
MW/BW2 (g/g * 100%)	P < 0.01 ↑	P < 0.001 ↑	P < 0.001	
TPT (ms)		P < 0.001 ↑	P < 0.001	
½RT (ms)	P < 0.05 * ↓			
FTW (N)	P < 0.02 ↑	P < 0.001 ↑		P < 0.02
FTET (N)	P < 0.01 ↑	P < 0.001 ↑	P < 0.001	
FTW/FTET		*	P < 0.005 *	P < 0.005 *
FTET/MW (N/mg)			P < 0.001	
FAT		P < 0.03 ↑	P < 0.05	

BW1: body weight at start of the experiment; ½RT: twitch half relaxation time; FTET/MW: tetanic force per muscle weight. For other abbreviations see table 1 and 2. *: These P-values different when omitting rats with atrophied fibres as seen after histochemical examination, all values remained significant except the training effect on ½RT (P = 0.053); FTW/FTET operation effect (P = 0.042) and MW train effect (P = 0.033) became significant.

Operation

The operation resulted in a 30% increase in muscle wet weight at all ages (table 4.3) which is also reflected in the increased muscle weight/body weight ratio (table 4.1, 4.3 and fig. 4.1). This increase in muscle weight is accompanied by an increased tetanic force (table 4.2 and 4.3). The Operation effects on twitch force will be described under the heading "Interactions". The operation resulted in a prolonged twitch time to peak tension (table 4.2, 4.3 and fig. 4.3). The Operated group also showed an increased Fatigue resistance (table 4.2, 4.3 and fig. 4.4).

Ageing

The muscle weight/body weight ratio was highest for the 5-month-old rats and did not differ significantly between the 13 and 25 month-old rats (table 4.1, 4.3 and fig. 4.1). This indicates that the increase in muscle weight was stabilised earlier than the increase in body weight and might reflect the reported increase in body fat content with ageing (Larsson and Edström, 1986). There were, however, no significant differences in muscle weight between the age groups (table 4.1). The tetanic force of the 13-month-old muscles was higher than

the tetanic force of both 25 and 5-month-old muscles (table 4.2 and 4.3). The tetanic force/muscle weight ratio was highest in the 13-month-old muscles and lowest in the 25-month-old muscles with the 5-month-old muscles in between (0.017 ± 0.003 (31); 0.020 ± 0.03 (34); 0.015 ± 0.03 (30); mean ± SEM (n) in N/mg of 5, 13 and 25-month-old muscles, respectively) (table 4.3 and fig. 4.2). The age effects on twitch/tetanus will be described under the heading "Interaction". The 5-month-old muscles showed a longer twitch time to peak tension as muscles of the other ages (table 4.2, 4.3 and fig. 4.3). The fatigue resistance was higher for 13-month-old, than for 5-month-old muscles, but both did not differ from the Fatigue resistance of 25-months-old muscles (table 4.3 and fig. 4.4).

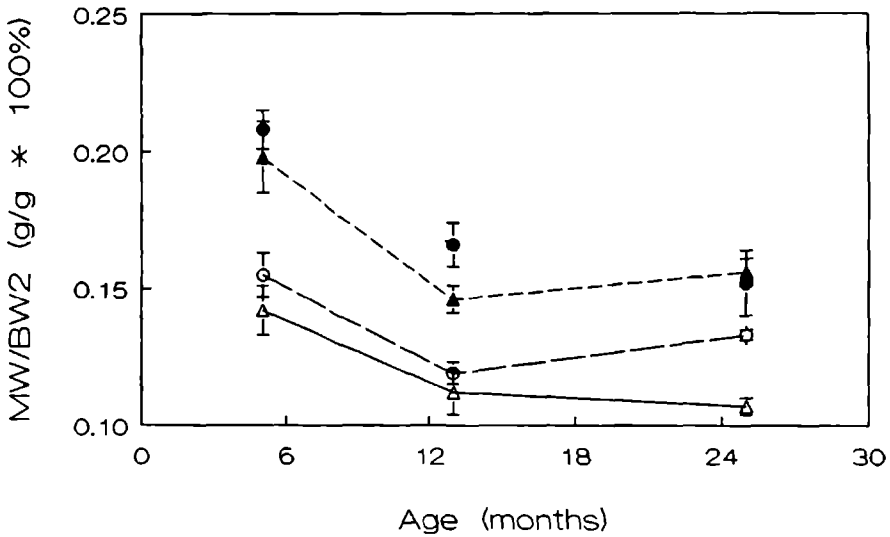

Fig. 4.1: Muscle weight/body weight ratios (MW/BW2) of rat m. plantaris of different age (mean ± SEM). The collecting line is drawn to point out the general age trend. Mind that for each age group different animals were used. CNT: Control Not Trained Δ——; CT: Control Trained O— —; ONT: Operated Not Trained ▲–; OT: Operated Trained ●... Operation effect ($P < 0.001$); Age effect ($P < 0.0001$); 5-months-old rats higher than other ages.

Interactions

Only age-operation interactions were found for twitch force and twitch/tetanus ratio (table 4.2 and 4.3). The twitch force only increased in the 5 and 25-month-old muscles but showed only minor changes in the 13-month-old muscles as a result of the operation. The twitch/tetanus ratio in 13-month-old muscles decreased as a result of the Operation, most probable due to the interaction for the twitch force as described.

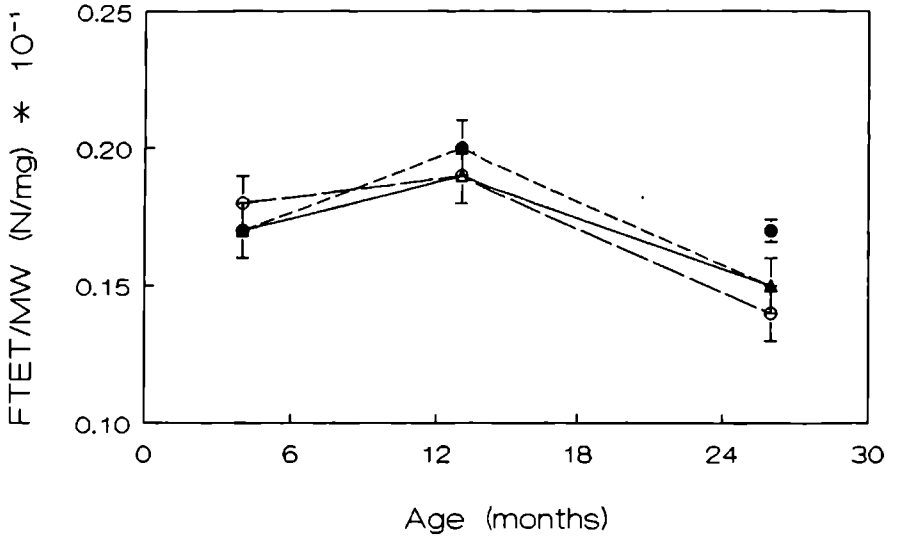

Fig. 4.2: Tetanic force/muscle weight (FTET/MW) of m. plantaris of different ages (Mean ± SEM). The collecting line is drawn to point out the general age trend. Mind that for each age group different animals were used. CNT: Control Not Trained Δ——; CT: Control Trained O— —; ONT: Operated Not Trained ▲--; OT: Operated Trained ●... Age effect ($P < 0.001$); 13-month-old muscles highest, 25-month-old muscles lowest and 5-month-old muscles in between.

DISCUSSION

Training

The applied training showed only minor effects. It may be relevant that the training program had also no significant effects on capillarisation, fibre type proportions and fibre cross-sectional areas of the m. plantaris of young rats (Degens et al., 1992).

Ageing

The present study compared data of 5, 13 and 25-month-old female Wistar rats.

The tetanic force/muscle weight was highest in the 13 and lowest in the 25-month-old muscles. Others reported also decreased tetanic force and tetanic force/muscle weight in 25-month-old muscles as compared to 11-month-old m. plantaris (Campbell et al., 1991) or described a decline in tetanic force and a decreased tetanic force/cross sectional muscle area from 9-month-old muscles to 24-month-old muscles (Klitgaard et al., 1989). Combination of the results of these investigations suggests that the force generating capacity per muscle mass of the m. plantaris has an optimum at the age of about 1 year. It also signifies the

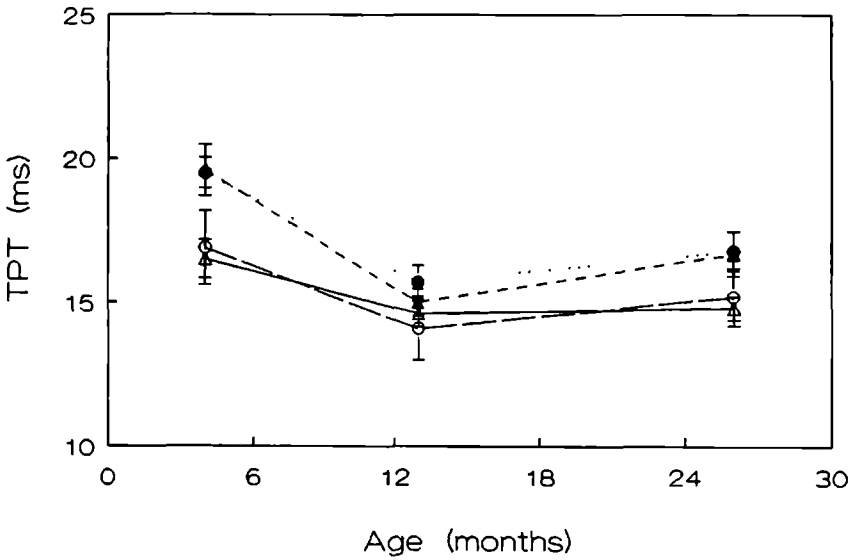

Fig. 4.3: Twitch Time to Peak Tension (TPT) of m. plantaris of different ages (Mean ± SEM). The collecting line is drawn to point out the general age trend. Mind that for each age group different animals were used. CNT: Control Not Trained Δ——; CT: Control Trained O— —; ONT: Operated Not Trained ▲--; OT: Operated Trained ●... Operation effect ($P < 0.001$); Age effect ($P < 0.001$); 5-month-old muscles longer than muscles of other ages.

importance of using more than two age groups in ageing studies. In contrast to the reported increased twitch contraction time of fast twitch muscles (Fitts et al., 1984) or fast twitch muscle fibres (Larsson and Salviati, 1989) with ageing we found a decrease. In a recent study on 9, 24 and 29-month-old rats no age associated changes in m. plantaris twitch contraction time were found (Klitgaard et al., 1989). Combination of our results and the results of Klitgaard et al. (1989) indicates that the Twitch contraction time of the m. plantaris is decreased in 9-month-old muscles as compared to 5-month-old muscles whereafter it remains constant. The similar myosin activity in the m. plantaris of 9, 24 and 29-month-old muscles, which correlated strongly to the twitch contraction time (Klitgaard et al., 1989) might explain the comparable twitch time to peak tension of 13 and 25-month-old plantaris muscles in our study also.

Our experiments showed that the fatigue resistance initially increased followed by no significant changes later on. It is reported that plantar flexors (i.c. m. soleus, m. plantaris plus m. gastrocnemius) show a reduction in the resistance to fatigue, as assessed by intermittent isometric tetanic contractions at 100 Hz, from the age of 12-24 months old (Irion et al., 1987), whereas the soleus showed no age effects with that stimulation pattern

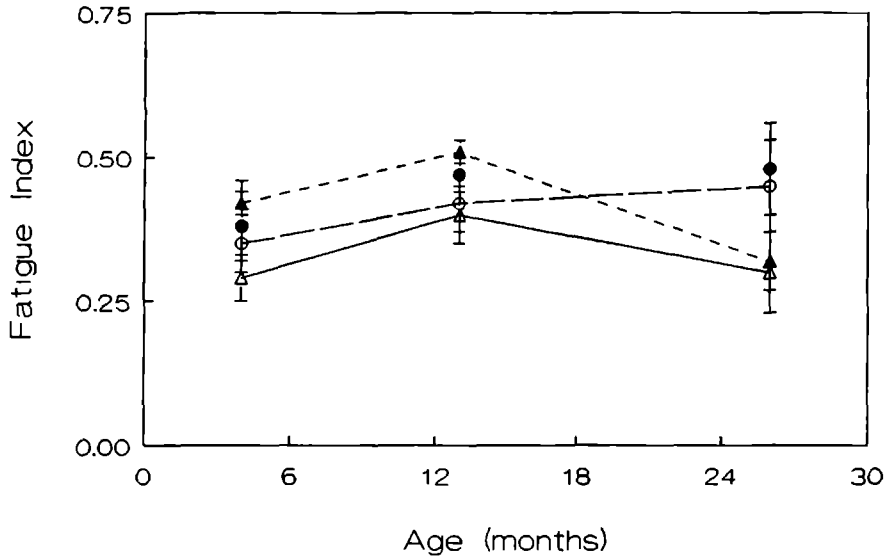

Fig. 4.4: Fatigue Index (FAT) of m. plantaris of different ages. The collecting line is drawn to point out the general age trend. Mind that for each age group different animals were used. CNT: Control Not Trained Δ——; CT: Control Trained O— —; ONT: Operated Not Trained ▲--; OT: Operated Trained ●... Operation effect ($P < 0.03$); Age effect ($P < 0.04$); 5-months-old muscles lower than 13-months-old muscles.

(Klitgaard et al., 1989). The difference between our findings and those studies concerning fatigue resistance changes with ageing might be due to the different fatiguing stimulation pattern. Pettigrew and Noble (1991) applying the same stimulation pattern, found an increase in S (slow) and transitional motor unit proportions, both with a high resistance against fatigue, which from 5 to 14 months occurred at the expense of FF (fast fast-fatiguable) and from 14 to 20 months at the expense of FINT (fast intermediate-fatiguable) units in the m. plantaris. This might explain our findings on the fatigue resistance of whole m. plantaris with ageing, showing an initial increase followed by no significant changes later on.

Operation

The results of the present study and the results of other studies (Roy et al., 1982; Pettigrew and Noble, 1991) on muscle weight show that compensatory hypertrophy is not limited by age. The not significantly changed tetanic force/muscle weight, indicates that the increase in muscle weight with compensatory hypertrophy can be ascribed to an identical relative increase in contractile material and non-contractile material. Indeed, the connective tissue/muscle ratio was found to be similar in compensatorily hypertrophied m. plantaris of

5-month-old rats and their age-matched controls, and was even lower in compensatorily hypertrophied old muscles than in their age-matched controls (Tomanek and Woo, 1970). The twitch force, however, did not increase with compensatory hypertrophy in the 13-month-old muscles, thus resulting in a decreased twitch/tetanus ratio in muscles of this age. No explanation could be found for this finding. Nevertheless, it indicates that the twitch force is not necessarily related to muscle weight.

Consistent with earlier findings in young muscles (Degens et al., 1990; Michel et al., 1989; Roy et al., 1982) compensatorily hypertrophied muscles of older ages also had prolonged twitch contraction times. This might be due to an increased amount of type I fibres (Degens et al., 1992) and an increased proportion of transitional motor units with a simultaneous decreased proportion FF units as was found in young muscles (Pettigrew and Noble, 1991). In 14-month-old muscles an increased proportion of S motor units (Pettigrew and Noble, 1991), might explain the increased twitch contraction time with compensatory hypertrophy. In 20-month-old muscles, however, no significant changes in motor unit proportions occurred as a result of compensatory hypertrophy (Pettigrew and Noble, 1991). Thus this could not explain the increased twitch contraction time with compensatory hypertrophy in senescent muscles. It might be that the increased twitch contraction time could be put down to an increase in connective tissue. This, however, does not seem to be the case as the tetanic force per muscle weight did not significantly change with hypertrophy.

Compensatory hypertrophy resulted in an increased fatigue resistance in each age group, as was also reported in other studies on young m. plantaris (Michel et al., 1989; Roy et al., 1982). In young muscles this may result from an increased proportion of type I fibres and a decreased proportion of type IIb fibres, as was found at least in the deep region of the m. plantaris (Degens et al., 1992) and confirmed also for the other age groups (unpublished results). Furthermore, the findings in this study fit the reported decreased proportion of FF units with a simultaneous increase in transitional units with compensatory hypertrophy in young m. plantaris (Pettigrew and Noble, 1991). However, in 14-month-old muscles an increased proportion of S motor units and a simultaneous decreased proportion of FR (fast fatigue-resistant) and transitional units, all showing a high resistance against fatigue, were found with hypertrophy, while in 20-month-old muscles no changes were found with hypertrophy (Pettigrew and Noble, 1991). Thus in aged m. plantaris the increase in fatigue resistance with hypertrophy cannot be explained by the shifts in motor unit proportions.

Conclusion

In conclusion it appears that both muscle tetanic force and tetanic force/muscle weight at first increases and after a certain age declines. These findings signify the importance of using more than two age groups when studying age effects. Induction of compensatory hypertrophy is not limited by age. Furthermore, tension generating capacity enhances at all

ages after induction of compensatory hypertrophy such that the maximum tetanic force per muscle weight remains the same. The resistance against fatigue was higher in compensatorily hypertrophied muscles than in their age matched controls. Thus muscles of different age can adapt in a similar way to increased functional demands. The applied training showed only minor effects on the contraction characteristics in each age group.

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5. CAPILLARISATION AND FIBRE TYPES IN COMPENSATORILY HYPERTROPHIED M. PLANTARIS IN DIFFERENTLY AGED RATS

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SUMMARY

Influences of age and hypertrophy obtained through denervation of synergists and training on the capillarisation of the m. plantaris were investigated in rats of 5, 13 and 25 months old. The operation resulted in about 30% hypertrophy in all the age groups. Age effects were significant only in the deep (more oxidative) region of the muscle. From 5 to 13 months, the percentage of IIa fibres increased to the expense of the IIb fibres, while the fibre cross-sectional areas (FCSA) of each fibre type increased. From 13 to 25 months, the FCSA of IIb fibres decreased, as did the local capillary-to-fibre ratio (LCFR) of all the fibre types, indicating capillary loss. Also the capillary density for each fibre type (CFD) declined, indicating a decreased capillary supply per FCSA. Hypertrophy effects were identical for the deep and superficial regions and for each age group. With hypertrophy, the FCSA and LCFR of each fibre type increased, while the CFD decreased. This suggests that even in old age, capillary proliferation occurred with hypertrophy, although it lagged behind the increases in the FCSAs. Training showed only minor effects.

INTRODUCTION

The age-related decline in exercise and respiratory capacity of rat hindlimb muscles (Cartee and Farrar, 1987) might be due to changes in fibre type composition. Most studies on ageing describe an age-associated decrease in the number of fibres (Ishihara and Araki, 1988) and age-related fibre atrophy in rat muscles (Ishihara and Araki, 1988; Klitgaard et al., 1989; Kovanen, 1989). Furthermore, in the m. rectus femoris, the percentage of type IIa and I fibres increases and the percentage of type IIb fibres decreases with age (Kovanen, 1989). In the m. plantaris of the rat, the percentage of type IIb increases in the deep part of the muscle but decreases in the superficial part with increasing age (Klitgaard et al., 1989). Others, however, have found no changes in fibre cross-sectional areas, fibre number and distribution with age (Eddinger et al., 1985).

Glycolytic muscles have lower capillary densities than oxidative muscles (Egginton et al., 1988; Ripoll et al., 1979). This relation holds true on the cellular level in 5-month-old rats, showing that the capillary supply per fibre cross-sectional area is higher for oxidative than for glycolytic fibres (Degens et al., 1992). Furthermore, the capillary supply to a fibre is positively related to its fibre cross-sectional area (Degens et al., 1992; Egginton et al., 1988; Gray and Renkin, 1978; Ripoll et al., 1979). Thus, age-associated changes in fibre type composition and their fibre cross-sectional area, as described above, might be related to changes in the capillary supply to a fibre and to whole muscle capillarisation.

In a previous study, capillary proliferation was found with compensatory hypertrophy in the m. plantaris of 5-month-old rats (Degens et al., 1992). Similar hypertrophy could be obtained in aged m. plantaris (Tomanek and Woo, 1970). In this study, capillarisation was not estimated, so no indication of capillary proliferation with compensatory hypertrophy was obtained in aged muscle.
The aim of the present study was to assess age-associated changes in capillarisation related to fibre types. Fibre type composition and fibre cross-sectional area in aged rat m. plantaris were investigated and the influence of compensatory hypertrophy of aged m. plantaris was determined on these variables. We also studied the influence of training added to compensatory hypertrophy on the determined variables. It is shown here that the number of capillaries was lowest in the muscles of old rats. However, capillary proliferation with compensatory hypertrophy was similar in each age group, even in the muscles of old rats.

MATERIALS AND METHODS

Animals and treatment allocation

Female Wistar rats were used. During the experimental period the rats were kept, one to a cage, in a room maintained at 22°C with 12 h light and 12 h darkness per 24 h period. Food and water were provided ad libitum. The rats were assigned at random to four treatments within the age groups 5, 13 and 25 months old at the time of the contraction measurements. The four treatment groups were: Control Not Trained (CNT), Control Trained (CT), Operated Not Trained (ONT) and Operated Trained (OT). Animals were excluded from the experiments if they had large tumours, were apparently sick, moribund or had extremely atrophied m. plantaris, i.e. muscle weight of less than 50% of the mean muscle weight in the age group.

Operation

The rats were anaesthetised with pentobarbital sodium (60 mg/kg i.p.). The operation resulting in compensatory hypertrophy of the left m. plantaris consisted of denervation of the soleus and gastrocnemius muscles. The nerves were cut as far distally as possible. As the tendon of the m. plantaris of the rat is not part of the Achilles tendon but ends in the plantar aponeurosis, its distal tendon was transplanted to the tendon of the m. gastrocnemius. In this way the m. plantaris could take over the function of the triceps surae better. Regeneration of the nerves was prevented by attaching them with ligatures into the m. semimembranosus or the m. biceps femoris (Binkhorst, 1969). At the time of the contraction measurements, no reinnervation was found.

Training

The day after the operation, both the CT and OT rats were placed on a treadmill set at an inclination of 30° and at zero speed for 15 min to make them familiar with the treadmill. Over the next four days, the time of running at a speed of 320 m/h with an inclination of 30° was gradually increased from 20 min on the second day after the operation to 2 h. This training programme was carried out 5 days a week for the next five weeks. The objective of

this training was to put additional stress on the plantaris muscle to obtain further hypertrophy and greater endurance capacity.

Muscle preparation
Directly following isometric contraction measurements under pentobarbital anaesthesia as described elsewhere (Degens et al., 1993), the plantaris muscles were excised, mounted at their L_0 on cork and frozen in isopentane cooled in liquid nitrogen. L_0 was defined as the length at which the muscle produces its maximal twitch force with a minimal twitch duration. The muscles were stored at -25°C or less until processed. Cross-sections (12 μm) were cut in a cryostat at -25°C and stored at -135°C until staining.

Histochemistry
Sections of the m. plantaris were treated at room temperature for myofibrillar ATP-ase activity at pH 9.4 after 5 min preincubation between pH 4.3 and pH 4.35 (Guth and Samaha, 1970) to classify fibres as type I or II. Adjacent sections were assayed for SDH activity at 37°C (Pool et al., 1979) to subclassify type II fibres into IIa and IIb fibres. Classification of identical fibres in identical sections was repeated every half year during a period of two years and the estimations of the fibre type composition were compared to each other. The errors found in the mean percentage type IIa and IIb fibres varied between 2.9% to 4.4% and for type I fibres between 0.27% to 0.4%. Statistical analyses showed that there were no systematic errors and no significant differences in mean fibre type composition. Thus this method of fibre type classification is well reproducible.
In a consecutive section, the capillaries were stained by a modification of the combined staining method for AP (Alkaline Phosphatase) and DPP-IV (Dipeptidyl Peptidase IV). This method demonstrates both the arterial and venular portions of a capillary, whereas after AP staining alone, the venular portion of a capillary can remain unstained (Degens et al., 1992; Mrazkova et al., 1986).

Histological Observations
The morphometric analysis was performed on photomicrographs of the deep region near the head of the tendon, and the superficial region near a large blood vessel in the core of the plantaris muscle. Each photomicrograph contained 37 - 155 fibres and one photomicrograph was taken of each region of each muscle. For each photomicrograph, the fibre type (I, IIa and IIb) composition was assessed. Using a digitising tablet, fibre outlines of complete fibres were read into the computer as contour coordinates and capillary locations were read in as coordinates of the capillary centres. The overall capillary density (CD) was defined as the number of capillaries per square millimetre of tissue (muscle fibres plus intercellular space). Traditionally, the capillarisation of a muscle is described by the overall indices capillary density (CD) and capillary to fibre ratio (C/F). These indices do not take into account the presence of different fibre types in a muscle and therefore give little information about capillarisation in relation to fibre types. The heterogeneity in capillary spacing in a muscle is not indicated by these parameters either although this heterogeneity may markedly influence the oxygenation of a muscle (Turek et al., 1991). The method of capillary domains developed at our Department (Hoofd et al., 1985), estimates capillary density and

heterogeneity of capillary spacing. This method was expanded in order to relate these indices to fibre types. A capillary domain is an area bounded by lines perpendicular to the midpoint of lines between adjacent capillaries. This was done by a computer programme (Degens et al., 1992; Egginton et al., 1988; Hoofd et al., 1985). With this programme the surface area of each domain was calculated. Domain surface areas have a lognormal distribution (Egginton et al., 1988; Hoofd et al., 1985). Therefore, the logarithmic standard deviation of the domain areas (i.e., SD of log-transformed variates, σlog10x, usually denoted by the abbreviation LogSD) gives an indication of the heterogeneity of the capillary spacing. Fibre cross-sectional areas were derived from complete fibre contours on these photomicrographs. Where domains and muscle fibres overlapped, two indices were derived. First, the local capillary-to-fibre ratio (LCFR) was determined for each fibre. It was defined as the sum of the fractions of each domain area overlapping the fibre. As one domain constitutes the region geometrically supplied by one capillary, the local capillary-to-fibre ratio can be interpreted as the sum of fractions of capillaries geometrically interacting with a muscle fibre. Under the assumption of an unchanged fibre number, an increase in the local capillary-to-fibre ratio indicates capillary proliferation. On the other hand, a decrease indicates capillary loss. Secondly, the local capillary-to-fibre ratio divided by the cross-sectional area of that fibre will provide the capillary density for that particular fibre, the capillary fibre density (CFD). Thus these indices offer the opportunity of estimating capillarisation in relation to separate fibre types, type I, IIa and IIb fibres. The same indices have been used in other reports (Degens et al., 1992; Egginton et al., 1988) and can be seen as a refinement of the indices introduced by Gray and Renkin (1978).

Table 5.1. *Body weight (BW) and muscle weight (MW).*

Age/Group	5C	5O	13C	13O	25C	25O
BW (g)	225 ± 6 (20)	215 ± 3 (18)[a]	303 ± 7 (20)[b]	285 ± 6 (19)[a]	307 ± 8 (17)[b]	296 ± 11 (15)[a]
MW (mg)	329 ± 12 (19)	434 ± 13 (16)[a]	348 ± 14 (20)	446 ± 16 (18)[a]	357 ± 13 (13)	456 ± 19 (15)[a]

Groups: 5, 13, 25: age of rats in months; C: Control group; O: Operated group. Values are mean ± SEM; number of animals in parentheses. [a]: Operation different from Control; [b]: Different from 5 months.

Statistics

ANOVA was applied to test for training, age and operation effects and two-way interactions assuming the absence of three-way interactions. Any interaction would indicate that a combination of the respective effectors gave a different outcome than either of the effectors on their own (Wallenstein et al., 1980). When age effects were found by the ANOVA the *t*-test was applied to test for differences between these groups. Differences were considered to be significant at $P < 0.05$. A Bonferroni correction for multiple testing was applied (Wallenstein et al., 1980). Fibre type distribution, fibre cross-sectional areas, local capillary-to-fibre ratio and capillary fibre density were tested. Capillary fibre density was calculated as the mean local capillary-to-fibre ratio divided by the mean corresponding fibre

area for each photomicrograph. The data for the fibre cross-sectional area and capillary fibre density were log-transformed, because of the skewness of the raw data, to make them suitable for application of the ANOVA. Unless stated otherwise, mean values are given ± SEM.

RESULTS

Table 5.1 shows the body and muscle weights. Table 5.2 gives the data on capillary density and heterogeneity of capillary spacing. Table 5.3 shows the data for which operation training interactions were found. The other P values obtained by ANOVA are given in table 5.4.

Table 5.2. *Capillary density (CD) and capillary spacing (LogSD) in control and operated (i.e. hypertrophied) m. plantaris at different ages.*

Age/ Group	A: CD (mm^{-2})	B: CD (mm^{-2})	A: LogSD	B: LogSD
5C	882 ± 32 (15)	430 ± 38 (16)	0.158 ± 0.007 (15)	0.194 ± 0.008 (16)
5O	802 ± 41 (15)	438 ± 31 (13)	0.164 ± 0.004 (15)	0.205 ± 0.010 (13)
13C	755 ± 45 (20)[b]	399 ± 22 (18)	0.167 ± 0.004 (20)	0.204 ± 0.007 (18)
13O	657 ± 32 (19)[a]	354 ± 17 (19)	0.161 ± 0.004 (19)	0.200 ± 0.010 (19)
25C	652 ± 24 (16)[b,c]	434 ± 35 (16)	0.165 ± 0.007 (16)	0.202 ± 0.008 (16)
25O	548 ± 30 (15)[a]	345 ± 21 (15)	0.170 ± 0.004 (15)	0.198 ± 0.009 (15)

A: Deep region; B: Superficial region. Groups: 5, 13, 25: age of rats in months; C: Control group; O: Operated group. Values are mean ± SEM; number of animals in parentheses. [a]: Operation different from Control; [b]: Different from 5 months; [c]: Different from 13 months.

Ageing

There was no change in muscle weight with ageing (table 5.1). The deep region of the muscles of the 5-month-old rats contained a smaller percentage of type IIa fibres than those of other ages. Likewise the percentage of type IIb fibres in this region was higher in the muscles of the 5-month-old rats than in those of 13 months old (fig. 5.1a). No differences were found in the fibre type distribution of the muscles of the 13 and 25-month-old rats (fig. 5.1a and b).

The cross-sectional areas of fibres of each type in the deep region and of type I fibres in the superficial region were smaller in the muscles of the 5-month-old rats than in those of 13 months (fig. 5.2). The cross-sectional areas of only IIb fibres were decreased in both regions of the muscle between 13 and 25 months of age (fig. 5.2). Consequently, the cross-sectional areas of type I and IIa fibres in the deep region were larger in the muscles of the 25-month-old rats than in those of 5 months old (fig. 5.2a). Although an age effect was

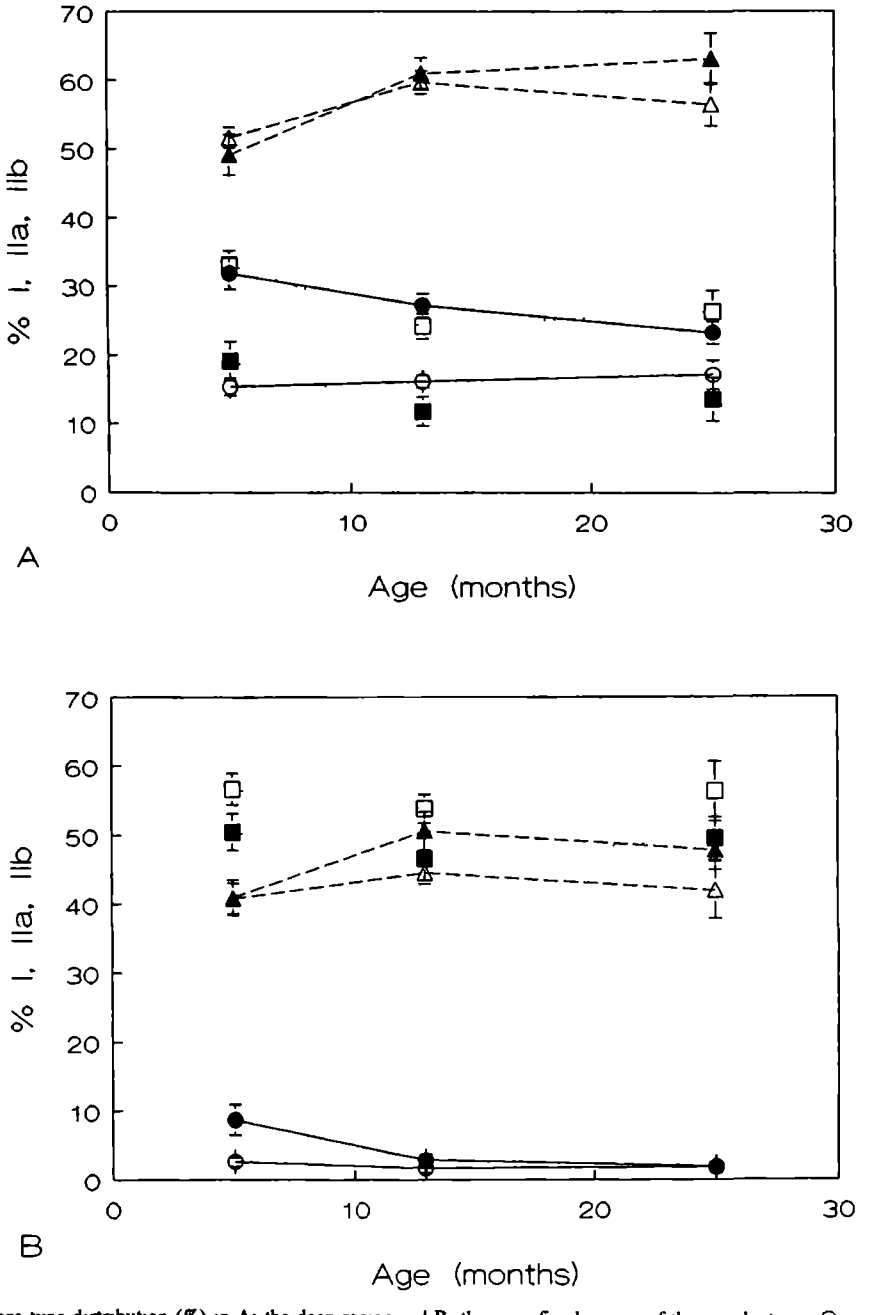

Fig. 5.1: Fibre type distribution (%) in A: the deep region and B: the superficial region of the m. plantaris. ○—— Control Type I; ●—— Operated Type I; Δ-- Control Type IIa; ▲ -- Operated type IIa; □··· Control type IIb; ■··· Operated type IIb. Values are mean ± SEM Significances are given in table 5.4

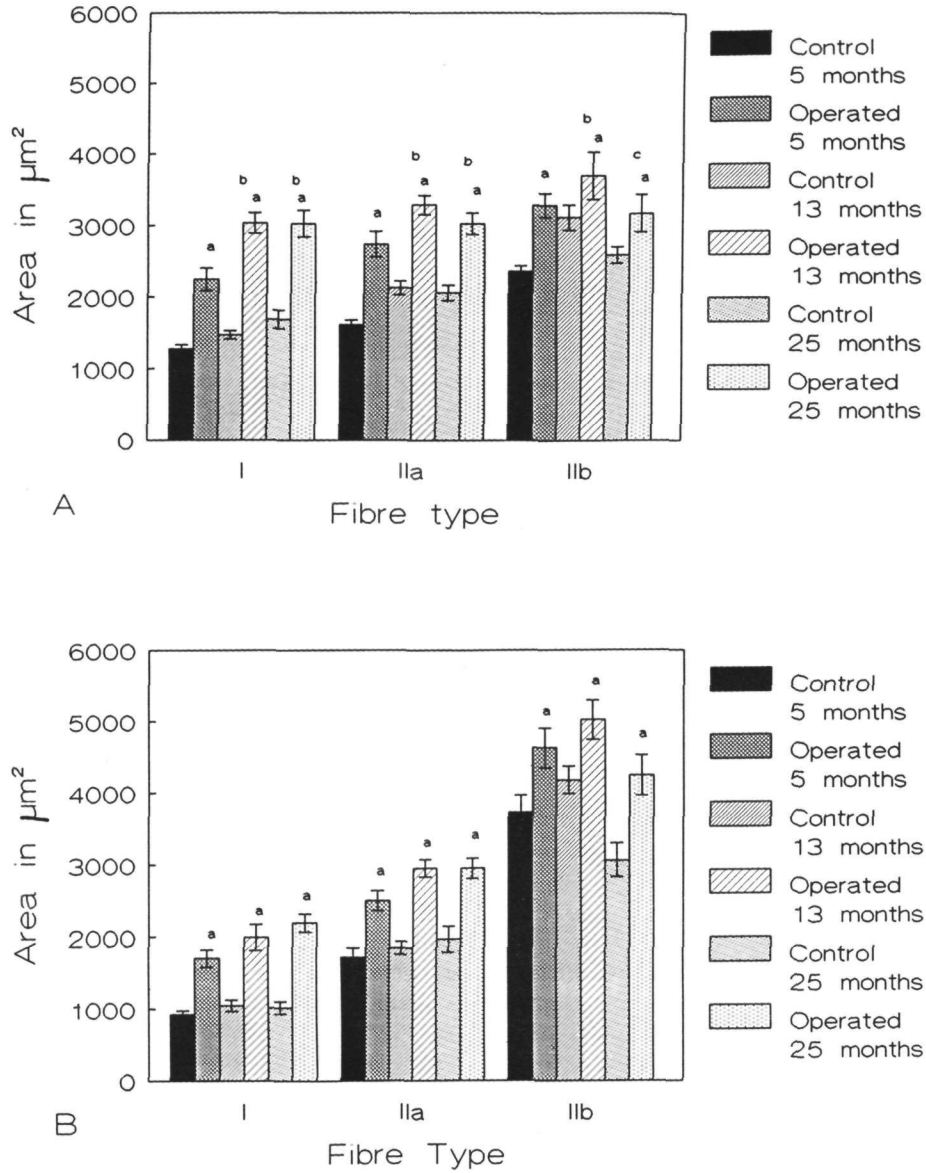

Fig. 5.2: Fibre cross-sectional areas (μm^2) in A: the deep region and B: the superficial region of the m. plantaris. Values are mean ± SEM. [a]: Operated different from Control; [b]: Different from 5 months; [c]: Different from 13 months.

detected on the type IIa cross-sectional area in the superficial region by the ANOVA (table 5.4), no differences were found between groups.
The capillary density of the deep region was highest in the muscles of the 5-month-old rats and lowest in those of 25 months old. No age effect was found for the capillary density in the superficial region of the muscle (tables 5.2 and 5.4). Also the heterogeneity of capillary spacing (LogSD) was not significantly affected by age. It was noticeable that at all ages, the capillary density was higher and the LogSD was smaller in the deep region than in the superficial region of the muscle.
While the local capillary-to-fibre ratio for each fibre type in both regions was not significantly different for the muscles of the 5-month-old and 13-month-old rats (fig. 5.3), the muscles of 25-month-old rats had a lower local capillary-to-fibre ratio of type IIb fibres in both regions and of type IIa fibres in the deep region than those of the younger animals (fig. 5.3). However, there was no difference in the local capillary-to-fibre ratio of type I fibres in both regions and of type IIa fibres in the superficial region of the muscles of the 5 and 25-month-old rats.
The capillary fibre density of type I and IIa fibres in the deep region was highest in the muscles of the 5-month-old rats and lowest in those of 25 months old; the density in the muscles of the 13-month-old animals was in between (fig. 5.4a). Also the capillary fibre density of IIb fibres in the deep region of the muscles of the 5-month-old rats was higher than that of the 25-month-old rats, but no significant differences were found between the muscles of the 5 and 13-month-old rats (fig. 5.4a). In the superficial region, age-associated differences were found in capillary fibre density but no differences were found between the age groups by the *t*-test with Bonferroni correction (fig. 5.4b).

Table 5.3. *Summary of values of variables showing significant Operation-Training interactions.*

	CNT	CT	ONT	OT	Interaction
B:%S	2.7 ± 0.7 (26)	1.2 ± 0.3 (24)	3.1 ± 0.9 (26)	5.6 ± 1.4 (22)	$P < 0.03$
B:FCSA I	1042 ± 61 (15)	937 ± 52 (11)	1708 ± 115 (11)	2105 ± 116 (17)	$P < 0.03$
B:LCFR I	0.696 ± 0.075 (12)	0.472 ± 0.052 (7)	0.629 ± 0.080 (9)	1.122 ± 0.090 (12)	$P < 0.03$
B:CFD I	675 ± 57 (12)	559 ± 73 (7)	383 ± 61 (9)	553 ± 43 (12)	$P < 0.03$

CNT: Control Not Trained; CT: Control Trained; ONT: Operated Not Trained; OT: Operated Trained; FCSA: fibre cross-sectional area in μm^2; LCFR: local capillary-to-fibre ratio; CFD: capillary fibre density in mm^2. Values are mean ± SEM; number of animals is given in parentheses.

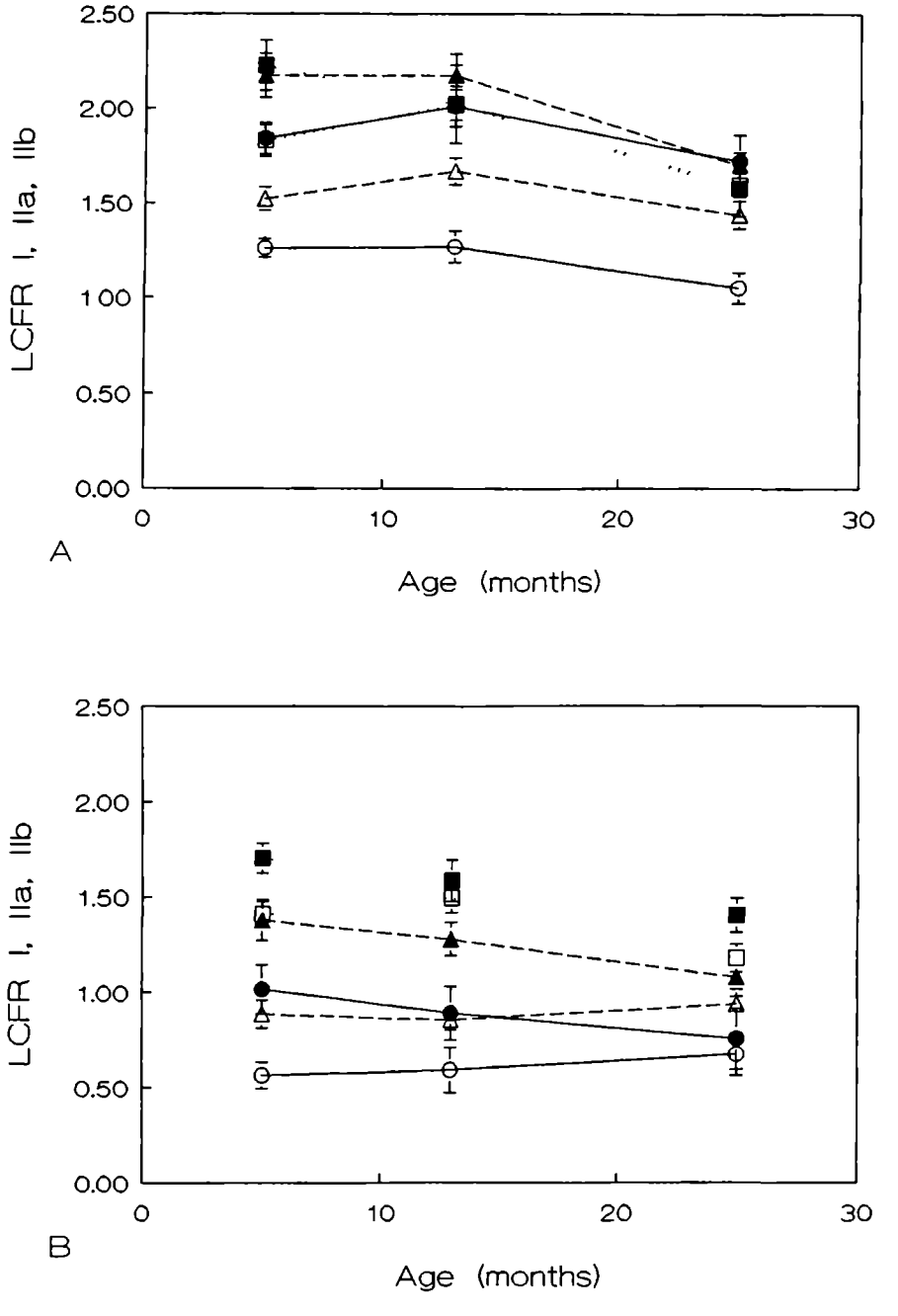

Fig. 5.3: LCFR (Local capillary-to-fibre ratio) for each fibre type in A: the deep region and B: the superficial region of the m. plantaris. Values are mean ± SEM. Significances are given in table 5.4.
For explanation of symbols see fig. 1.

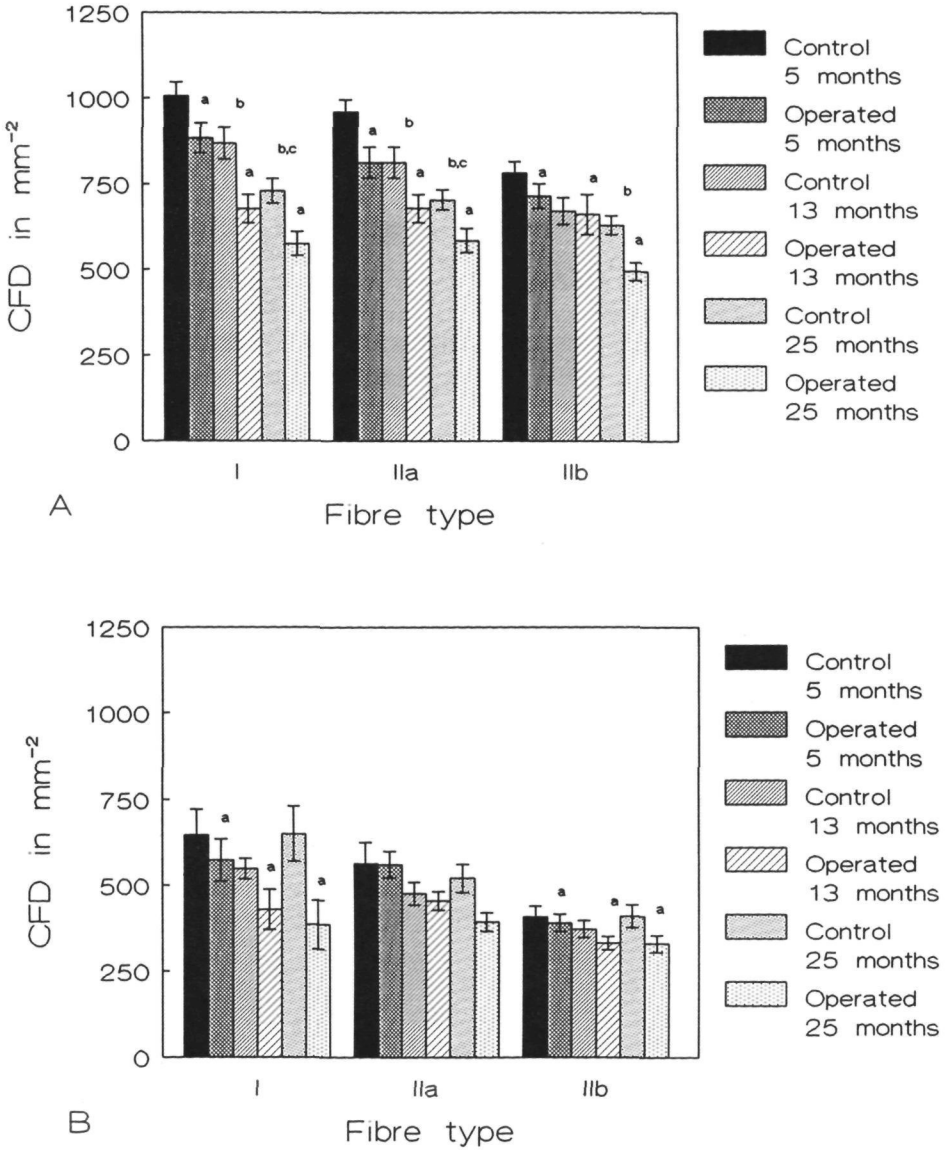

Fig. 5.4: CFD (Capillary fibre density in capillaries per mm^2) per fibre type in A: the deep region and B: the superficial region of the m. plantaris. Values are mean ± SEM. [a]: Operated different from Control; [b]: Different from 5 months; [c]: Different from 13 months.

Operation

The amount of hypertrophy of the plantaris muscle due to operation was about 29% ($P < 0.001$) (table 5.1). Operation resulted in an increased percentage of type I fibres and a decreased percentage of type IIb fibres in both regions of the muscle (fig. 5.1). The percentage of type I fibres in the deep region was increased to 207%, 168% and 136% of the corresponding control values for the muscles of the 5 ,13 and 25-month-old rats, respectively (fig. 5.1a). In the superficial region, this was 301%, 167% and 103% for the muscles of the 5, 13 and 25-month-old rats, respectively (fig. 5.1b).

Table 5.4 *P values as obtained by ANOVA for Operation (Oper) and Age effects on each of the determined variables.*

	A: Oper	A: Age	B: Oper	B: Age
% type I	$P < 0.001$ ↑	N.S.	$P = 0.008$ ↑	$P = 0.001$
% type IIa	N.S.	$P < 0.001$	N.S.	N.S.
% type IIb	$P < 0.001$ ↓	$P = 0.005$	$P = 0.006$ ↓	N.S.
FCSA I	$P < 0.001$ ↑	$P < 0.001$	$P < 0.001$ ↑	$P = 0.042$
FCSA IIa	$P < 0.001$ ↑	$P < 0.001$	$P < 0.001$ ↑	$P = 0.035$
FCSA IIb	$P < 0.001$ ↑	$P = 0.003$	$P < 0.001$ ↑	$P = 0.001$
CD	$P = 0.002$ ↓	$P < 0.001$	N.S.	N.S.
LogSD	N.S.	N.S.	N.S.	N.S.
LCFR I	$P < 0.001$ ↑	$P = 0.03$	$P = 0.003$ ↑	N.S.
LCFR IIa	$P < 0.001$ ↑	$P < 0.001$	$P < 0.001$ ↑	N.S.
LCFR IIb	N.S.	$P = 0.001$	$P = 0.008$ ↑	$P = 0.006$
CFD I	$P < 0.001$ ↓	$P < 0.001$	$P = 0.013$ ↓	N.S.
CFD IIa	$P < 0.001$ ↓	$P < 0.001$	N.S.	$P = 0.024$
CFD IIb	$P = 0.044$ ↓	$P < 0.001$	$P = 0.037$ ↓	N.S.

A: Deep region; B: Superficial region; The direction of the arrows indicates the direction of the operation effect; N.S.: Not Significant; FCSA: Fibre Cross-sectional Area in μm^2; CD: Capillary density in mm^{-2}; LogSD: Logarithmic standard deviation of Domain Areas, as index of heterogeneity; LCFR: Local capillary-to-fibre ratio; CFD: Capillary fibre density in mm^2.

In both regions of the muscle, the cross-sectional areas of fibres of each type were increased with operation at all ages (fig. 5.2). The capillary density was decreased with operation at all ages in the deep region of the muscle only, while no significant changes in the heterogeneity of the capillary spacing were found (table 5.2).

At all ages, the local capillary-to-fibre ratio of each fibre type in both regions, except that

of IIb fibres in the deep region, were higher in the muscles of Operated than in Control rats (fig. 5.3).
Operation resulted in a lower capillary fibre density of each fibre type in both regions as compared to the age-matched controls, except for the capillary fibre density of IIa fibres in the superficial region which did not change (fig. 5.4).

Training
Muscles of Operated Trained rats (OT) had a higher percentage of type I fibres with larger cross-sectional areas than the muscles of Operated Not Trained rats (ONT) (table 5.3).
The OT group showed a higher local capillary-to-fibre ratio for type I fibres in the superficial region of the muscle than the other groups (table 5.3). The training effect found by ANOVA ($P < 0.05$) was therefore only present in the operated groups. In addition, training alone (CT) seemed to decrease the local capillary-to-fibre ratio of type I fibres in the superficial region (table 5.3). Training increased the local capillary-to-fibre ratio of type IIa fibres in the superficial region of muscles in both the control and operated groups (CNT: 0.883 ± 0.049 (24); CT: 0.898 ± 0.043 (24); ONT: 1.133 ± 0.057 (25); OT: 1.366 ± 0.084 (22)) ($P < 0.05$).
The decrease in the capillary fibre density of type I fibres in the superficial region of the muscles due to the operation (ONT), was counteracted when training (OT) was added (table 5.3).

DISCUSSION

In the present study the influence of age, compensatory hypertrophy and training on capillarisation in relation to fibre types was investigated in the m. plantaris of 5, 13 and 25-month-old female rats.

Ageing
Fibre type Distribution
Our data showed that the percentage of type IIa fibres in the deep region was increased to the expense of IIb fibres in the muscles of the 13-month-old rats compared to the 5-month-old rats. In the superficial region of the muscles the percentage of type I fibres was higher in the 5-month-old rats than in the rats of other ages. The fibre type composition of the muscles of the 13-month old and the 25-month-old rats did not differ significantly. Thus in our study a change in fibre type composition was found in the period from 5 to 13 months of age. In accordance with this an increase in FOG fibres to the expense of FG fibres was found in the m. plantaris of rats from 76 days (2.5 months) old to 360 days (12 months) old (Maltin et al., 1989). Klitgaard et al. (1989) studied the m. plantaris of 9, 24 and 29-month-old rats. They found that the muscles of the 29-month-old rats contained a higher percentage of type IIb fibres in the deep region and a higher percentage of type I fibres in the superficial region than the muscles of the younger rats. Thus these changes in their study took place after the age of 24 months (Klitgaard et al., 1989). Furthermore they found an increase in the percentage of type IIa and a decrease in the percentage of type IIb

fibres in the superficial region from the age of 9 to 24 months, whereas the percentage of type IIa fibres in the deep region decreased during this period (Klitgaard et al., 1989). This does not agree with our findings as we did not find any significant age related changes in the fibre type composition of the superficial region but we did find an increase in the percentage of type IIa in the deep region of the muscle in the period between 5 and 13 months. This disparity might be explained by the different subclassification of type II fibres in their and our study. They subclassified type II fibres from an ATP-ase staining pattern, whereas we subclassified them from the SDH staining pattern. Therefore, different results can be obtained, as changes in the SDH staining pattern do not necessarily coincide with changes in the ATP-ase staining pattern.

Fibre Cross-Sectional Areas

The cross-sectional areas of each fibre type were larger in the muscles of the 13-month-old rats than the 5-month-old rats. IIb fibres were smaller (most probably atrophied) in the muscles of the 25-month-olds as compared to the 13-month-old animals. This corresponds with the finding that the maximum isometric tetanic force was higher for the muscles of the 13-month-olds than the 5 and 25-month-old rats (Degens et al., 1993). In another study it was also found that the cross-sectional areas of each fibre type in the m. plantaris increased during the first year of life (Maltin et al., 1989). This was corroborated in the m. rectus femoris and in addition it was shown that IIb fibres tended to atrophy after the age of 10 months (Kovanen, 1989). Klitgaard et al. (1989) reported an age-related reduction in the cross-sectional areas of each fibre type in the m. plantaris from 9 months on. Combining our findings with those found in these studies might indicate an increase in the fibre cross-sectional areas up to about the age of 1 y whereafter at least the IIb fibres become smaller.

Capillary density

The capillary density in the deep region of the muscle decreased with increasing age, whereas in the superficial region no significant changes with ageing were found. Furthermore, the heterogeneity in capillary spacing was not significantly affected by ageing. Ripoll et al. (1979) found that in the rat capillary density was negatively related to the fibre cross-sectional area. The decreased capillary density in the deep region of the muscles of the 13-month-olds as compared to 5-month-old rats could thus be explained by the increased cross-sectional areas of each fibre type. However, the decreased cross-sectional area of the IIb fibres and unchanged cross-sectional areas of fibres of other types in the muscles of the 25-month-old rats versus those of the 13 month olds do not explain the lower capillary density in the deep region of the muscles of 25-month-old versus the 13-month-old rats and the similar capillary density in the superficial region of the muscle. Neither is the decreased capillary density in the deep region and the similar capillary density in the superficial region of the muscles of the 25-month-olds as compared to the 13-month-old rats explainable by a changed fibre type composition as this did not differ significantly. This indicates that there is an age-associated capillary loss at least in the deep region of the muscle.

Local capillary-to-fibre ratio

The local capillary-to-fibre ratios of the IIa fibres in the deep region and of IIb fibres in both regions of the muscle were decreased in the 25-month-old rats when compared to the rats of other ages. Furthermore the local capillary-to-fibre ratio of type I fibres in the deep region was higher in the 13-month-old than in the 25-month-old muscles. No significant

differences were found between the 5 and 13-month-old muscles. Under the assumption of an unchanged fibre number, this indicates capillary loss with advancing age at least in the deep region of the muscle. Even when an age-related decrease in fibre number occurs, the decreased local capillary-to-fibre ratio for fibre types after the age of 13 months suggests that capillary loss occurs with advancing age. During denervation atrophy of human muscles, a loss of capillaries has also been found which could be ascribed to capillary necrosis (Carpenter and Karpati, 1982). Therefore the capillary loss as observed in our study in the period between 13 and 25 months might also be due to capillary necrosis.
In a previous study on the rat, a positive correlation was found between the fibre cross-sectional area and the local capillary-to-fibre ratio (Egginton et al., 1988). Thus an increased local capillary-to-fibre ratio in the 13 month olds as compared to the 5-month-old muscles was to be expected, whereafter the local capillary-to-fibre ratio remained stable. However, the fibre cross-sectional area of the fibres of each fibre type was significantly increased in the muscles of the 13-month-old animals as compared to those of the 5-month-old rats, whereas the local capillary-to-fibre ratio did not change significantly. Also the muscles of the 25-month-old rats had a lower local capillary-to-fibre ratio for all the fibre types than the muscles of the 13-month-old rats, whereas the fibre cross-sectional areas did not show any significant differences, or even decreased as was the case for the IIb fibres. These results suggest that the positive correlation between the fibre cross-sectional area and the local capillary-to-fibre ratio, as described previously (Degens et al., 1992; Egginton et al., 1988) is modulated by age. It is, however, still present as the induction of compensatory hypertrophy (i.e. an increase in fibre cross-sectional area) in aged muscles was accompanied by an increase in the local capillary-to-fibre ratio for each fibre type which was similar to the data reported for younger muscles (Degens et al., 1992).

Capillary fibre density

The decreased capillarisation with ageing in the deep region of the muscle was also reflected in a decreased capillary fibre density. Nevertheless the capillary fibre density of oxidative fibres was higher than that of glycolytic fibres in each age group. Also both the local capillary-to-fibre ratio and the capillary fibre density of all the fibre types were higher in the deep (more oxidative) than in the superficial (more glycolytic) region for each age group. This implies that similar relations between the capillary supply to a fibre and its cross-sectional area, metabolic type and the metabolic type of the surrounding fibres as found in young muscles (Degens et al., 1992; Gray and Renkin, 1978) are also applicable to aged muscles, although the capillary supply per fibre cross-sectional area for each fibre type is decreased.

Hypertrophy

It has been reported that hypertrophy of the rat m. plantaris can be obtained even in old age with strength-training (Klitgaard et al., 1989). In the present study the operation resulted in hypertrophy of fibres of each type in all the age groups. Thus at all ages compensatory hypertrophy can be induced as has also been reported by others (Tomanek and Woo, 1970).
The fibre type distribution showed an increased proportion of type I fibres and a decreased proportion of type IIb fibres in each age group in both regions of the muscle. This might offer an explanation for the increased twitch time to peak tension observed and the

increased fatigue resistance with compensatory hypertrophy in each age group (Degens et al., 1993).
The capillary density decreased only in the deep region of the muscle. No change was found in the heterogeneity of capillary spacing. This is in agreement with previous findings in young rats (Degens et al., 1992).
Gollnick et al. (1981), who used a similar model, found that the number of fibres did not change with compensatory hypertrophy in adult rats. Binkhorst (unpublished results), who used the same model as that used in this study, found only minimal numbers of branched fibres. These findings were observed in adult rats but not in old ones. Our findings indicate that the increase in muscle wet weight at all ages can be related to an increase in the fibre cross-sectional areas and thus does not give any indication of major fibre proliferation or branching with compensatory hypertrophy. If there was fibre proliferation with no change in the number of capillaries, this would result in a decreased local capillary-to-fibre ratio. Hence, the increased local capillary-to-fibre ratio of each fibre type in both regions in each age group which accompanied the increases in fibre cross-sectional area of each fibre type, indicates capillary proliferation with compensatory hypertrophy, not only in young muscles (Degens et al., 1992) but also in aged muscles. The possibility remains that necrosis of some of the fibres occurs with hypertrophy in the muscles of old rats, which would be accompanied by disproportionate enlargement of the remaining fibres for attaining similar muscle hypertrophy. Necrosis of muscle fibres is expected to be accompanied by an increase in the collagen content. However, Tomanek and Woo (1970) did not find any increase in the collagen content in the hypertrophied m. plantaris of old rats. This makes the occurrence of muscle fibre necrosis unlikely. The inference with respect to capillary proliferation is also supported by the finding of a significantly increased local capillary-to-fibre ratio of all the fibres per animal pooled with hypertrophy in both regions at all ages (e.g. 1.410 ± 0.052 (16) vs. 1.700 ± 0.070 (15); mean $\pm$ SEM (number of animals); Control vs. Overload for the deep region of the muscles of the 25-month-old rats). Pooling the local capillary-to-fibre ratio of all the fibres circumvents the possible influences of changes in fibre type composition or fibre type specific changes in the fibre cross-sectional area on the local capillary-to-fibre ratio.
However, capillary proliferation lagged somewhat behind the increases in fibre cross-sectional areas as can be deduced from the decreased capillary fibre density. The decreased capillary fibre density in the deep region of the hypertrophic muscles is expected to result in increased diffusion distances from the capillary to the interior of a fibre. This, however, did not have any apparent detrimental effects on either the maximal isometric tetanic force or the fatigue resistance (Degens et al., 1993).
These results indicate that the effects of the operation, which led to hypertrophy, on capillarisation were qualitatively similar in each age group. Therefore, these results also stress the fact that muscles have a high degree of plasticity even in old age.

Training
The training applied increased the local capillary-to-fibre ratio of type IIa fibres in the superficial region. The training also increased the local capillary-to-fibre ratio of type I fibres in this region in hypertrophic muscles. In a study by Ishihara et al. (1991) it was

observed that the capillarisation of the m. plantaris varied little with voluntary exercise on a wheel-cage, with only an increase in the number of capillaries around the type S fibres in the superficial region (Ishihara et al., 1991). But when the ratio was expressed as the number of capillaries around a type S fibre per type S cross-sectional area there was no difference (Ishihara et al., 1991). In terms of capillary fibre density this was also found in our study for both type I and IIa fibres.

Conclusion

The present study showed that there is an age-related increase in the percentage of IIa fibres to the expense of IIb fibres, up to the age of 13 months followed by no significant changes later on. The cross-sectional areas of fibres of each type increased until the age of 13 months, whereas there was IIb fibre atrophy in the period between 13 and 25 months of age. The capillary supply to muscle fibres was lowest in the 25-month-old rats, both in terms of capillaries per fibre and capillaries per fibre cross-sectional area, thus indicating an age-associated capillary loss. Furthermore, the relation between capillary supply and fibre types as found in younger muscles persisted, although the number of capillaries was lower. Finally, our data suggest that muscles of all ages are capable of adapting in a similar way to increased functional demands through hypertrophy of fibres of all fibre types and by capillary proliferation, although capillary proliferation lags behind the increases in fibre cross-sectional areas.

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6. FATIGUE RESISTANCE IN COMPENSATORILY HYPERTROPHIED M. PLANTARIS IN DIFFERENT AGED RATS: RELATION TO METABOLIC CAPACITY, FIBRE TYPE AREA AND CAPILLARISATION

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SUMMARY

The plantaris muscle of the rat was used to investigate the influence of age, compensatory hypertrophy, obtained by denervation of its synergists, and training on the fatigue resistance. Muscles of the 13-month-old rats showed a higher fatigue resistance than those of the 5-month-old rats, but the muscles of the 25-month-old rats did not differ significantly from the muscles of younger aged rats. The capillary density decreased with age and was lower in hypertrophied muscles than in control muscles. Fatigue resistance increased with hypertrophy at all ages. Concomitant with the increase in fatigue resistance, the cross-sectional area of muscle occupied by type I and IIa fibres also increased. Weak positive correlations were found between the fatigue resistance and the muscle cross-sectional area occupied by IIa fibres and citrate synthase activity, but not between the capillary density, the muscle cross-sectional area occupied by type I or IIb fibres and the activities of creatine kinase, phosphorylase, hexokinase and 3-hydroxyacyl-CoA dehydrogenase or the fatty acid-binding protein content. This indicated that the fatigue resistance, as assessed by the 'Burke test', is probably not closely related to the oxidative capacity of the muscle. The activities of citrate synthase and creatine kinase were decreased in the muscles of the 25-month-old rats.

INTRODUCTION

Fatigue can be defined as a decline in force during repeated isometric contractions at a constant stimulation pattern. It is generally accepted that there is a positive relationship between the oxidative capacity of muscle fibres or motor units and their fatigue resistance (Burke et al., 1973; Larsson et al., 1991). Thus fatiguability of whole muscle may be related to its fibre type composition and oxidative capacity.
The capillarisation of a muscle is coupled with its oxidative capacity (Saltin & Gollnick, 1983). Therefore, the capillarisation of a muscle may be related to its fatiguability as well.
Studies on ageing in rats have demonstrated an age-associated fibre atrophy (Klitgaard et

al., 1989; Kovanen, 1989; Degens et al., 1993[b]). Furthermore, the fibre type distribution is reported to change towards an increased percentage of IIa (oxidative) fibres with increasing age (Boreham et al., 1988; Kovanen, 1989; Degens et al., 1993[b]). In some studies, however, an age-related decrease in the activities of mitochondrial enzymes was found (Cartee and Farrar, 1987 (m. gastrocnemius)), whereas others did not find any age-related change in the activities of mitochondrial enzymes (Klitgaard et al., 1989 (m. plantaris)). The number of capillaries has been reported to increase with age (Boreham et al., 1988), but also to decrease (Degens et al., 1993[b]).

With compensatory hypertrophy, the percentage of type I fibres increases and the percentage of IIb fibres decreases in the rat plantaris muscle (Degens et al., 1992), whereas the oxidative capacity remains unchanged (Ianuzzo & Chen, 1979; Riedy et al., 1985). Also the number of capillaries per fibre increases with hypertrophy (Degens et al., 1992).

Changes with ageing and hypertrophy may have an influence on the fatiguability of muscle. Therefore we investigated whether there were any relationships between fatiguability and fibre type composition, capillarisation and metabolic capacity in the plantaris muscle of the rat and whether these factors were influenced by age, compensatory hypertrophy and training.

METHODS

Animals and treatment allocation

Female Wistar rats were used. They were kept 10 to a cage until the beginning of the experimental period, when they were housed individually. The environment was maintained at 22°C with 12 h light and 12 h darkness. Food and water were provided ad libitum. The rats were assigned at random to four treatments within the age groups of 5, 13 and 25 months at the time of the contraction measurements. The four treatment groups were: Control Not Trained (CNT), Control Trained (CT), Operated Not Trained (ONT) and Operated Trained (OT). The body weights of the animals at the time of the experiments are given in table 6.1. Animals were excluded from the experiments if they had large tumours, were apparently sick, moribund or had extremely atrophied m. plantaris, i.e. muscle weight of less than 50% of the mean muscle weight in the age group.

Operation

The rats were anaesthetised with pentobarbital sodium (60 mg/kg i.p.). The operation which resulted in compensatory hypertrophy of the left m. plantaris consisted of denervation of the m. soleus and m. gastrocnemius by cutting the nerves as distally as possible. As the tendon of the m. plantaris of the rat is not part of the Achilles tendon, but ends in the plantar

aponeurosis, its distal tendon was transplanted to the tendon of the m. gastrocnemius. In this way the m. plantaris was better able to take over the function of the denervated muscles. To prevent reinnervation of the m. soleus and m. gastrocnemius, their nerves were attached to the m. semimembranosus or the m. biceps femoris using ligatures (Binkhorst, 1969). At the time of the contraction measurements, no reinnervation was found.

Training

The objective of the training was to put additional stress on the muscle to obtain further hypertrophy and an increased endurance capacity. Therefore, the day after the operation both the CT and OT rats were placed on a treadmill set at an inclination of 30° and at zero speed for 15 min to make them familiar with the treadmill. During this period the rats were not forced to walk, which gave them a recovery period of approximately 48 h. Over the next four days, the time of running at a speed of 320 m/h at an inclination of 30° was gradually increased from 20 min on the second day after the operation, to 2 h. Rats which had problems running uphill during these days were taken from the belt before the end of a session, but all the rats were able to fulfill the 2 h run after this period. This training programme was carried out 5 days a week for the next 5 weeks. The rats were forced to run by electrical or mechanical stimulation.

Contraction measurements

After this training period, the rats were anaesthetised with pentobarbital sodium (60 mg/kg i.p.) and in situ isometric contractions were elicited. The body temperature and muscle temperature were maintained at 35 $\pm$ 1°C. The m. plantaris was prepared free, keeping the blood supply intact. The leg was fixed by screwing the condyli of the femur to a support to ensure isometric contractions of the m. plantaris. The distal tendon of the muscle was connected to a force transducer (Statham Transducing cell UC2, load cell accessory UL4-5; resonance frequency of the whole set up was 786 Hz). Stimulation and on-line data sampling were done with a computer. The isometric contractions were elicited by supramaximal stimulation of the muscle with double pulses via the n. ischiadicus. Each pulse was a square pulse of 0.2 ms duration and an amplitude of 3 V for the 5 and 13-month-old rats and 5 V for the 25-month-old rats. There was a time lapse of 0.05 ms between the double pulses to prevent any back-response (Brown & Matthews, 1960). Five V stimulation was applied to the 25-month-old rats because 3 V stimulation was not always sufficient for maximal contractions in these rats. The muscle was set at its optimal length (L_0), defined as the length at which the muscle produced its maximal twitch force with a minimal twitch duration. During the entire experiment, a twitch was elicited every 30 s to keep the preparation in a steady state. The first five twitch contractions were analysed. We measured the twitch force, twitch time-to-peak tension and twitch half-relaxation time. After this, two

tetani were elicited with a 5 min interval between them by a 185 Hz pulse train of 330 ms duration. The maximal tetanic force was then determined. If the force of the second tetanus was decreased by 5% or more compared to the first tetanus, the rat was excluded from the contraction experiments. The results are described in another study (Degens et al., 1993[a]). Each tetanus was followed by a 5-min rest period with only one twitch every 30 s. We then measured the fatigue sensitivity of the muscle. This was done by stimulating the muscle with 330 ms bursts of 40 Hz every second for 4 min (Burke et al., 1973). A fatigue index (FAT) was calculated as the ratio between the peak force of the contraction encountered 2 min after the strongest contraction and the peak force of the strongest contraction during the test. This fatigue index is the same as the fatigue index A of Kernell et al. (1987). Then the muscle was rapidly excised and the wet weight was determined on an analytical balance. The rats were killed under anaesthesia by injection of KCl into the heart or by excision of the heart.

Muscle preparation

Directly following the contraction measurements, the muscles were excised, mounted at their L_0 on cork and frozen in isopentane cooled in liquid nitrogen. The muscles were stored at -25°C or less until being processed. Cross-sections (12 μm) were cut in a cryostat at -25°C and stored at -135°C until required for staining. The remaining muscle tissue was stored at -80°C.

Histochemistry

Sections were stained at room temperature for myofibrillar ATP-ase activity at pH 9.4 after 5 min preincubation between pH 4.3 and pH 4.35 for the young rats (Guth & Samaha, 1970). Adjacent sections were stained for succinate dehydrogenase activity at 37°C (Pool et al., 1979). Fibres were classified as type I or II based on the m-ATP-ase staining and type II fibres were subclassified into oxidative (IIa) and glycolytic (IIb) based on SDH staining. Classification of identical fibres in identical sections was repeated every half year and the estimations of the fibre type composition were compared to each other. The errors found in the mean percentage of type IIa and IIb fibres varied between 2.9% to 4.4% and for type I fibres between 0.27% to 0.4%. Statistical analyses showed that there were no systematic errors and no significant differences in the mean fibre type composition. Thus this method of fibre type classification was well reproducible over the entire study period (i.e. 5 to 25 months).

In a consecutive section, the capillaries were stained by a modification of the combined staining method for alkaline phosphatase and dipeptidyl peptidase IV. This method depicts both the arterial and venular portions of a capillary, whereas after alkaline phosphatase staining alone, the venular portion of a capillary remains unstained (Mrazkova et al., 1986;

Degens et al., 1992).

Histological observations

The analysis of the histochemical cross-section was done on photomicrographs of the deep region near the head of the tendon, and the superficial region near a large blood vessel in the core of the muscle, as these regions have different fibre type composition and capillarisation. Each photomicrograph contained 37 - 155 fibres and one photomicrograph was taken of each region of each muscle. The fibre type (I, IIa and IIb) composition was assessed in each photograph. Using a digitizing tablet, fibre outlines of complete fibres were read into the computer as contour coordinates, and capillary locations were read in as coordinates of the capillary centres. FCSAs (Fibre cross-sectional areas) were derived from complete fibre contours on these photographs. In a previous study it was found that IIb fibres had the largest FCSA and type I fibres had the smallest FCSA (Degens et al., 1992). Consequently, the contribution of IIb fibres was underestimated and that of type I fibres overestimated, both in terms of their contribution to the total tension generated by a muscle and its oxidative potential if the data are expressed as percentage of incidence of the fibre population. Therefore, we calculated the area occupied by a fibre type in a muscle region, expressed as a percentage:

$$\frac{(\%*FCSA)_x}{(\%*FCSA)_I+(\%*FCSA)_{IIa}+(\%*FCSA)_{IIb}}*100\%$$

where % = percentage of incidence and *FCSA* = mean FCSA for that fibre type. *I, IIa, IIb*: fibre types; *x*: fibre type of interest. The % area occupied by a fibre type in the whole muscle was then calculated as:

(% area occupied by a fibre type deep + % area occupied by a fibre type superficial)/2.

The Capillary Density (CD) was defined as the number of capillaries per square millimetre of tissue (muscle fibres plus intercellular space). In whole muscle it was calculated as the mean value of the capillary density of the deep and superficial region. Although the analyses were performed on both regions of the muscle, only the data from the whole muscle are discussed. The histochemical data could then be related to fatigue resistance and biochemical data which were performed on whole muscle.

Biochemistry

As parameters of metabolic capacity, we determined the activities of creatine kinase, of the glycogenolytic enzyme phosphorylase, of the glycolytic enzyme hexokinase and the mitochondrial enzymes citrate synthase and 3-hydroxyacyl-CoA dehydrogenase. The content of cytosolic fatty acid-binding protein was assayed, because muscular activity and fatty acid

oxidation may be related to this parameter (Veerkamp et al., 1991).
Segments of frozen plantaris muscles were thawed in ice-cooled buffer containing 250 mM sucrose, 2 mM EDTA and 10 mM Tris-HCl (pH 7.4). In this buffer muscle homogenates (5% w/v) were prepared by hand homogenisation, using a Potter-Elvehjem glass-teflon homogeniser.
The assays for metabolic enzymes were performed spectrophotometrically. Citrate synthase activity was determined at 25°C as described by Shepherd and Garland (1969) and was expressed as μmol coenzyme A formed/min·g tissue. All the other measurements were done at 37°C. Creatine kinase activity was determined with the Boehringer CK-NAC activated test kit (Jacobs et al., 1987) and expressed in mmol NADPH formed/min·g tissue. Hexokinase activity was determined with 2.2 mM D-glucose (Jacobs et al., 1990). Phosphorylase (a + b) activity was assayed by a method described previously (Jacobs et al., 1992). Phosphorylase and hexokinase activities were expressed as μmol NADPH formed/min·g tissue. 3-Hydroxyacyl-CoA dehydrogenase activity was assessed at 50 μM acetoacyl-CoA as described by Bass et al. (1969) and expressed in nmol NADH oxidised/min·g tissue.
The quantitation of fatty acid-binding protein was assayed by an ELISA (Paulussen et al., 1989). The content was expressed as pmol/mg cytosolic protein. The homogenates for the assay were centrifuged for 10 min at 600 x g and subsequently for 90 min at 105000 x g.

Statistics

ANOVA was applied to test for age, operation and training effects. Three-way interactions were not tested for. Any interaction in the ANOVA would indicate that a combination of the respective effectors gave a different outcome than these effectors on their own (Wallenstein et al., 1980). If age effects were found a *t*-test was applied to test for differences between the age groups. Differences were considered to be significant at $P < 0.05$. A Bonferroni correction was done (Wallenstein et al., 1980). The FAT, muscle cross-sectional area occupied per fibre type, CD and enzyme activities were tested for. The data for the cross-sectional area of muscle occupied by type I fibres were logtransformed to make them suitable for analysis in the ANOVA. Pearson's correlation coefficients were calculated between FAT as dependent variable and the area occupied by a fibre type, the enzyme activities or CD as independent variables. Unless stated otherwise, mean values are given ± SEM.

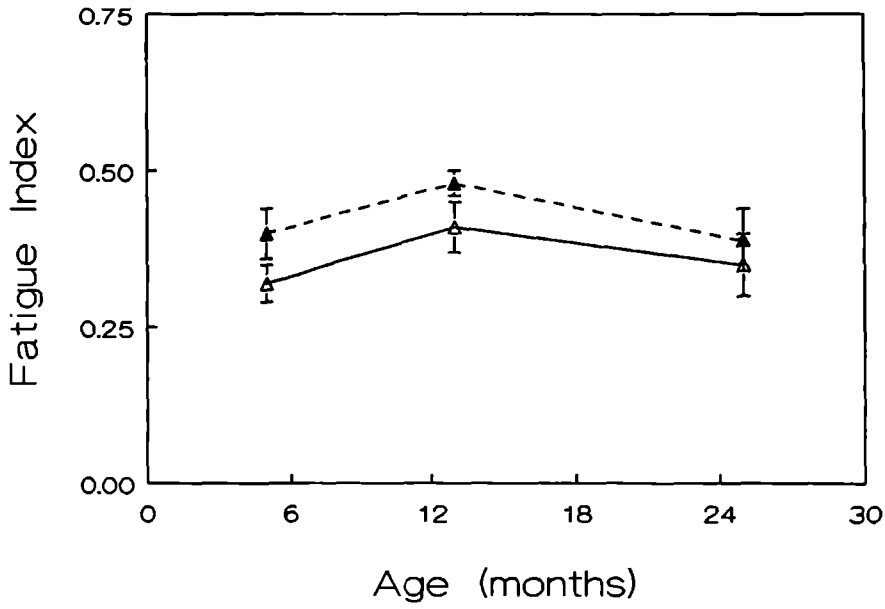

Fig. 6.1: Fatigue index in m. plantaris of control and operated m. plantaris of rats of different ages.

Δ——: Control; ▲--: Operated. Values are mean ± SEM. Significances are given in table 6.4.

RESULTS

Table 6.1 shows the body and muscle weights. In table 6.2 the enzyme activities and fatty acid-binding protein contents are presented. Table 6.3 gives the correlation coefficients between fatigue resistance and the other variables. The P values obtained by ANOVA are given in table 6.4. The significant differences will be described below.

Age

The fatigue index was lower in the muscles of the 5-month-old rats than in those of the 13-month-old rats, but the fatigue index of the muscles of the 25-month-old rats did not differ from that of the muscles of the other aged rats (fig. 6.1).

There were no differences between the muscles of rats of different ages regarding the area occupied by type I fibres (fig. 6.2). The area occupied by IIa fibres in the muscles of the 5-month-old rats was lower than that in the muscles of the other rats (fig. 6.2). No differences were found between the muscles of the 13 and 25-month-old rats. In the muscles of 5-month-old rats, the area occupied by IIb fibres was higher than in the muscles of the

Table 6.1. *Body and muscle weights.*

	5 MONTHS				13 MONTHS				25 MONTHS			
	CNT	CT	ONT	OT	CNT	CT	ONT	OT	CNT	CT	ONT	OT
MW (mg)	329 ± 18 (10)	330 ± 16 (9)	422 ± 22 (8)	446 ± 13 (8)	313 ± 24 (10)	364 ± 15 (10)	431 ± 21 (9)	461 ± 26 (9)	332 ± 12 (10)	400 ± 17 (6)	461 ± 28 (9)	447 ± 24 (6)
BW (g)	233 ± 9 (10)	216 ± 6 (10)	216 ± 4 (9)	214 ± 4 (9)	300 ± 12 (10)	306 ± 6 (10)	295 ± 8 (9)	276 ± 9 (10)	312 ± 12 (10)	301 ± 10 (7)	294 ± 13 (9)	283 ± 9 (6)
MW/BW (g/g * 100%)	0.142 ± 0.009 (10)	0.155 ± 0.008 (9)	0.198 ± 0.013 (8)	0.208 ± 0.007 (8)	0.112 ± 0.008 (10)	0.119 ± 0.004 (10)	0.146 ± 0.005 (9)	0.166 ± 0.008 (9)	0.107 ± 0.003 (10)	0.133 ± 0.002 (6)	0.156 ± 0.005 (9)	0.152 ± 0.012 (6)

CNT: Control Not Trained; CT: Control trained; ONT: Operated Not Trained; OT: Operated trained. Values are mean ± SEM; number of animals is given in parentheses.
MW: Wet weight of m. plantaris; BW: body weight at time of contraction measurements; MW/BW: muscle weight/body weight.

Table 6.2. *Activities of several enzymes of various metabolic pathways and the contents of fatty acid-binding protein (FABP) in m. plantaris of rat.*

	5 MONTHS				13 MONTHS				25 MONTHS			
	CNT	CT	ONT	OT	CNT	CT	ONT	OT	CNT	CT	ONT	OT
CK	10.16 ± 1.56 (4)	13.40 ± 1.40 (5)	2.96 ± 0.84 (6)	6.44 ± 0.64 (4)	16.36 ± 1.44 (5)	14.00 ± 1.88 (3)	4.84 ± 0.64 (4)	15.04 ± 0.24 (4)	3.64 ± 0.64 (4)	5.12 ± 0.68 (4)	3.96 ± 0.40 (4)	2.84 ± 0.44 (4)
PHOS	39.4 ± 6.9 (4)	29.9 ± 4.2 (5)	58.1 ± 3.5 (3)	37.9 ± 2.6 (4)	63.4 ± 4.4 (5)	72.5 ± 8.1 (2)	64.6 ± 4.2 (3)	53.6 ± 2.4 (4)	50.9 ± 3.7 (4)	60.0 ± 2.1 (5)	45.9 ± 1.7 (5)	32.9 ± 3.5 (4)
HK	1.83 ± 0.15 (4)	1.84 ± 0.37 (4)	0.75 ± 0.12 (4)	2 70 ± 0.13 (4)	1.90 ± 0.11 (5)	2 27 ± 0.12 (3)	0.73 ± 0.05 (6)	2.12 ± 0.11 (4)	1.15 ± 0.26 (4)	2.34 ± 0.04 (4)	2.00 ± 0.15 (4)	2.00 ± 0.48 (4)
CS	15.3 ± 1.9 (6)	10.4 ± 1.6 (9)	12.0 ± 1.7 (6)	13.8 ± 1.0 (7)	13.8 ± 0.7 (8)	11.6 ± 1.5 (6)	16.2 ± 0.9 (7)	15.7 ± 0.9 (10)	8.0 ± 0.7 (9)	10.1 ± 0.7 (7)	5.7 ± 0.7 (8)	9.2 ± 2.0 (5)
HADH	869 ± 58 (4)	1081 ± 81 (4)	1170 ± 66 (3)	798 ± 40 (4)	826 ± 128 (4)	828 ± 28 (4)	1378 ± 90 (4)	1096 ± 76 (4)	1146 ± 124 (4)	982 ± 78 (3)	892 ± 46 (3)	866 ± 38 (2)
FABP	417 ± 29 (7)	699 ± 26 (9)	546 ± 62 (8)	576 ± 25 (7)	417 ± 25 (10)	802 ± 69 (8)	539 ± 20 (9)	399 ± 63 (10)	705 ± 74 (9)	435 ± 29 (7)	597 ± 42 (8)	683 ± 59 (5)

CNT: Control Not Trained; CT: Control trained; ONT: Operated Not Trained; OT: Operated Trained. CK: creatine kinase activity in mmol NADPH formed/min·g tissue; PHOS: phosphorylase (a + b) activity in μmol NADPH formed/min·g tissue; HK: hexokinase activity in μmol NADPH formed/min·g tissue; CS: citrate synthase activity in μmol coenzyme A formed/min·g tissue; HADH: 3-hydroxyacyl-CoA dehydrogenase activity in nmol NADH oxidised/min·g tissue; FABP:fatty acid-binding protein content in pmol/mg cytosolic protein. Values are mean ± SEM: Number of animals in parentheses.

Table 6.3. *Correlation (R) between fatigue resistance and area occupied by a fibre type (in %), capillary density and activities of metabolic enzymes respectively of all rat m. plantaris pooled.*

	%I	%IIa	%IIb	CD	CK	PHOS	HK	CS	HADH	FABP
R value	0.08 (72)	0.26* (72)	-0.20 (72)	-0.12 (79)	-0.01 (41)	0.16 (40)	0.09 (41)	0.24* (75)	-0.18 (34)	-0.19 (81)

%: percentage area occupied by a fibre type in the muscle; CD: capillary density in mm^{-2}; For other abbreviations see table 6.2. *: $P < 0.05$.

other aged rats (fig. 6.2). The capillary density was highest in the muscles of the 5-month-old rats and lowest in the muscles of the 25-month-old rats (fig. 6.3).

Only the CNT groups were compared for age effects on the enzyme activities and fatty acid-binding protein content (table 6.4). The creatine kinase activity was highest in the muscles of the 13-month-old rats and lowest in those of the 25-month-old rats (table 6.2). Furthermore, the phosphorylase (a + b) activity was higher in the muscles of the 13-month-old rats than in those of the 5 and 25-month-old rats (table 6.2). The citrate synthase activity was lowest in the muscles of the 25-month-old rats (table 6.2). No differences were found in the activities of hexokinase and 3-hydroxyacyl-CoA dehydrogenase and the content of fatty acid-binding protein in the muscles of the different aged rats (table 6.2).

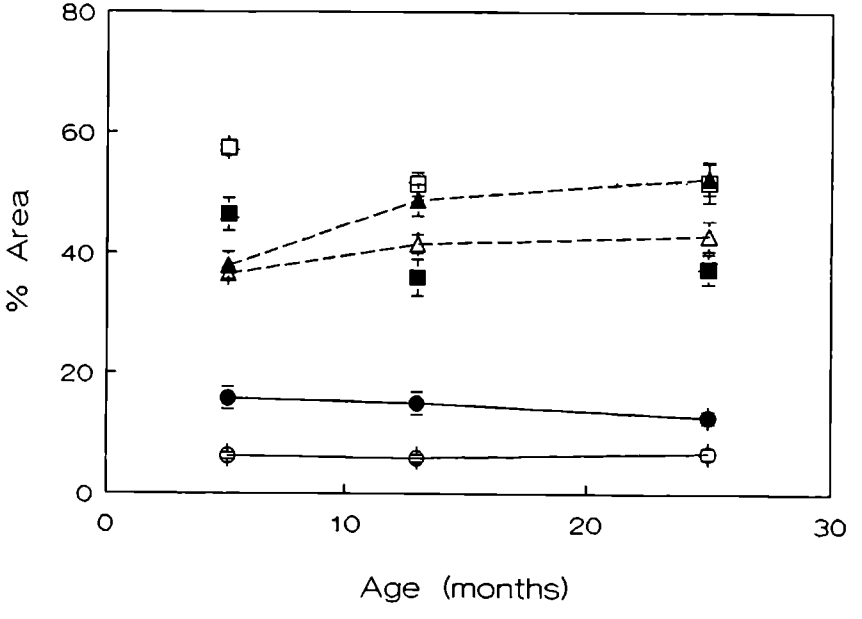

Fig. 6.2: Percentage of area occupied by different fibre types in m. plantaris of control and operated rats of different age. ○—— Control Type I; ●—— Operated Type I; Δ-- Control Type IIa; ▲ -- Operated IIa; □··· Control IIb; ■··· Operated IIb. Values are mean ± SEM. Significances of age and operation are given in table 6.4.

Operation

The amount of hypertrophy of the m. plantaris expressed as wet weight was about 30% ($P < 0.001$) at all ages (table 6.1). The muscles of the operated rats had a higher fatigue index than those of the age-matched controls (fig. 6.1). The area occupied by type I fibres and the

area occupied by IIa fibres of whole muscle were higher in the operated groups (fig. 6.2), while concomitantly the area occupied by IIb fibres decreased at all ages. The capillary density was lower in the muscles of the operated rats than in those of the age-matched controls (fig. 6.3).

The ONT groups were compared to the CNT groups for operation effects on the enzyme activities and fatty acid-binding protein content (table 6.4). The creatine kinase activity decreased as a result of the operation in the muscles of the 5 and 13-month-old rats (table 6.2). Although the operation resulted in a decreased phosphorylase activity in the muscles of the 25-month-old rats, its activity increased with operation in the muscles of the 5-month-old rats (table 6.2). The hexokinase activity was decreased in the muscles of the 5 and 13-month-old operated rats, but increased in the muscles of the oldest operated group (table 6.2). In the muscles of the 13-month-old rats, the citrate synthase activity was increased, while it was decreased in the muscles of the 25-month-old operated rats (table 6.2). As a result of the operation, the 3-hydroxyacyl-CoA dehydrogenase activity was increased in the muscles of the 5 and 13-month-old rats (table 6.2).

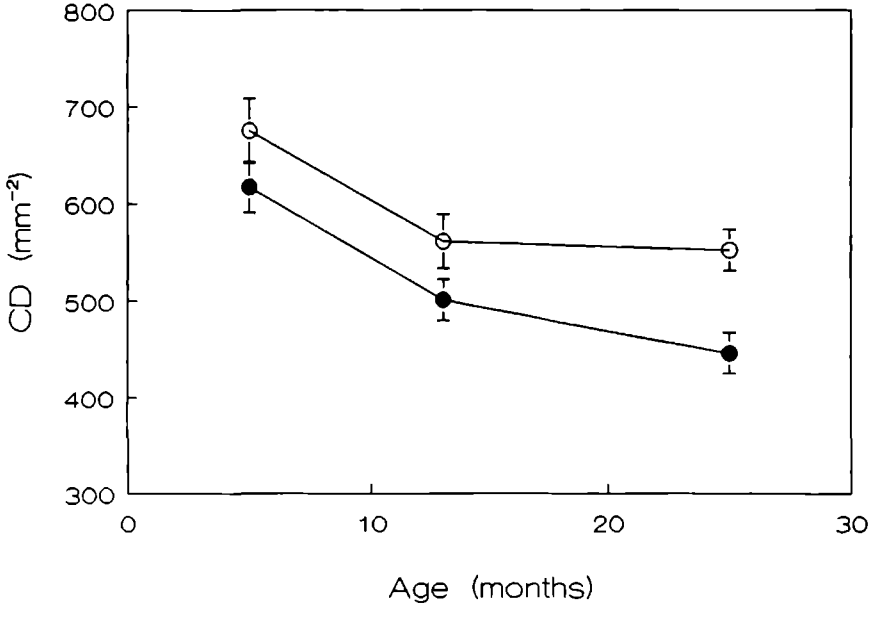

Fig. 6.3: Capillary density in the m. plantaris of control and operated rats of different age. ○—— Control; ●—— Operated. Values are mean ± SEM. Significances are given in table 6.4.

Training

Training did not significantly affect the fatigue index, the fibre type composition or capillary density. The activity of creatine kinase increased with training except in the control muscles of the 13-month-old rats and the muscles of the operated 25-month-old rats (table 6.2). However, no interactions were detected with ANOVA (table 6.4). The hexokinase activity increased with training in all cases (table 6.2). The phosphorylase and 3-hydroxyacyl-CoA dehydrogenase activities decreased with training only in the muscles of the operated rats in all the age groups (table 6.2). Training increased the citrate synthase activity only in the control and operated muscles of 25-month-old rats (table 6.2). The fatty acid-binding protein content was increased in the control muscles of the 5- and 13-month-old rats, but decreased in those of the 25-month-old rats (table 6.2).

Table 6.4. *P values obtained by ANOVA for age, operation and training effects and operation-age and operation-training interactions for each of the determined variables in m. plantaris of the rat.*

	Age	Operation	Training	Operation-Age	Operation-Train
FAT	$P < 0.05$	$P = 0.03$	N.S.	N.S.	N.S.
%I total	N.S.	$P < 0.001$	N.S.	N.S.	N.S.
%IIa total	$P < 0.001$	$P = 0.001$	N.S.	N.S.	N.S.
%IIb total	$P = 0.005$	$P < 0.001$	N.S.	N.S.	N.S.
CD	$P < 0.001$	$P = 0.001$	N.S.	N.S.	N.S.
CK	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P = 0.01$	N.S.
PHOS	$P < 0.001$	N.S.	$P = 0.02$	$P = 0.031$	$P = 0.001$
HK	N.S.	N.S.	$P < 0.001$	$P = 0.043$	N.S.
CS	$P < 0.001$	N.S.	N.S.	$P = 0.025$	$P = 0.024$
HADH	N.S.	N.S.	N.S.	$P < 0.001$	$P = 0.014$
FABP	N.S.	N.S.	N.S.	N.S.	$P = 0.002$

FAT: fatigue index; %: percentage area occupied by a fibre type in the muscle; CD: capillary density in mm^{-2}. For other abbreviations see table 6.2. Age-Training interactions for FABP ($P = 0.001$) and CS ($P = 0.016$).

Relationship between fatigue and the other variables

Correlation coefficients for all the data pooled and for each group separately (data not shown) were calculated between the fatigue index and the muscle cross-sectional area occupied by type I, IIa or IIb fibres, the capillary density, the activities of creatine kinase,

phosphorylase, hexokinase, citrate synthase or 3-hydroxyacyl-CoA dehydrogenase and the content of fatty acid-binding protein, respectively (table 6.3). This was done to assess whether an increase in fatigue resistance with age or the operation was related to an increase in these parameters. Significant correlations were only found with the cross-sectional area of muscle occupied by IIa fibres and the citrate synthase activity.

DISCUSSION

Age

We found that the creatine kinase activity was highest in the muscles of the 13-month-old rats and lowest in those of the 25-month-old rats. The phosphorylase activity was higher in the muscles of the 13-month-old rats than in those of the 5 and 25-month-old rats. This fits in with the decreased lactate dehydrogenase activity in the m. plantaris of 24 and 29-month-old rats compared to those of 9-month-old rats found by Klitgaard et al. (1989). Furthermore, they found that the hexokinase activity decreased between 24 and 29 months of age, while the citrate synthase activity in the m. plantaris did not show an age-associated change (Klitgaard et al., 1989). This may explain the absence of any significant age effect on hexokinase activity in our study, as the oldest rats were 25 months old. However, we found that the citrate synthase activity was lower in the muscles of the 25-month-old rats than in those of the other aged rats. Other authors who studied the m. gastrocnemius, also found a decrease in the citrate synthase activity with increasing age (Cartee & Farrar, 1987). In addition, they described an age-associated decrease in 3-hydroxyacyl-CoA dehydrogenase activity in contrast to our observations. Nevertheless, the absence of any significant change in the fatty acid-binding protein is in line with the unchanged 3-hydroxyacyl-CoA dehydrogenase activity, as both are related to fatty acid oxidation.

Hypertrophy

The overload resulted in a 30% compensatory hypertrophy in terms of increase in the muscle wet weight in each age group. The cross-sectional areas of all the fibre types increase with compensatory hypertrophy (Riedy et al., 1985; Degens et al., 1992). Also the number of type I fibres have been shown to increase with hypertrophy (Ianuzzo & Chen, 1979; Riedy et al., 1985; Degens et al., 1992), while the number of IIb fibres have been reported to decrease (Degens et al., 1992). In this study the cross-sectional area of the muscle occupied by type I fibres and type IIa fibres was increased, whereas that of IIb fibres was decreased with hypertrophy. This indicates that the proportion of oxidative fibres increased with compensatory hypertrophy. In a previous study on 5, 13 and 25-month-old rats, capillary proliferation was found with compensatory hypertrophy. However, this

proliferation lagged behind the increase in the fibre cross-sectional areas and resulted in a decreased capillary density per fibre cross-sectional area for each fibre type (Degens et al., 1993[b]). In the present study, we found a decreased overall capillary density in hypertrophied muscles, which is in agreement with our previous findings. This also suggests that the increase in cross-sectional area of muscle occupied by oxidative fibres is not accompanied by an identical relative increase in the number of capillaries.

No other studies have reported on creatine kinase activities in compensatorily hypertrophied muscles as far as we know. We found a significantly decreased creatine kinase activity in hypertrophied muscles of 5 and 13-month-old rats, but not in those of 25-month-old rats. The unchanged phosphorylase activity found for the muscles of the 13-month-old rats in our study corresponds with the findings from other studies (Ianuzzo & Chen, 1979; Riedy et al., 1985). However, the phosphorylase activity increased in muscles of 5 and decreased in muscles of 25-month-old rats. The hexokinase activity increased with hypertrophy in the muscles of the 25-month-old rats concurring with other findings on adult rats (Ianuzzo & Chen, 1979; Riedy et al., 1985). On the other hand, we found a decrease in hexokinase activity with hypertrophy in the muscles of 5 and 13-month-old rats. In agreement with the studies which reported unchanged succinate dehydrogenase activity (Ianuzzo & Chen, 1979; Riedy et al., 1985) we did not find any change in the citrate synthase activity in the muscles of 5-month-old rats. The citrate synthase activity increased, however, in those of the 13-month-old rats. Furthermore, Baldwin et al. (1976) reported a decrease in the citrate synthase activity in hypertrophied plantaris muscles, as we observed in the muscles of the 25-month-old rats. Ianuzzo and Chen (1979) reported that the 3-hydroxyacyl-CoA dehydrogenase activity was slightly increased in hypertrophied muscles, which indicated a slightly larger oxidative capacity for fatty acids. We found this in the muscles of the 5 and 13-month-old rats. However, these changes in the 3-hydroxyacyl-CoA dehydrogenase activity were not accompanied by changes in the fatty-acid binding protein content. In addition, the activity of 3-hydroxyacyl-CoA dehydrogenase was decreased with hypertrophy in the muscles of the 25-month-old rats. These results indicate that the effects of hypertrophy on the activities of enzymes of the anaerobic and aerobic pathways, are not uniform with ageing. It also seems that there is no relationship between the changes in activities of these enzymes and the increases in the cross-sectional area of muscle occupied by type I and IIa fibres and the decrease in the cross-sectional area of muscle occupied by IIb fibres.

Training

The citrate synthase activity decreased in hypertrophied plantaris muscle, but increased to the normal control level as a result of training (Baldwin et al. 1976) which was of a similar intensity as the training applied in our study. In another study in which more intense

training was applied, an increased succinate dehydrogenase activity was found with no significant changes in the phosphorylase and hexokinase activity in either normal or hypertrophied plantaris muscle (Riedy et al., 1985). An increased hexokinase activity has also been observed in the trained m. plantaris by others (Turcotte & Belcastro, 1991). In our study, training increased the citrate synthase activity only in the control and hypertrophied muscles of 25-month-old rats. Training increased the hexokinase and decreased the phosphorylase activity in hypertrophied muscles but did not affect their activities in control muscle. No explanation can be given for the discrepancies, except that the training applied in our study may not have been intense enough to induce changes in the citrate synthase activity in the muscles of the younger rats. Electrostimulation induced a rise in the fatty acid-binding protein concentration in the tibialis anterior muscle of the rat (Kaufmann et al., 1989). In our study, training produced the same effect in the control plantaris muscle of 5 and 13-month-old rats. However, this was not accompanied by an increase in the 3-hydroxyacyl-CoA dehydrogenase activity.

Fatigue resistance

The fatigue resistance was modulated by age. The muscles of the 13-month-old rats showed a higher fatigue resistance than the muscles of the younger rats, but it was not significantly different from that in the muscles of the older rats. Hypertrophied muscles on the other hand showed a significantly increased fatigue resistance at all ages as was also found by Frischknecht and Vrbova (1991) for the EDL (extensor digitorum longus muscle) of young adult rats. These increases in fatigue resistance may be due to the concomitant increase in the cross-sectional area of muscle occupied by oxidative fibres and suggests that although the capillary density decreases with age and hypertrophy the capillary supply was still sufficient. The training applied did not significantly affect the resistance against fatigue as assessed by the so-called "Burke Test", nor the fibre type composition and capillary density. Burke et al. (1973) found a close correlation between the fatigue resistance of motor units and their oxidative capacity. Since then, this correlation has been confirmed biochemically on the motor unit level (Hamm et al., 1988) and by quantitative histochemistry on the single fibre level (Larsson et al., 1991), although the latter used other stimulation patterns to induce fatigue. In other studies, an increase in the fatigue resistance due to various interventions, was associated with an increase in the incidence of oxidative fibres (Egginton, 1987; Itoh et al., 1990). The increase in fatigue resistance with ageing or hypertrophy in the present study, however, was not always accompanied by an increase in the activities of oxidative enzymes. For the EDL, the increase found in oxidative enzymes appeared to be only slight considering the large increase in fatigue resistance with hypertrophy (Frischknecht and Vrbova, 1991). Only weak positive correlations were found between the fatigue resistance and the cross-sectional area of muscle occupied by IIa fibres or the citrate

synthase activity, but no significant correlations were found with other enzyme activities, the cross-sectional area occupied by other fibres or the capillary density. No tight coupling was found between the histochemically ascertained activity of succinate dehydrogenase and fatigue resistance of whole muscle in some other studies also (Kernell et al., 1987; Gardiner, 1992). One explanation might be the finding that the mechanical responses of the motor units (which have different biochemical and contractile properties) during the fatigue test do not summate linearly during whole muscle contraction (Gardiner & Olha, 1987). This is probably due to metabolic changes during the activity of the whole muscle which are not as severe as during single motor unit activity. These metabolic changes may affect the neuromuscular propagation of excitation (Gardiner & Olha, 1987). Kernell et al. (1987) suggested that the "Burke test" primarily tests for other endurance-related factors, such as excitation-contraction coupling. These studies and our findings indicate that the fatigue resistance of the whole muscle is not closely related to the activities of oxidative enzymes, or to the area occupied by oxidative fibres or to capillarisation, but probably to other factors such as an impaired propagation of action potentials or impaired excitation-contraction coupling. However, some relationship with the oxidative metabolism exists as fatigue resistance was positively related to citrate synthase activity. In addition, the increased fatigue resistance of hypertrophied muscles and of the muscles of the 13-month-old rats, as compared to those of 5 months old, are accompanied by an increased area occupied by IIa fibres.

Conclusion

The increases in fatigue resistance with age and hypertrophy were accompanied by an increase in the muscle cross-sectional area occupied by oxidative fibres, but not always by increases in the activities of oxidative enzymes. However, as only weak correlations were found between the fatigue resistance (as assessed by the "Burke test") and the muscle cross-sectional area occupied by IIa fibres or the citrate synthase activity, the former parameter is probably not closely related to the oxidative capacity of the muscle. Training did not affect fatiguability.

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7. GENERAL DISCUSSION

In this thesis the m. plantaris of 5, 13 and 25-month-old rats was used to investigate the effects of ageing, compensatory hypertrophy and training on isometric contraction characteristics and fatigue resistance, fibre type composition (I, IIa and IIb) and capillarisation.

Ageing

Muscle weight did not show any age-associated changes. The maximum tetanic force per muscle weight was highest in the muscles of the 13-month-old rats and lowest in those of the 25-month-old rats. Others found a higher maximum tetanic force per muscle weight in the m. plantaris of 9 than in 24-month-old rats (Klitgaard et al., 1989). This suggests that the force generating capacity per muscle weight of the m. plantaris is optimal between the age of 9 and 24 months. The twitch contraction time was longer in the muscles of the 5-month-old rats than in those of other ages. Combining this finding with the observation of an unchanged twitch contraction time in the m. plantaris of 9, 24 and 29-month-old rats (Klitgaard et al., 1989), indicates that the twitch contraction time is shorter in the muscles of 9-month-old rats than in 5-month-old rats, whereafter it remains constant. The findings on maximum tetanic force per muscle weight, twitch contraction time and the higher fatigue resistance in the muscles of the 13-month-old rats compared to those of the 5-month-old rats (see section "fatigue") indicate that the m. plantaris reaches its optimal capacity between the age of 9 and 24 months. This was also reflected in the age-related changes in fibre type composition and fibre cross-sectional areas, described below.

The percentage of incidence of type I fibres did not change with ageing, while the percentage of type IIa fibres increased to the expense of the percentage of type IIb fibres in the muscles of the 13-month-old rats compared to the of 5-month-old rats. The fibres of each type had smaller cross-sectional areas in the muscles of the 5-month-old rats than the muscles of the 13-month-old rats, while only the cross-sectional areas of IIb fibres were larger in the muscles of the 13- as compared to those of 25-month-old rats. This is in agreement with a study on the m. rectus femoris of the rat, in which it was found that the fibre cross-sectional areas increased up to the age of 10 months, whereafter IIb fibres tended to atrophy (Kovanen et al., 1989). Thus, a general pattern emerges, which indicates an increased percentage of IIa (oxidative-glycolytic) fibres to the expense of IIb (glycolytic) fibres, with concomitant age-related fibre hypertrophy in the first year of life, followed by no changes in the fibre type composition and selective atrophy of IIb fibres at advanced age. The increase in the fibre cross-sectional areas in the muscles of the 13-month-old rats as compared to those of 5 months old was not accompanied by an increase in the local capillary-to-fibre ratio, thus resulting in a decreased capillary fibre density. The capillary

fibre density was further decreased in the m. plantaris of the 25-month-old rats. This suggests that the capillary supply per fibre cross-sectional area decreased with increasing age. In addition, all the fibre types of the muscles of the 25-month-old rats had a lower local capillary-to-fibre ratio than those of the 5 and 13-month-old rats, which indicated capillary loss at advanced age. The capillary loss observed during denervation atrophy of human muscles, could be ascribed to capillary necrosis (Carpenter and Karpati, 1982). Therefore it is possible that the capillary loss with ageing observed in our study was also due to capillary necrosis. The absolute blood flow in the triceps surae muscle of the rat has been found to decrease with increasing age (Irion et al., 1987), which might have also played a role in the process of capillary necrosis in our study.

Compensatory hypertrophy

The degree of hypertrophy, expressed as an increase in the muscle wet weight, was about 30% for the rats in each age group. This was similar to the findings of Binkhorst in rats of approximately 14 weeks old (1969). Compensatory hypertrophy was characterised by enlargement of the existing fibres. The increase in muscle weight was accompanied by an increase in the maximum tetanic force, such that the maximum tetanic force per muscle weight did not change significantly. This is in accordance with a similar relative increase in contractile and non-contractile material (Tomanek and Woo, 1970). The increased twitch contraction time, consistent with other reports (Roy et al., 1982) might be related to the increased number of type I fibres and the decreased number of IIb fibres, as in general, type I fibres are considered to be slow fibres and IIb fibres to be fast fibres (See Introduction).

Hypertrophy might result in increased diffusion distances from capillary to the interior of a fibre by pushing apart the existing capillaries. A change in the distribution of the capillaries might alter the heterogeneity of the capillary spacing. Both phenomena might affect tissue oxygenation (Turek et al., 1991). In the present study we found that both these effects of hypertrophy were partially counteracted by concomitant capillary proliferation, even in the muscles of the 25-month-old rats. This capillary proliferation was observed in all the fibre types as an increased local capillary-to-fibre ratio. Owing to the fact that this capillary proliferation lagged behind the increase in fibre cross-sectional areas, the overall capillary density and capillary fibre density were decreased.

It has been suggested that increased blood flow is an important factor in the induction of capillary proliferation (Hudlicka and Price, 1990). Therefore an increase in absolute blood flow with compensatory hypertrophy (Armstrong et al., 1986) might have been the initiating process for the capillary proliferation observed in our study.

The effects of hypertrophy were similar in each age group. This indicates that muscles of various ages can adapt in a similar way to increased functional demands.

Training

Endurance training can induce an increase in the oxidative capacity and the number of capillaries (Saltin and Gollnick, 1983; Booth and Thomason, 1991), thereby increasing the endurance capacity. However, the training applied in our study did not significantly affect fatiguability, as assessed by repetitive isometric tetanic stimulation, or capillarisation, fibre type composition and activities of oxidative enzymes in either the control or hypertrophied muscles. This might indicate that the training was too mild to induce changes in these parameters.

Fatigue

The fatigue resistance of the plantaris muscles of the 13-month-old rats was higher than that of plantaris muscles of the 5-month-old rats. In each age group, the fatigue resistance was increased with compensatory hypertrophy. As the capillary density decreased with ageing and hypertrophy, this suggests that within the limits of our observations, the capillary supply was not related to fatiguability. Close correlations between the fatigue resistance of motor units and their oxidative capacity have been reported (Burke et al., 1973). The increase in fatigue resistance with ageing and compensatory hypertrophy may thus be related to an increase in the percentage of muscle cross-sectional area occupied by type IIa fibres with ageing and an increase in the percentage of type IIa and type I fibres with compensatory hypertrophy. In this thesis also correlations between the percentage of muscle cross-sectional area occupied by IIa fibres and citrate synthase activity, and fatigue resistance of the whole muscle were found. However, these correlations were weak ($r < 0.3$), but they were significant ($P < 0.04$; $n = 72$). In addition, there were no significant correlations between the fatigue resistance and the percentage of muscle cross-sectional area occupied by type I or IIb fibres (negative relation expected) and the activities of other metabolic enzymes. These results indicate that there is no close coupling between fatigue resistance, as assessed by the "Burke test", and capillarisation or the metabolic capacity of a muscle. Similar results have also been obtained by others (Kernell et al., 1987). They suggested that the "Burke test" primarily tests for other endurance-related properties, such as an impaired excitation contraction coupling.

Conclusions

The maximum tetanic force measured in our experiments on the plantaris muscle showed an optimum at the age of 13 months, whereafter the force generating capacity decreased, both in absolute terms and in force per muscle weight. The cross-sectional areas of all the fibre types were largest in the 13-month-old rats. Capillary loss occurred with increasing age. The muscles of the different aged rats reacted in a similar way to the induction of

compensatory hypertrophy. This hypertrophy was accompanied by capillary proliferation. Fatigue resistance was weakly related to oxidative capacity and related variables.

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SUMMARY

In the study described in this thesis, the m. plantaris, a skeletal muscle, of the rat was used to investigate the influences of ageing, compensatory hypertrophy and training on contraction characteristics, fibre type composition (I, IIa and IIb), capillarisation and fatigue resistance. Rats of different ages were studied to assess age-related changes. Compensatory hypertrophy of the m. plantaris was obtained by denervation of its synergists, and training was given on a treadmill (Chapter 2). With a computerised method, capillarisation was related to fibre types (Chapter 3). Fatigue resistance was related to fibre type composition, capillarisation and the activities of metabolic enzymes (Chapter 6).

Age

Plantaris muscles of 5, 13 and 25-month-old rats were studied. No age-related changes in muscle weight were found. However, the higher muscle weight/body weight ratio in the 5-month-old animals than in those of other age, indicates that the increase in muscle weight is stabilised earlier than the increase in body weight. The muscles of the 13-month-old rats produced a larger maximum tetanic force per muscle weight than the muscles of the rats of other ages. The twitch contraction time was longer in the muscles of the 5-month-old rats than of those of other age (Chapter 4). The heterogeneity in capillary spacing did not change with ageing. Although there was no difference in the local capillary-to-fibre ratio (number of capillaries geometrically supplying a fibre) of each fibre type between the muscles of the 5 and the 13-month-old rats, the overall capillary density (capillaries/mm^2) and capillary fibre density (number of capillaries per mm^2 of cross-sectional area of a particular fibre type) were found to be decreased, due to the increased fibre cross-sectional areas. In the muscles of the 25-month-old rats, the capillary fibre density and overall capillary density were further decreased, but the fibre cross-sectional areas did not change or were even decreased, as was also the case for IIb fibres. In addition, the local capillary-to-fibre ratio of fibres of each type in the muscles of the 25-month-old rats was lower than in the muscles of the younger rats (Chapter 5). This indicates capillary loss with ageing. Citrate synthase (a marker of oxidative capacity) and creatine kinase activities were lowest in the muscles of the 25-month-old rats (Chapter 6).

Compensatory hypertrophy

Compensatory hypertrophy of the m. plantaris, obtained by denervation of its synergists, was quantitatively similar in the muscles of each age group. This increase in muscle weight was characterised by enlargement of the cross-sectional areas of fibres of each type (Chapter 5). The maximum tetanic force increased such that the maximum tetanic force per muscle weight did not change significantly. This indicated that the increase in muscle

weight could be ascribed to a similar relative increase in contractile and non-contractile material. Hypertrophied muscles had a prolonged twitch contraction time (Chapters 2 and 4) which might be related to an increase in the percentage of cross-sectional muscle area occupied by type I fibres, where in addition the area occupied by IIb fibres was decreased. In chapters 3 and 5, the effect of compensatory hypertrophy on capillarisation is described. The heterogeneity in capillary spacing was not affected by compensatory hypertrophy. Concomitant with the increase in fibre cross-sectional areas, the local capillary-to-fibre ratio of each fibre type increased. However, the capillary density and capillary fibre density decreased. These results indicate that capillary proliferation occurred with compensatory hypertrophy, though lagging behind the increase in fibre cross-sectional areas (Chapter 5). The activity of creatine kinase was decreased with compensatory hypertrophy, whereas the activities of several metabolic enzymes of other pathways did not change significantly (Chapter 6).

Training

Training had only minor effects, such as a slight increase in the muscle weight, maximum tetanic force and hexokinase, phosphorylase (markers for glycolytic and glycogenolytic capacity) and creatine kinase activities. The training applied was possibly too mild to induce any marked changes in the oxidative capacity and capillarisation.

Fatigue

In chapter 6, changes in fatigue resistance are discussed and related to fibre type composition and metabolism, as fatigue resistance of a muscle is generally believed to be related to its oxidative capacity. The increased fatigue resistance in compensatorily hypertrophied muscles and in the muscles of the 13-month-old rats compared to those of 5 months old, thus may be explained by the increase in the percentage of muscle cross-sectional area occupied by oxidative fibres. The fatigue resistance in the muscles of the 13 and 25-month-old rats was similar. As the overall capillary density decreased and the fatigue resistance increased or remained the same with hypertrophy and ageing, this suggests that within the limits of our observations, the capillary density does not significantly affect fatiguability. The correlations between fatigue resistance and percentage muscle cross-sectional area occupied by IIa fibres or citrate synthase activity was weak, but significant. There were, however, no significant relationships between fatigue resistance and the activities of other metabolic enzymes, capillary density or the percentage area occupied by type IIb fibres (Chapter 6). These results suggest that the fatigue resistance of a muscle, as assessed with the Burke test, is not closely coupled to its capillarisation or metabolic capacity.

1. Maximum tetanic force per muscle weight was higher in the muscles of the 13-month-old rats than in those of the 5 and 25-month-old rats. This indicates that there is a certain age at which the force generating capacity is optimal.
2. Between 5 and 13 months of age, the percentage of incidence of IIa fibres in the m. plantaris increased, while that of IIb fibres decreased, followed by no significant changes between 13 and 25 months of age. This indicates a change in fibre type composition towards IIa (oxidative-glycolytic) fibres to the expense of IIb (glycolytic) fibres until a certain age after which no further changes occur.
3. The fibre cross-sectional areas of all fibre types increased between 5 and 13 months of age, while the cross-sectional areas of the IIb fibres were decreased in the muscles of the 25-month-old rats. This indicates age-related hypertrophy of fibres of each type until a certain age, followed by selective IIb fibre atrophy at advanced age.
4. The age-related decrease in the local capillary-to-fibre ratio indicates an age-related capillary loss.
5. For each age group, similar compensatory hypertrophy effects were found. This suggests that the muscles of different aged rats can adapt to increased functional demands in a similar way.
6. At all ages, compensatory hypertrophy was accompanied by increases in the local capillary-to-fibre ratios, which indicates capillary proliferation.
7. The decreased overall capillary density and capillary fibre density in compensatorily hypertrophied muscles, however, indicate that the capillary proliferation lagged behind the increases in fibre cross-sectional area.
8. Only minor training effects were found, possibly because the training was too mild.
9. Fatigue resistance of the m. plantaris, as assessed by the "Burke test", is not closely related to its oxidative capacity.

In dit proefschrift zijn de resultaten beschreven van een onderzoek over de invloeden van veroudering, compensatoire hypertrofie en training op de contractie karakteristieken, vezeltype samenstelling (I, IIa and IIb), capillarisatie en weerstand tegen vermoeidheid van de m. plantaris van de rat. Veranderingen gerelateerd aan veroudering, werden bestudeerd in ratten van verschillende leeftijd. Compensatoire hypertrofie werd geïnduceerd door de synergisten van de m. plantaris te denerveren. Training werd gegeven door de ratten op een lopende band te laten lopen. Vezels werden geclassificeerd en capillairen aangetoond met behulp van histochemische kleuringen op cryostaat coupe's. Met behulp van een computer-programma werd de capillarisatie gerelateerd aan de verschillende vezeltypes.

Veroudering

De effecten van veroudering werden onderzocht in de m. plantaris van 5, 13 en 25 maanden oude ratten. Het spiergewicht veranderde niet met het ouder worden. Toch was de spiergewicht:lichaamsgewicht-ratio hoger voor de 5 maanden oude ratten dan voor de oudere ratten. Dit geeft aan dat de toename in spiergewicht zich eerder stabiliseert dan de toename in lichaamsgewicht. De spieren van de 13 maanden oude ratten produceerden een hogere maximale tetanische kracht per eenheid van spiermassa dan de spieren van ratten van andere leeftijden. Deze ratten hadden dus spieren die, wat kracht genererende capaciteit betreft, in een betere conditie verkeerden dan die van ratten van jongere of oudere leeftijd. De twitch-contractietijd was langer in spieren van 5 maanden oude ratten dan in die van ratten van andere leeftijd (Hoofdstuk 4). De heterogeniteit van de capillairenverdeling over een dwarsdoorsnede van de spier vertoonde geen verandering met toenemende leeftijd. Hoewel de local capillary-to-fibre ratio (aantal capillairen die geometrisch gezien bij een spiervezel horen) van elk vezeltype geen verschil liet zien tussen de spieren van 5 en 13 maanden oude ratten, waren zowel de globale capillaire dichtheid (capillairen/mm^2) als de capillary fibre density (aantal capillairen per mm^2 dwarsdoorsnede van een bepaald vezeltype) afgenomen. Dit was het gevolg van een toename in oppervlakte van de dwarsdoorsnede van de spiervezels. De capillaire dichtheid en de capillary fibre density waren nog verder afgenomen in de spieren van de 25 maanden oude ratten. Dit terwijl de oppervlakte van de dwarsdoorsnede van de spiervezels niet was veranderd, of zelfs afgenomen, in het geval van IIb vezels, tussen 13 en 25 maanden. Ook de local capillary-to-fibre ratio van vezels van elk type was lager in spieren van de 25 maanden oude ratten dan in die van jongere ratten. Deze bevindingen geven aan dat er met het ouder worden verlies van capillairen optreedt (Hoofdstuk 5). De activiteiten van citraat synthase (een indicator van de oxidatieve capaciteit) en creatine kinase waren het laagst in de spieren van de 25 maanden oude ratten (Hoofdstuk 6).

Compensatoire hypertrofie

De mate van compensatoire hypertrofie van de m. plantaris, geïnduceerd door denervatie van synergisten, was vergelijkbaar voor ratten van verschillende leeftijd. Deze toename in spiergewicht werd gekenmerkt door een oppervlaktetoename van de dwarsdoorsnede van de spiervezels (Hoofdstuk 5). De maximale tetanische kracht nam eveneens toe, en wel in die mate dat de ratio maximale tetanische kracht:spiergewicht geen verschil vertoonde tussen controle en compensatoir gehypertrofiëerde spieren. Dit geeft aan dat de toename in spiergewicht toegeschreven kan worden aan een vergelijkbare relatieve toename in contractiel en niet-contractiel materiaal. Gehypertrofiëerde spieren hadden een langere twitch-contractietijd dan controle spieren (Hoofdstukken 2 en 4). Dit zou gerelateerd kunnen zijn aan het toegenomen percentage van de oppervlakte van de dwarsdoorsnede van de spier dat ingenomen werd door type I vezels, terwijl bovendien de oppervlakte ingenomen door IIb vezels was afgenomen. In hoofdstukken 3 en 5 wordt de invloed van compensatoire hypertrofie op de capillarisatie van de spier beschreven. De heterogeniteit van de capillairenverdeling over een dwarsdoorsnede van de spier vertoonde geen verandering als gevolg van hypertrofie. De toename in oppervlakte van de dwarsdoorsnede van de spiervezels van elk type werd vergezeld door een toename in local capillary-to-fibre ratio voor elk vezeltype. De globale capillaire dichtheid en de capillary fibre density namen echter af. Deze resultaten geven aan dat er proliferatie van capillairen optrad met compensatoire hypertrofie, welke echter achterbleef bij de toename in dwarsdoorsnede van de vezels (Hoofdstuk 5). De activiteit van creatine kinase was afgenomen in de gehypertrofiëerde spieren, terwijl de activiteit van verscheidene andere metabole enzymen geen significante veranderingen vertoonde (Hoofdstuk 6).

Training

De gegeven training had slechts minimale effecten, zoals een geringe toename in het spiergewicht en de maximale tetanische kracht, de hexokinase, phosphorylase (indicatoren voor respectievelijk de glycolytische en glycogenolytische capaciteit) en creatine kinase activiteit. De training was mogelijk te mild om grote veranderingen in de oxidatieve capaciteit en capillarisatie te induceren.

Weerstand tegen vermoeidheid

In hoofdstuk 6 worden de veranderingen in de weerstand tegen vermoeidheid van de m. plantaris bediscussieerd. Deze wordt gerelateerd aan de vezeltype-samenstelling en activiteit van metabole enzymen, daar algemeen wordt aangenomen dat er een relatie bestaat tussen de oxidatieve capaciteit van een spier en zijn weerstand tegen vermoeidheid. De hogere weerstand tegen vermoeidheid van compensatoir gehypertrofiëerde spieren ten opzichte van controle spieren, en van spieren van 13 maanden oude ratten ten opzichte van die van 5

maanden oude, zou dan ook verklaard kunnen worden door het toegenomen percentage van de oppervlakte van de dwarsdoorsnede van de spier dat ingenomen wordt door oxidatieve vezels. De weerstand tegen vermoeidheid was vergelijkbaar voor spieren van 13 en 25 maanden oude ratten. Een toename of gelijk blijven van de weerstand tegen vermoeidheid ten gevolge van ouder worden of compensatoire hypertrofie ging gepaard met een afname van de globale capillaire dichtheid. Dit suggereert dat binnen de grenzen van onze waarnemingen de weerstand tegen vermoeidheid niet beïnvloed wordt door de globale capillaire dichtheid. De correlaties tussen weerstand tegen vermoeidheid enerzijds, en het percentage van de dwarsdoorsnede van de spier dat ingenomen wordt door IIa vezels dan wel de citraat synthase activiteit anderzijds, waren zwak, maar wel significant. Er werden echter geen significante relaties gevonden tussen de weerstand tegen vermoeidheid enerzijds, en de activiteiten van de enzymen creatine kinase, phosphorylase, hexokinase en 3-hydroxyacyl-CoA dehydrogenase, het fatty acid-binding protein gehalte, de globale capillaire dichtheid en percentage van de oppervlakte van de dwarsdoorsnede van een spier ingenomen door IIb vezels anderzijds (Hoofdstuk 6). Deze resultaten wekken de indruk, dat de weerstand tegen vermoeidheid van een spier, als bepaald volgens de Burke-test, slechts zwak gekoppeld is aan zijn oxidatieve capaciteit of capillarisatie.

Conclusie

De maximale tetanische kracht van de m. plantaris vertoonde in onze experimenten een optimum op de leeftijd van 13 maanden, waarna de kracht genererende capaciteit afnam, zowel absoluut alsook uitgedrukt als kracht per spiergewicht. Ook de oppervlakten van de dwarsdoorsneden van de spiervezels waren het grootst in de spieren van de 13 maanden oude ratten. Met het ouder worden trad capillairenverlies op. Spieren van ratten van elke leeftijd reageerden op overeenkomstige wijze op de inductie van compensatoire hypertrofie. Deze hypertrofie ging gepaard met capillairenproliferatie. De weerstand tegen vermoeidheid van een spier was zwak gerelateerd aan zijn oxidatieve capaciteit en daaraan gerelateerde variabelen.

CURRICULUM VITAE

Hans Degens werd op 3 augustus 1964 te Amsterdam geboren. In 1982 werd aan het Chr. Lyceum te Apeldoorn het Gymnasium ß diploma behaald. In datzelfde jaar is begonnen aan de studie Biologie aan de Landbouwuniversiteit te Wageningen, alwaar hij in 1988 afstudeerde. Van september 1988 tot september 1992 was hij aangesteld als AIO aan de afdeling Fysiologie van de Katholieke Universiteit Nijmegen. Thans is hij als vrijwilliger werkzaam aan de afdeling Biochemie van de Katholieke Universiteit Nijmegen.

STELLINGEN

I

De capillarisatie in een gemengde spier kan goed gerelateerd worden aan de verschillende vezeltypen met behulp van de Domeinen methode (*Dit proefschrift*).

II

Compensatoire hypertrofie gaat gepaard met capillairen proliferatie (*Dit proefschrift*).

III

De weerstand tegen vermoeidheid van een spier als bepaald met de Burke-test is niet sterk gerelateerd aan haar oxidatieve capaciteit (*Dit proefschrift*).

IV

De uitgestelde verouderingsatrofie van het hart, in ratten met een dieetrestrictie, speelt mogelijk een belangrijke rol bij de verlenging van de levensduur van de rat welke optreedt bij dieetrestrictie. (Goldspink et al., 1986; *Cardiovasc. Res. 20:672-678*).

V

Wanneer niet alleen in skeletspieren (Wu and Thomas, 1991; *Geront. 37:317-325*), maar ook in het hart de met veroudering optredende achteruitgang van antioxidante functies wordt tegengegaan door duurtraining, leidt dit mogelijk tot een verlengde levensduur.

VI

Door de aanwezigheid van specifieke waterkanalen in de plasmamembraan van cellen gespecialiseerd in snel osmotisch watertransport is de bijdrage van plasmamembraanproteïnen aan de waterpermeabiliteit fysiologisch van geen betekenis.
(Dempster et al., 1992;*NIPS* 7:172-176).

VII

Voor het maken van onderscheid tussen degeneratie en regeneratie van spiervezels tijdens myopathie verdient onderzoek naar de expressie van cytoskelettaire eiwitten aanbeveling.

VIII

De term "Bouwplan" in de vergelijkende morfologie impliceert een "Architect".

IX

Naarmate de levensverwachting van AIDS patiënten beter wordt, stijgt de maatschappelijke belangstelling voor deze ziekte.

X

Het broeikaseffect zal resulteren in een verandering in de geografische verspreiding van diersoorten.

XI

Het verplicht stellen van standaardfietsverlichting waarvoor niet alleen geldt dat "Je gezien wordt" maar ook dat "Je zien kan" zal fietsers beter op het "rechte" pad houden.

XII

Een goede stelling vraagt om een tegen-stelling.