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ORIGINAL ARTICLE

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Strength training with partial ischaemia stimulates microvascular remodelling in rat calf muscles

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Abstract The effects of strength training with partial tourniquet ischaemia on skeletal muscle capillarity were examined, particularly in terms of the distribution of arteriolar and venular capillaries and their capillary domain area, in male Wistar rats. A tourniquet applied around the knee joint induced partial ischaemia. Repeated isometric contractions of calf muscles, 1 s on/1 s off for 3 min, induced by electrical stimulation (100 Hz), were conducted 2 days/week for 6 weeks as training. Morphologic data were obtained from four groups; non-treatment control (C), ischaemic (IS), non-ischaemic training (NIT) and ischaemic training (IT). In the superficial portion of gastrocnemius (GASs) muscle, the total capillary density of arteriolar capillaries was significantly greater in the IT-leg than in the C-leg ($P < 0.05$). In the plantaris (PL) muscle, these values were significantly greater in the IT-leg than in both the C- and NIT-legs ($P < 0.05$). Only in the GASs was the capillary-to-fibre ratio significantly greater in the IT-leg than in the C-leg ($P < 0.05$). In GASs and PL, the capillary domain area (CDA) was smaller in the IT-leg than in the C- and NIT-legs. In all muscles examined, mean fibre cross-sectional area was not significantly changed by the experimental treatment. These findings suggest that adaptive changes in the microvascular network, identified as an increase in the arteriolar capillary area and a reduction in diffusion distance, occur in the skeletal muscles after strength training with partial ischaemia. These adaptive changes probably improve the supply of oxygen and nutrients to skeletal muscle tissues.

Key words Capillarity · Ischaemia · Lactate dehydrogenase · Skeletal muscle · Succinate dehydrogenase

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Introduction

It is well documented that endurance exercise training induces a marked increase in capillarity in skeletal muscles (Hermansen and Wachtlova 1971; Cabric and James 1983; Hoppeler et al. 1985; Poole et al. 1989; Gute et al. 1994). For strength training, however, changes in capillarity after the training are equivocal. A recent study showed an increase in the number of capillaries per fibre after 12 weeks of resistance training (McCall et al. 1996). It is thus possible that increased capillarity is beneficial not only to endurance exercise but also to strength exercise for increasing the capacity to exchange oxygen, substrates and metabolites between capillaries and muscle tissues.

Tissue hypoxia may stimulate, either directly or indirectly, capillary angiogenesis associated with exercise training (Hudlicka 1982; Burton and Barclay 1986). In hypoxic tissues, increased production of vascular endothelial growth factor (VEGF) (Shweiki et al. 1992; Landoux and Frelin 1993; Gustafsson et al. 1999) and elevated shear stress in capillaries (Hudlicka et al. 1992; Hudlicka 1994) associated with metabolic vasodilation (Meininger et al. 1988) may facilitate capillary angiogenesis. Studies of patients with intermittent claudication showed a tendency to increased capillarity (Henriksson et al. 1980; Jansson et al. 1988). A significant increase in the number of capillaries in contact with type IIa fibres was observed in the claudicating legs (Hammarsten et al. 1980). Makitie (1977) showed, in patients with intermittent claudication, the severer the disease, the greater the capillarization. Esbjornsson et al. (1993) reported that endurance exercise training with ischaemia,

reducing muscle blood flow by 16%, caused an increase in the number of capillaries per fibre, whereas non-ischaemic training did not. After a single bout of this type of exercise, the mRNA expression of hypoxia-inducible factor 1 (HIF-1) and VEGF was markedly increased, and the degree of expression tended to be greater in the ischaemic than in the non-ischaemic conditions (Gustafsson et al. 1999). These observations suggest that the degree of capillary angiogenesis may be in proportion to the severity of tissue hypoxia. According to the "metabolic hypothesis" described by Adair et al. (1990), the blood vascular system adapts its structure to meet the maximum oxygen needs of the tissue cells rather than the average tissue requirements. Therefore, it is possible that severe hypoxia induced by exercise with partial ischaemia facilitates capillary angiogenesis, even though daily exercise lasts for only a short duration.

In our previous investigation, the number of arteriolar portions of capillaries was found to be increased in the soleus muscle (Suzuki et al. 1997a) as well as in the left ventricle (Gao et al. 1994) of rats endurance-trained for 6 weeks. The arterial blood, which has abundant oxygen and substrates, flows through arteriolar capillary portions. Thus, it is possible that an increase in arteriolar capillarity may improve the supplement of oxygen and substrates to muscle tissues during endurance exercise as well as during resistance exercise.

The distribution of arteriolar and venular capillaries can be examined with a double staining method, which distinguishes the arteriolar portions from the venular portions of the capillaries (Lojda 1979). The staining method is based on the hypothesis that the endothelial cells of capillaries sited on the arteriolar side contain alkaline phosphatase (AP) and those on the venular side contain dipeptidyl peptidase IV (DPP IV) (Lojda 1979). Validation of the double staining method was examined by infusing microspheres (10 μm in diameter) into the coronary artery. The microspheres were sustained only in AP-positive capillary portions, suggesting that the AP-positive capillary portions are located on the arteriolar side and the DPP IV-positive ones on the venular side of capillaries (for a review; Koyama et al. 1998). This method has been utilized in studies of capillarity in skeletal muscles (Mrazkova et al. 1986; Grim and Carlson 1990; Suzuki et al. 1997a, b) as well as in the myocardium (Batra et al. 1989, 1991; Gao et al. 1994).

In the present study, we undertook experiments designed to examine whether strength training with partial ischaemia causes adaptive changes in the capillary geometry, especially the distribution of arteriolar and venular capillaries and their capillary domain area (CDA), in skeletal muscles.

Methods

Animals

Fifteen male Wistar rats (9 weeks old) were purchased from Charles River Japan (Tokyo, Japan). After the rats were fed for 7 days to allow adaptation to the new environment, they were divided into control ($n = 7$) and training ($n = 8$) groups. All rats were housed under conditions of control temperature [24 (1)°C] and a relative humidity of about 50%. Lighting (7:00–19:00 hours) was controlled automatically. All rats were given commercial laboratory chow (MF-type, Oriental East, Tokyo, Japan) and tap water ad libitum. The animals were cared for in accordance with the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences" of the Physiological Society of Japan. The rationale of this study was approved by the Animal Care and Use Committee of Hokkaido University of Education.

Experimental procedures

All rats were given the following treatments for 6 weeks. The treatments were performed 2 days a week, on Monday and Thursday. Under ether anaesthesia, the rats were placed in the supine position on a blanket electrically warmed at 37 °C. To maintain a constant level of anaesthesia, the rats were allowed to breath ether mixed with air, spontaneously as required. Partial reduction of blood flow to the leg muscles was induced by a tourniquet, an unstretched plastic band 3.5 mm wide and 1 mm thick, applied around the knee joint of right hindlimb. The tourniquet was applied for 8 min. The blood flow to the leg before and during the tourniquet application was measured using a laser-Doppler flowmeter (ALF-21RD, Advance, Tokyo, Japan). A laser probe was attached to the skin surface, excluding visible large veins, around the lower and medial portion of the calf. Hair around this portion, an area 5 mm × 5 mm, was shaved at least 24 h before the experiment. During the first minute of ischaemia, the intensity of the tourniquet was adjusted so as to reduce blood flow by 60–70%. In the present study, the tourniquet application reduced the blood flow by 64.7 (8.4)% [mean (SD)].

For the training group, repeated isometric contractions, 1 s on/1 s off for 3 min, of calf muscles were induced by electrical stimulation (100 Hz, 2 ms, 40 V). Paired electrodes were placed on the bellies of both the medial and the lateral head of the gastrocnemius muscles. In the right leg of the training group, the muscle contractions were performed during tourniquet ischaemia, and the exercise was started 3 min after the onset of the tourniquet application.

The four groups of legs in this study were non-treatment control (C) and ischaemic (IS) legs from control rats, and non-ischaemic training (NIT) and ischaemic training (IT) legs from training rats.

Skeletal muscle samples

Under pentobarbital anaesthesia (50 mg/kg i.p.), bilateral plantaris (PL) and gastrocnemius (GAS) muscles were excised and weighed. The muscles were fixed at the length measured when both the knee joint and the plantar were maximally extended. Then the muscles were placed in embedding medium, O.C.T. compound (Miles, Elkhart, Ind., USA), and rapidly frozen in isopentane cooled to its freezing point (−160 °C) with liquid nitrogen. The muscle samples were stored at −80 °C until histochemical analyses. Serial transverse sections, 10 μm thick, were cut from the mid-belly of the muscle using a cryotome (CM-1500; Leica Japan, Tokyo, Japan) at −20 °C. Morphological analyses were obtained from the deep portion of the PL and deep (GASd) and superficial (GASs) portions of the medial gastrocnemius. The total muscle area used for each morphological measurement was 0.61 mm^2 per sample.

Determination of capillary profiles

The staining protocol is described elsewhere (Batra et al. 1989; Grim and Carlson 1990). Briefly, tissue sections were incubated in a mixture reactive to DPP IV in the capillary endothelium, which stained the venular portions of capillaries red. The sections were then transferred to a mixture reactive to alkaline phosphatase (AP),

which stained the arteriolar portions of capillaries blue. The transitional zone along the capillary length that demonstrated both AP and DPP IV activity was stained a violet colour. Some examples of colour images are given in a previous study (Suzuki et al. 1997b). The validity of this double staining method for differentiation between arteriolar and venular capillary portions has been confirmed previously (for a review; Koyama et al. 1998). The images of incubated sections were digitized by a video scanner (U-VPT-N, Olympus, Tokyo, Japan) attached to a light microscope (BX-SO, Olympus) and were stored on computer disk (Apple Japan, Tokyo, Japan). The capillary profiles were identified as either arteriolar (blue), venular (red) or intermediate (violet). Most parameters of the measurements are described in detail elsewhere (Gao et al. 1994). Briefly, the parameters were: the capillary density (the number of capillaries per mm^2); the capillary-to-fibre ratio (the number of capillaries per mm^2 divided by the number of muscle fibres per mm^2); the CDA [a voronoi polygonal area of muscle to which one capillary supplies oxygen (Heron and Rakusan 1994)].

Determination of succinate and lactate dehydrogenase (SDH and LDH) activities

The activities of dehydrogenases were measured using the staining procedure described previously (Van Noorden and Frederiks 1992) with some modifications. The modifications include the use of 8-dimethylamino-2,3-benzophenoxazine (meldola blue) instead of 1-methoxyphenazine methylsulfate (mPMS) as the exogenous electron carrier (Kugler and Wrobel 1987). Serial sections were incubated at 37 °C in a medium containing 100 mM phosphate buffer, pH 7.6; 18% (W/V) polyvinyl alcohol (Sigma, P1763); 0.75 mM sodium azide; 0.1 mM meldola blue; 1.5 mM nitroblue tetrazolium (NBT). Incubation media for demonstrating SDH activity contained 5 mM EDTA (disodium salt) and 48 mM succinate (disodium salt). For the demonstration of LDH, sections were incubated with 150 mM sodium lactate and 3 mM NAD. The non-specific reduction of NBT was determined by incubating serial sections in the above medium without substrate and coenzyme. The images of incubated sections were digitized and stored on computer disk as described above except that the light source of the microscope was restricted to 570 nm (the peak absorbance wavelength of diformazan; reaction indicator) using a narrow-pass interference filter (Asahi Spectra, Tokyo, Japan). The video image of the muscle section was composed of a matrix of 640×480 pixels (each pixel had an area of 0.503 μm^2). The optical density (OD) of the images was analysed using the NIH image program (written by Wayne Rasband at the U.S. National Institute of Health). The grey levels of the video system were calibrated for OD units using neutral density filters (Melles Griot Japan, Tokyo, Japan). From the digitized image, the boundaries of individual muscle fibres were outlined and then the average OD for all pixels within the outlined fibre and the fibre cross-sectional area were determined. This averaging procedure reduced possible errors resulting from areas of higher SDH staining density within a muscle fibre (Siek et al. 1986). The final OD for each section was derived by subtracting the mean value for the blank sections (without substrate) from the mean value for tissue sections with substrate present.

Determination of histochemical fibre type composition

Serial sections were stained for myofibrillar ATPase after pre-incubation at both pH 10.3 and 4.6 to classify type I, IIa, and IIb muscle fibres (Gollnick et al. 1983). The images of incubated sections were digitized and stored on computer disk as described in the capillary profile measurements.

Statistical analyses

All results are expressed as means (SE). Using the Kolmogrov–Smirnoff test, we first tested the distribution of all parameters to determine whether it was compatible with a normal distribution. Unpaired Student's *t*-test was used for parametric two-sample comparison. The two-way analysis of variance (ANOVA) was used to test the effects of ischaemia, training and their interaction. If the two-way ANOVA was significant, differences among the four groups were analysed using one-way ANOVA and the Fisher PLSD post-hoc test. Differences were considered to be statistically significant at $P < 0.05$.

Results

Body weights at the end of the treatment were not significantly different between control [474.1 (13.6) g] and trained [461.9 (9.7) g] rats. Muscle weights and the muscle weight-to-body weight ratio showed no significant difference among the four groups (Table 1).

Total capillary density and the density of each capillary portion are shown in Fig. 1. In the GASs, the total capillary density and density of arteriolar capillaries were significantly greater in the IT-leg than in both C- and IS-legs ($P < 0.05$). In the PL, the total capillary density and density of arteriolar capillaries were significantly greater in the IT-leg than in the other three types of leg ($P < 0.05$). However, in the GASd, the capillary densities remained unchanged after the experimental treatment. Only in the GASs was the capillary-to-fibre ratio significantly greater in the IT-leg than in either C- or IS-legs ($P < 0.05$; Fig. 2).

Table 2 shows the CDA in each leg. In the GASd, the CDA did not change after the experimental treatment. In the GASs, however, the CDA for the arteriolar capillary portion and total capillaries was significantly lower in the IT leg than in the C-leg ($P < 0.05$). The CDA values for arteriolar and venular capillary portions and total capillaries in PL were significantly less in the IT-leg than in the respective portions in the C-leg ($P < 0.05$).

Table 1 Muscle weights and muscle weight-to-body weight ratio values. Values are means (SE). (*I* ischaemia, *NS* not significant, *TR* training)

	C ($n = 7$)	IS ($n = 7$)	NIT ($n = 8$)	IT ($n = 8$)	Significance (two-way ANOVA)		
					I	TR	I × TR
Muscle weights (mg)							
Gastrocnemius	2440.7 (67.6)	2352.2 (48.2)	2320.7 (70.7)	2393.2 (78.8)	NS	NS	NS
Plantaris	547.5 (20.7)	547.2 (12.8)	509.9 (21.9)	530.8 (18.9)	NS	NS	NS
Muscle/body weight ratio (mg g^{-1})							
Gastrocnemius	5.17 (0.20)	4.99 (0.18)	5.03 (0.14)	5.18 (0.14)	NS	NS	NS
Plantaris	1.16 (0.03)	1.16 (0.03)	1.10 (0.03)	1.15 (0.04)	NS	NS	NS

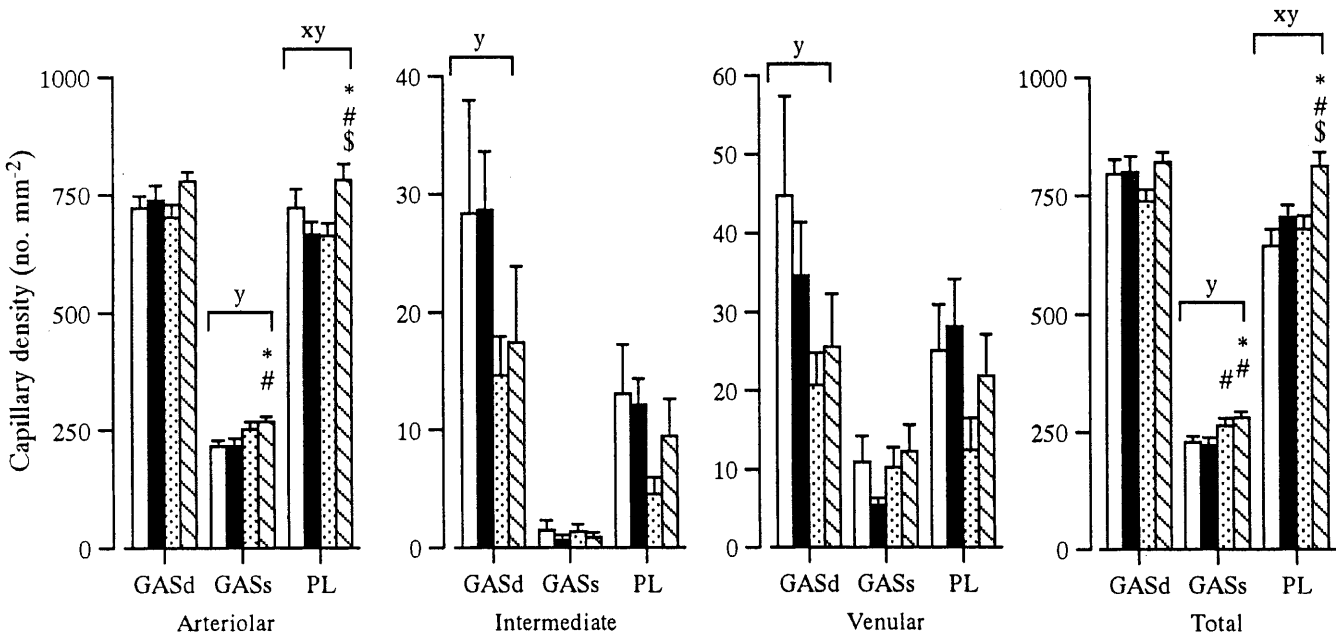

Fig. 1 The density of arteriolar, intermediate and venular capillaries and total capillary density in the deep gastrocnemius (*GASd*), superficial gastrocnemius (*GASs*) and plantaris (*PL*) muscles. All data are represented as means (SE). [*Open bar* non-treated control leg (*C*), *solid bar* ischaemic leg (*IS*), *stippled bar* non-ischaemic-trained leg (*NIT*), *hatched bar* ischaemic-trained leg (*IT*)]. $^{*,\#,\$}$Significantly different from C, IS and NIT, respectively ($P < 0.05$). *x* and *y* indicate ischaemia and training effects, respectively, were significantly different at $P < 0.05$ (two-way ANOVA)

Mean fibre cross-sectional area (FCSA) and fibre type composition are shown in Table 3. In all muscles and all fibre types examined, the FCSA was not significantly altered by the experimental treatment. There was no significant difference in fibre type composition among the four groups in any of the muscle portions examined.

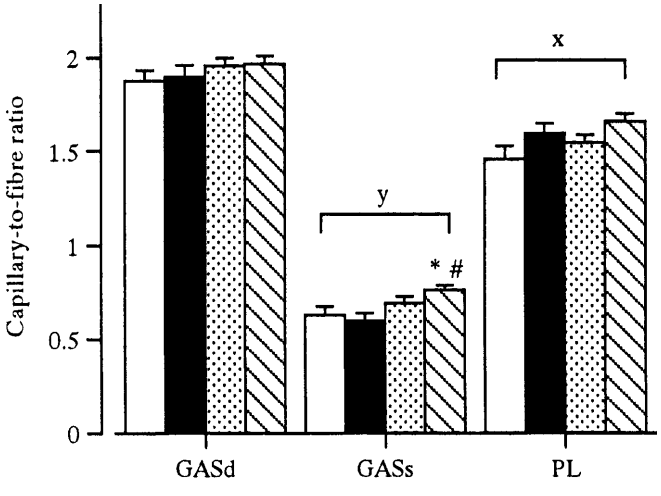

Fig. 2 Changes in the capillary-to-fibre ratio in deep (*GASd*) and superficial (*GASs*) portions of the gastrocnemius and plantaris (*PL*) muscles after the experimental treatment. Data are represented as means (SE). Groups are the same as in Fig. 1. $^{*,\#}$Significantly different from C and IS, respectively ($P < 0.05$)

Figure 3 shows the SDH activity in each fibre type. In type IIb fibres of the GASs, SDH activity was significantly greater in the IT-leg compared to the other three leg types ($P < 0.05$). In the PL, SDH activities in type IIa and IIb fibres were significantly greater in the IS-leg than in the respective fibre types in C- and NIT-legs ($P < 0.05$). In type IIb fibres of the PL, SDH activity was significantly greater in the IT-leg than in both C- and NIT-legs ($P < 0.05$). SDH activity in type IIa fibres of GASd was significantly greater in the IT-leg than in the C- and IS-legs ($P < 0.05$).

LDH activity showed a significant difference only in the GASs (Fig. 4). In type IIb fibres of GASs, the activity was significantly greater in the IT-leg than in the other three leg types ($P < 0.05$).

Discussion

This study shows that adaptive changes in capillary geometry, identified as an increase in the number of arteriolar capillaries and a reduction in the CDA, were observed in strength-trained muscles with partial reduction of blood flow, even though the duration of exercise per day was only a few minutes.

In the present study, the calf muscles were exercised by electrical stimulation at 100 Hz. The stimulating frequencies of 100 Hz may produce maximal tetanic responses in muscles mainly composed of fast-twitch fibres (Agbenyega and Wareham 1990; Murphy et al. 1996). Increases in SDH and LDH activities, in the present study, were observed in type IIa and IIb fibres, but not in type I fibres (Figs. 3 and 4), suggesting that type IIa and IIb fibres were mainly recruited.

An increase in the arteriolar portion of the capillaries was observed in the GASs and PL muscles of the IT-leg after training (Fig. 1). The capillary-to-fibre ratio

Table 2 Capillary domain area values. Values are means (SE). (*GASd* Deep portion of the gastrocnemius, *GASs* superficial portion of the gastrocnemius, *I* ischaemia, *NS* not significant, *PL* plantaris muscle, *TR* training)

	C	IS	NIT	IT	Significance (two-way ANOVA)		
					I	TR	I × TR
GASd							
Arteriolar	1230.3 (45.7)	1246.1 (50.7)	1330.8 (48.3)	1175.2 (33.7)	NS	NS	NS
Venular	1163.4 (47.9)	1271.7 (54.3)	1199.8 (58.1)	1151.9 (58.3)	NS	NS	NS
Total	1232.9 (45.4)	1251.6 (50.9)	1322.5 (47.0)	1176.0 (33.4)	NS	NS	NS
GASs							
Arteriolar	4077.3 (212.1)	4125.0 (286.6)	3596.6 (177.2)	3406.8 (126.2)*#	NS	§	NS
Venular	2553.4 (302.6)	3212.4 (469.6)	3799.6 (480.5)	3050.1 (334.8)	NS	NS	NS
Total	4048.0 (212.4)	4092.7 (277.2)	3555.0 (167.3)	3409.1 (131.1)*#	NS	§	NS
PL							
Arteriolar	1534.4 (90.7)	1364.4 (42.6)	1451.4 (63.8)	1226.8 (54.6)*$	NS	§	NS
Venular	1865.5 (133.9)	1447.4 (85.3)*	1540.3 (102.6)	1099.9 (61.4)*#$	§	§	NS
Total	1544.9 (96.0)	1369.1 (43.2)	1454.8 (63.4)	1259.5 (64.9)*#	§	NS	NS

*,#,$ Significantly different from C, IS and NIT, respectively at $P < 0.05$. § Significantly different at $P < 0.05$

Table 3 Fibre cross-sectional area (μm^2) and fibre type composition (%) values. Values are means (SE). (*I* Ischaemia, *TR* training, *NS* not significant)

		C	IS	NIT	IT	Significance (two-way ANOVA)		
						I	TR	I × TR
		GASd						
Type I	Area	2892.5 (273.9)	3004.7 (140.1)	3159.9 (196.6)	2584.9 (120.2)	NS	NS	NS
	%	17.8 (1.9)	17.0 (0.8)	19.0 (2.5)	18.8 (1.8)	NS	NS	NS
Type IIa	Area	2378.0 (256.5)	2288.4 (66.9)	2660.2 (78.5)	2326.0 (89.8)	NS	NS	NS
	%	23.4 (0.6)	22.9 (1.4)	24.6 (1.2)	19.8 (1.7)	NS	NS	NS
Type IIb	Area	3073.4 (239.8)	2958.7 (147.5)	3276.2 (114.6)	3120.8 (124.6)	NS	NS	NS
	%	58.8 (2.0)	60.1 (1.8)	56.3 (3.3)	61.4 (1.8)	NS	NS	NS
		GASs						
Type IIb	Area	3147.3 (132.8)	3213.6 (129.8)	3147.8 (136.4)	3243.7 (144.2)	NS	NS	NS
	%	100.0	100.0	100.0	100.0	NS	NS	NS
		PL						
Type I	Area	1932.4 (89.3)	2234.3 (97.5)	1990.6 (120.8)	1995.2 (106.1)	NS	NS	NS
	%	7.0 (0.8)	10.0 (1.3)	7.8 (1.6)	9.3 (2.0)	NS	NS	NS
Type IIa	Area	1989.6 (68.6)	2172.9 (65.8)	1972.3 (95.7)	1879.4 (77.7)	NS	§	NS
	%	21.1 (2.3)	23.7 (1.7)	18.3 (1.7)	19.2 (1.7)	NS	NS	NS
Type IIb	Area	2705.8 (50.3)	2770.8 (95.5)	2815.9 (106.8)	2558.0 (93.3)	NS	NS	NS
	%	71.9 (3.0)	66.3 (2.7)	73.9 (2.2)	71.5 (3.0)	NS	NS	NS

§ Significantly different at $P < 0.05$

increased only in the GASs of the IT-leg after training (Fig. 2). These adaptive changes may be attributed to the acceleration of capillary proliferation. Exercise-induced capillary angiogenesis may be induced mainly by tissue hypoxia (Adair et al. 1990). Sundberg and Kaijser (1992) have reported that, during aerobic cycling exercise with partial ischaemia (reduced blood flow by 13–20%), venous oxygen saturation was around 12% lower than that during non-ischaemic exercise. After 4 weeks of training under these conditions, they found increased capillarity in ischaemic, but not in non-ischaemic legs (Esbjornsson et al. 1993). After a single bout of this type of exercise the mRNA expression of HIF-1 and VEGF was markedly increased, and the degree of expression tended to be greater in the ischaemic than in the non-ischaemic conditions (Gustafsson et al. 1999). In the present study, strength exercise with reduction of blood flow by 65% might cause severe hypoxia. It is therefore possible that the production of VEGF was facilitated in the leg muscles mainly recruited in the present exercise. Since elevated shear stress in capillaries may also facilitate capillary angiogenesis (Hudlicka et al. 1992; Hudlicka 1994), reactive hyperaemia after release of the tourniquet may be partly involved in microvascular remodelling. It has been reported that skeletal muscle ischaemia and reperfusion may stimulate the production of oxygen radicals (for a review; Pang et al. 1993), which injure the skeletal muscle microvasculature (Suval et al. 1987). The injury to the microvasculature can facilitate angiogenetic process (Hudlicka et al. 1992; Hudlicka 1994). However, it is not known whether the production of oxygen radicals is

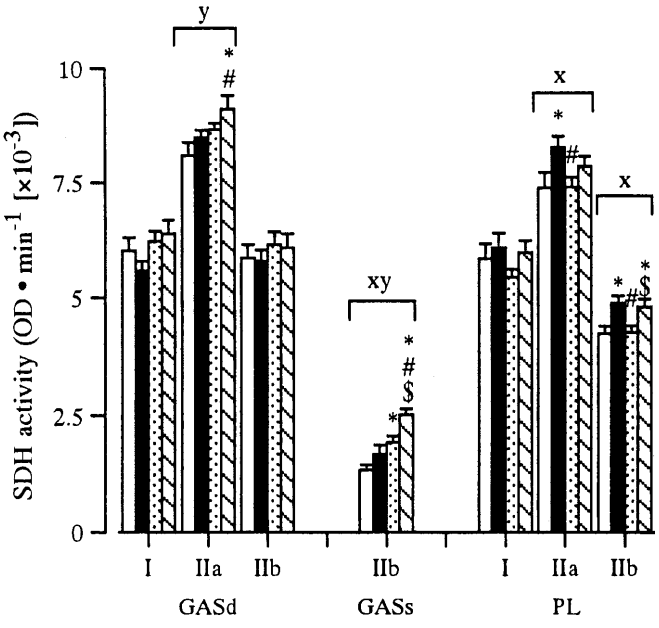

Fig. 3 Succinate dehydrogenase (*SDH*) activity in each fibre type. Data are represented as means (SE). Groups are the same as in Fig. 1. $^{*,\#,\$}$ Significantly different from C, IS and NIT, respectively ($P < 0.05$). *x* and *y* indicate ischaemia and training effects, respectively, were significantly different at $P < 0.05$ (two-way ANOVA)

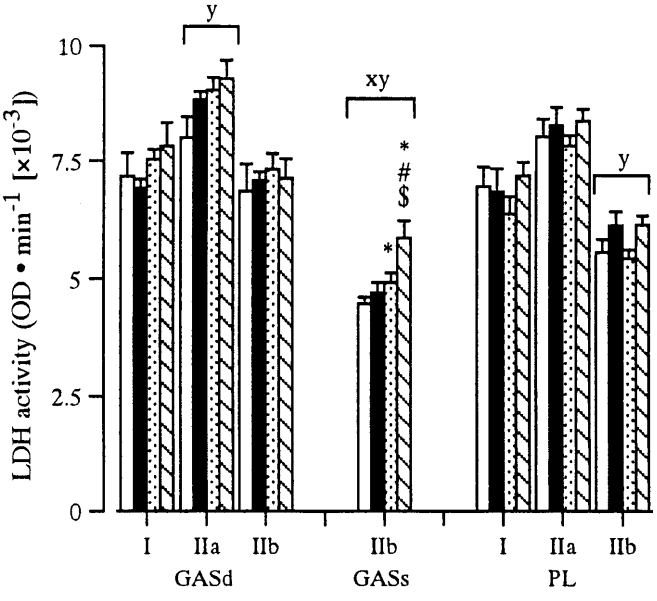

Fig. 4 Lactate dehydrogenase (*LDH*) activity in each fibre type. Data are represented as means (SE). Groups are the same as in Fig. 1. $^{*,\#,\$}$ Significantly different from C, IS and NIT, respectively ($P < 0.05$). *x* and *y* indicate ischaemia and training effects, respectively, were significantly different at $P < 0.05$ (two-way ANOVA)

increased in the present type of ischaemia and reperfusion.

Another cause of an increase in the arterial portion of the capillary may be arterialization. Price et al. (1994) reported that, in rat gracilis muscle, the smooth muscle cells in the terminal arterioles "proceed" towards the venular side along capillary pathways as the animals grow. Those authors postulated that the terminal arteriolar growth is facilitated by elevated circumferential wall stress (Price and Skalak 1995). Reduced perfusion pressure and metabolite accumulation during the tourniquet-induced ischaemia may cause arteriolar vasodilation that increases their circumferential wall stress. Hogan and Hirshmann (1984) demonstrated tremendous proliferation of arterioles by ligating the feed artery of the rat cremaster muscle, even though arterial ligation causes vasodilation with decreased blood flow. We postulate, therefore, that the elongation of terminal arterioles, with a subsequent elongation of arteriolar capillary portions, may be facilitated by strength exercise with the tourniquet-induced ischaemia performed in the present study. Furthermore, the superoxide radical, which is produced during reperfusion after ischaemia (Pang et al. 1993) and facilitates proliferation of vascular smooth muscle cells (Li et al. 1997), may also contribute to the elongation of terminal arterioles.

In this study, strength exercises lasted for only a short time (3 min), with partial ischaemia induced microvascular remodellings. No previous studies have observed microvascular adaptation after training that consisted of such a short duration of daily exercise. Our present findings support the hypothesis that the blood vascular system adapts its structure to meet the maximum oxygen needs of the tissue cells rather than the average tissue requirements (Adair et al. 1990). This hypothesis was also supported by the study of Burton and Barclay (1986), who reported that the proliferation of vascular endothelial cells increased significantly when the incubator oxygen tension was changed from 130 to 35 mmHg for only 1 h each day.

Strength training with partial ischaemia, in the present study, caused microvascular remodellings in the GASs and PL, but not in the GASd. However, SDH activity in type IIa fibres of GASd was significantly increased after ischaemic training (Fig. 3), which indicates that muscle fibres around GASd were recruited. This discrepancy can be explained by the facts that the GASd portion has greater capillary networks (Figs. 1 and 2), a smaller domain area (Table 2) and a greater aerobic capacity (Fig. 3) than the GASs and PL. Therefore, in the GASd, strength training with ischaemia in this study may not have induced severe enough hypoxia to stimulate vascular angiogenesis.

The CDA is the cross-sectional area of muscle tissue that one capillary supplies with oxygen. A reduction in the CDA is therefore beneficial for oxygen supply to muscle tissues. In this study, the CDA was significantly diminished in the IT-leg in both the GASs and PL ($P < 0.05$; Table 2). The reduction in the CDA may be caused by an increase in capillarity and/or by a reduction in the FCSA. In the present study, the FCSA was not significantly changed by the experimental treatment in any of the muscles or fibre types examined (Table 3). Therefore, the CDA reduction observed in this study

was mainly due to an increase in capillarity and partly due to the small but insignificant reduction in the FCSA. In the rat myocardium, CDA values for each capillary were greater in arteriolar than in venular side (Batra et al. 1991). This finding implies that the arteriolar capillaries are responsible for supplying oxygen to a greater area of myocardial tissue compared to the venular capillaries. In this study, the CDA values for the IT-leg followed that order in all muscle portions examined (Table 2). These changes in the CDA may also contribute to improving the oxygen supply to muscle tissues.

Recently, Shinohara et al. (1998) reported the efficacy of tourniquet ischaemia for strength training with low resistance. They used isometric knee extension exercise lasting for 3 min and conducted on 3 days per week for 4 weeks as training and found significant increases in muscle strength and maximal rate of torque development in ischaemic, but not in non-ischaemic legs. Taken together with our present findings, it is possible that strength training with ischaemia effectively increases muscle capillarity as well as muscle strength, even though the daily exercise lasts for only a few minutes.

In conclusion, the findings of the present study demonstrate adaptive changes in the microvascular network in response to strength training with partial ischaemia in skeletal muscles. The adaptive changes were identified as an increase in the number of arteriolar capillaries and a reduction in the CDA. These adaptive changes probably improve the supply of oxygen and nutrients to skeletal muscle tissues.

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