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Abstract

We observed differences in the capillary architecture of the skeletal muscles that have different fiber metabolism. The soleus, the vastus intermedius and the tibialis anterior muscles of adult Wistar rats were prepared using two different techniques. Samples for adenosine triphosphatase (ATPase) staining were prepared following Dubovitz's method, and the distributions of fiber type, Types 1, 2A and 2B, were analyzed. Then, corrosion casts of capillary architecture of these muscles prepared following Murakami's method were observed with a scanning electron microscope (SEM) and compared with the fiber distribution. The fiber type composition of the soleus muscle showed Type 1 (slow-twitch) dominance and that of the vastus intermedius and the tibialis anterior muscle showed Type 2 (fast-twitch) dominance. The capillaries of the soleus muscle were tortuous, and this was thought to be advantageous for blood supply. In contrast, the capillaries of the vastus intermedius and tibialis anterior muscles had a relatively parallel pattern. Additionally, two different patterns of capillary architecture that appeared to correspond to certain metabolic characteristic of different muscle fiber types were preserved with corrosion casting. In conclusion, comparative studies on capillary architecture of the skeletal muscles are useful for analyses of its function.

KEYWORDS: skeletal muscle, ATPase staining, muscle fiber type, corrosion cast, capillary architecture

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We observed differences in the capillary architecture of the skeletal muscles that have different fiber metabolism. The soleus, the vastus intermedius and the tibialis anterior muscles of adult Wistar rats were prepared using two different techniques. Samples for adenosine triphosphatase (ATPase) staining were prepared following Dubovitz's method, and the distributions of fiber type, Types 1, 2A and 2B, were analyzed. Then, corrosion casts of capillary architecture of these muscles prepared following Murakami's method were observed with a scanning electron microscope (SEM) and compared with the fiber distribution. The fiber type composition of the soleus muscle showed Type 1 (slow-twitch) dominance and that of the vastus intermedius and the tibialis anterior muscle showed Type 2 (fast-twitch) dominance. The capillaries of the soleus muscle were tortuous, and this was thought to be advantageous for blood supply. In contrast, the capillaries of the vastus intermedius and tibialis anterior muscles had a relatively parallel pattern. Additionally, two different patterns of capillary architecture that appeared to correspond to certain metabolic characteristic of different muscle fiber types were preserved with corrosion casting. In conclusion, comparative studies on capillary architecture of the skeletal muscles are useful for analyses of its function.

Key words: skeletal muscle, ATPase staining, muscle fiber type, corrosion cast, capillary architecture

Skeletal muscles can be divided on the basis of their appearance into red and white muscles that correspond to Types 1 and 2, respectively. Physiologically, the speed of contraction of the Type 1 fibers (oxidative muscle) is slow and that of the Type 2 fibers (glycolytic muscle) is fast. Various methods of histochemical staining

have been introduced to classify skeletal muscles into different types, but adenosine triphosphatase (ATPase) staining is the preferred way to characterize each muscle type physiologically. Brook (1) was able to define three fiber types in normal muscle on the basis of enhanced ATPase staining, with pre-incubation at pH 9.4, 4.6 and 4.3. In addition to Type 1 fibers (weak in ATPase at pH 9.4), there were two subtypes of the Type 2 fibers (strong in ATPase at pH 9.4), which they called type 2A (weak reaction at pH 4.6 and 4.3) and 2B (strong reaction at pH 4.6 but weak at 4.3). A third subtype 2, designated as type 2C, had a residual positive reaction at pH 4.3 that became negative at still lower pH. Myosin is the primary protein component of skeletal muscle. The myosin molecule is composed of two heavy chains and four light chains. The myosin heavy chain (MHC) type expressed by a muscle fiber also correlates with the histochemical ATPase staining characteristic of that fiber. Because the MHC plays a major role in defining the rate at which myosin can hydrolyze ATP, myofibrillar ATPase activity is a useful indicator of fiber type classifications based on myosin.

This study was conducted to characterize the capillary architecture in fast-and slow-twitch muscles, and to determine whether or not the architecture can be used as a means of characterizing muscle fibers. We used the corrosion cast technique to observe capillary vessels with a scanning electron microscope (SEM). In most studies done to date, ink was fused into the capillary vessels, allowing them to be observed (2-7). However, with the corrosion cast method described by Murakami (8), the vessels can be observed microscopically and three-dimensionally.

Materials and Methods

Male adult Wistar rats, 15 weeks of age and weighing about 300-350 g, were anesthetized with an ether-oxygen

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mixture. Bilateral pairs of the soleus, vastus intermedius and tibialis anterior muscles were used for this study.

Histochemical analysis, fiber type grouping (12 rats, $n = 24$). The samples were quickly frozen in dry ice and acetone and stored at -80°C until histochemical analysis. Tissue from the midsection of the muscle bellies prepared for histochemistry was placed in a cryostat at -20°C and serially sectioned at a thickness of $10\mu\text{m}$. Samples for ATPase staining were prepared following Dubovitz's method (9), and the distributions of fiber type (Types 1, 2A and 2B) were analyzed. Muscle fiber type (Types 1, 2A and 2B) was determined on the basis of ATPase staining after alkaline (pH 10.4) or acid (pH 4.3-4.6) preincubation as established by Brook and Kaiser (1). Over 200 fibers were counted for each animal.

Corrosion cast technique (10 rats, $n = 20$). The aorta and inferior vena cava were prepared and a No. 18 winged catheter was introduced into the artery almost to the iliac fork and fixed with two 1-0 silk ligatures. The inferior vena cava was opened to allow the outflow of perfusing fluids. The vascular bed was thoroughly rinsed with heparinized 0.9 % saline solution. Methacrylate resin (Mercox CL-2B) with a standard amount of its catalyzer, was injected at room temperature until it completely filled the vascular bed. During this experiment, both legs of the anesthetized animals were kept free until the Mercox was completely injected; then the legs turned blue and contracted maximally. At 15 min after polymerization, both legs were cut from the hip joints and put into 70°C water for 48 h to allow the polymers to solidify.

The samples were immersed in a 20 % NaOH solution at room temperature for 24 h. The samples were then rinsed for about 7 days in distilled water to free the casts from tissue, and dried at room temperature. Then the casts of the soleus, the vastus intermedius and the tibialis anterior muscles were excised. The soleus muscle was identified from the Achilles tendon, and the vastus intermedius was identified from its location on the femur. The samples were dissected out and small pieces (5 mm) were obtained under Nikon transformer microscope. The specimens were fixed to stubs with Dotite, covered with platinum-palladium and observed with a HITACHI S-2300 scanning electron microscope operating at 5 keV. To compare the tortuosity of the capillaries, we measured each length, height and diameter of capillary vessels according to Ito's (10) method (Fig. 1). Over 20 capillaries were counted for each animal.

In the second series of experiments, both ankles were

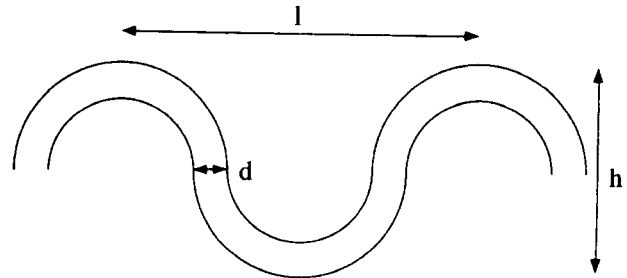


Fig. 1 The degree of the capillary tortuosity. We measured each length (l), height (h) and diameter (d) of capillary vessels according to Ito's method.

fully dorsiflexed, knees flexed and hips extended to extend the soleus and the vastus intermedius muscles. This position was maintained during every other phase of this experiment.

Student's t -test for independent samples was used to test for significant differences between pairs of group means. $P < 0.01$ was regarded as significant.

Results

Muscle fiber type distribution. The soleus muscle (Fig. 2) showed Type 1 dominance; Type 1 fibers comprised $76.1 \pm 8.1\%$ and Type 2A fibers $23.9 \pm 8.1\%$. No Type 2B fibers were observed. The vastus intermedius muscle (Fig. 3) showed Type 2 dominance; Type 1 fibers comprised $22.4 \pm 19.6\%$, Type 2A fibers $35.5 \pm 8.2\%$ and Type 2B fibers $41.3 \pm 14.7\%$. The tibialis anterior muscle (Fig. 4) showed Type 2 dominance; Type 1 fibers comprised $6.5 \pm 8.4\%$, Type 2A fibers $26.4 \pm 11.3\%$ and Type 2B fibers $67.2 \pm 16.3\%$. No Type 2C fiber was observed in these muscles.

Corrosion casts of the capillary architecture. The capillaries in the shortened soleus muscle (Fig. 5) had a tortuous pattern. The length, height and diameter of each wave were $15.7\mu\text{m}$, $15.2\mu\text{m}$ and $6.7\mu\text{m}$, respectively. The length and height are almost the same in the shortened soleus muscle. There were many transverse anastomoses between neighboring capillaries and the average distance between two anastomoses was $171.8\mu\text{m}$ ($n = 27$). Auto-loop anastomoses were seen in the soleus muscle and were thought to characterize this muscle. The capillary pattern in the extended soleus muscle (Fig. 6) was still tortuous, but to a lesser degree. The length and height of each wave were $37.2\mu\text{m}$, 15.7

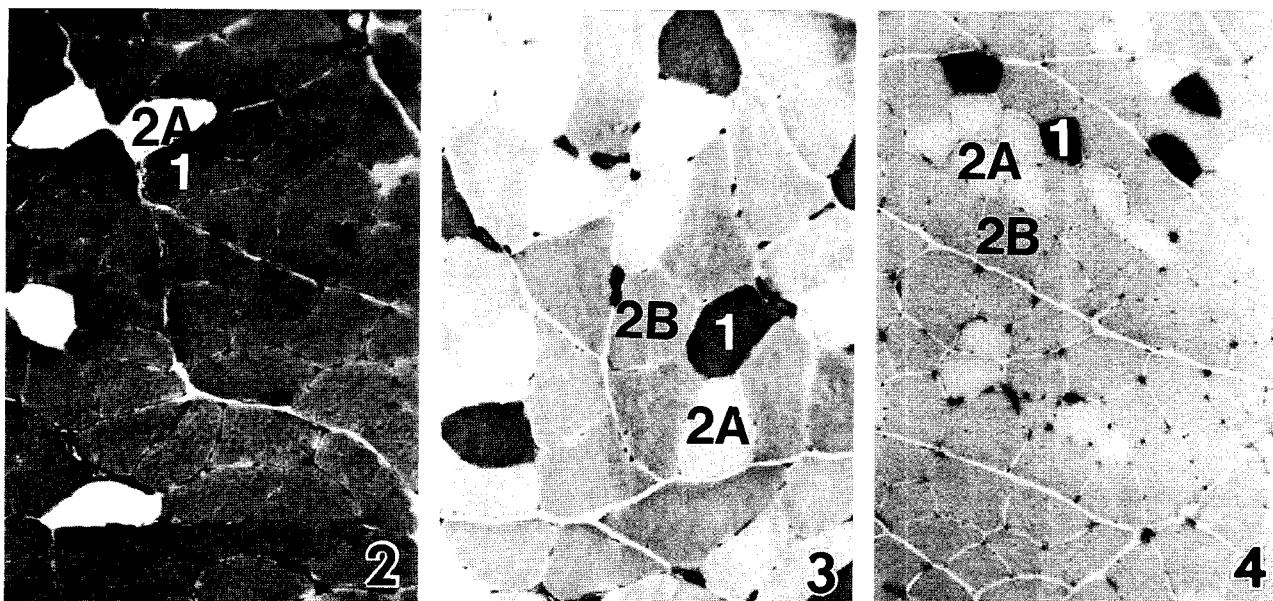


Fig. 2 The soleus muscle, ATPase staining $\times 100$ (pH 4.3). The soleus muscle shows Type 1 dominance.

Fig. 3 The vastus intermedius muscle, ATPase staining $\times 100$ (pH 4.3). The vastus intermedius muscle shows Type 2 dominance.

Fig. 4 The tibialis anterior muscle, ATPase staining $\times 100$ (pH 4.3). The tibialis anterior muscle shows Type 2 dominance and contains more Type 2 fibers than the vastus intermedius muscle.

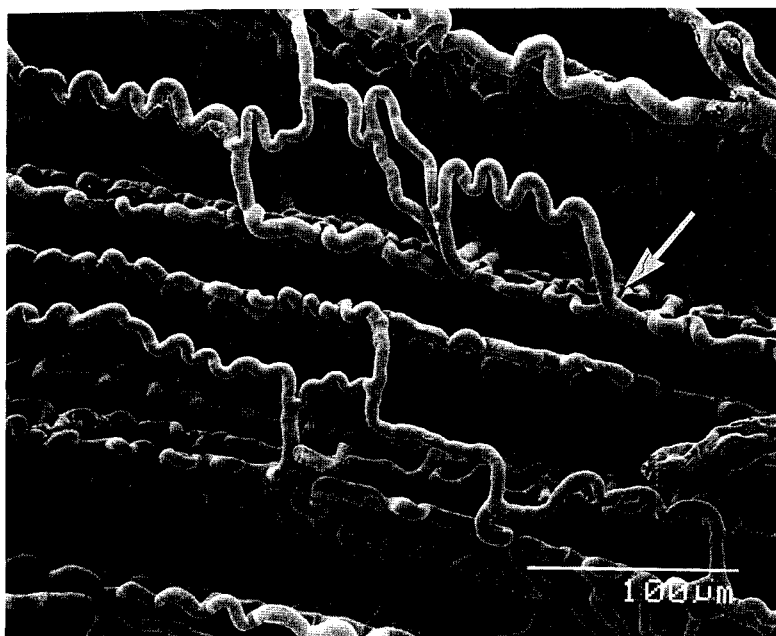


Fig. 5 Capillary architecture of the shortened soleus muscle. Bar = $100\mu\text{m}$. Its tortuous capillary pattern is thought to be advantageous for blood supply. Auto-loop anastomoses (arrow) are seen in the soleus muscle.

μm in the extended muscle, respectively. Only the length was longer than that of the shortened one.

The capillary pattern in the shortened vastus intermedius muscle (Fig. 2) was parallel but with a slightly

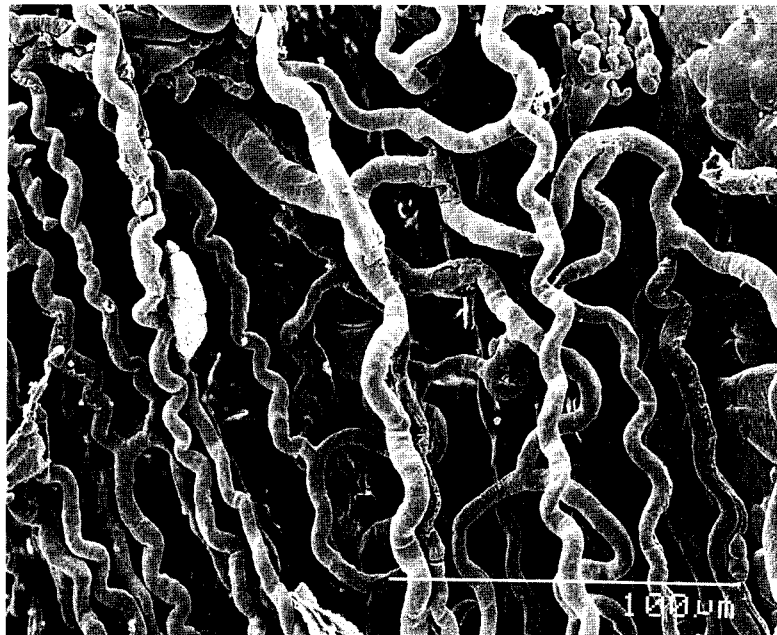


Fig. 6 Capillary architecture of the extended soleus muscle. Bar = 100 μ m. The capillary pattern in the extended soleus muscle is still tortuous, but the degree of tortuosity is smaller and capillary architecture is elongated longitudinally.

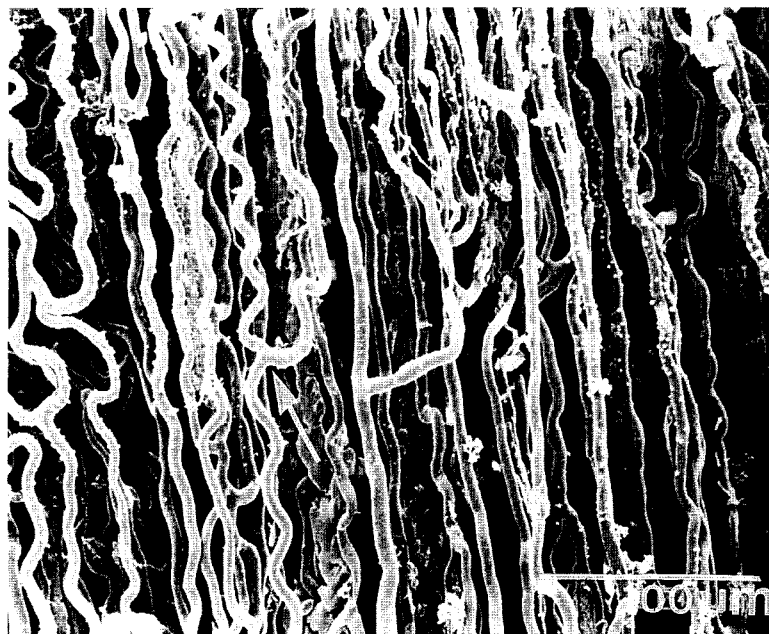


Fig. 7 Capillary architecture of the shortened vastus intermedius muscle. Bar = 100 μ m. The capillary pattern in the shortened vastus intermedius muscle is parallel with a slightly tortuous trend. Fork-division anastomoses (arrow) are sometimes seen in this muscle.

tortuous trend. The length, height and diameter of each wave were 29.3 μ m, 11.7 μ m and 6.1 μ m, respectively. The length was much longer than the height. There were

significant differences in the length and height between the shortened soleus and vastus intermedius muscles. The length of the vastus intermedius muscle was longer than

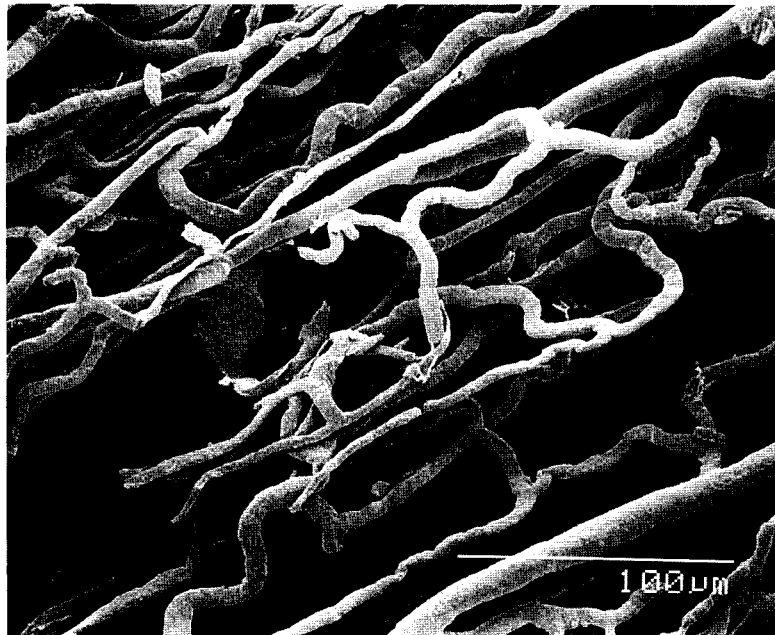


Fig. 8 Capillary architecture of the extended vastus intermedius muscle. Bar = 100. The capillary pattern in the extended vastus intermedius muscle is mostly parallel with transverse anastomoses forming a mesh.

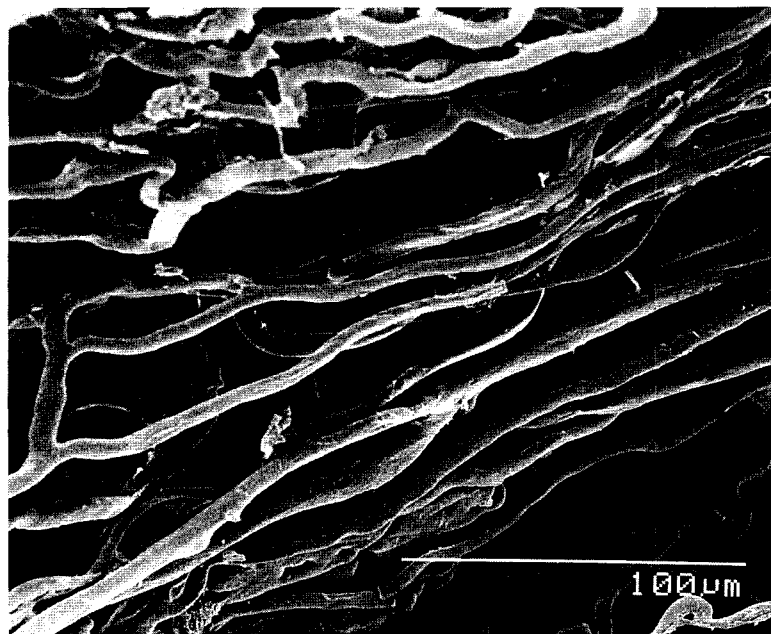


Fig. 9 Capillary architecture of the shortened tibialis anterior muscle. Bar = 100 μ m. The capillary pattern in the shortened tibialis anterior muscle is straighter than that of the vastus intermedius muscle.

that of the soleus muscle and the height was shorter than that of the soleus muscle, but there was no significant difference in the diameter. The average distance between two anastomoses was 292.1 μ m ($n = 29$) and was longer

than that of the soleus muscle. Fork-division anastomoses were sometimes seen in this muscle.

The capillary pattern in the extended vastus intermedius muscle (Fig. 8), was mostly parallel with trans-

verse anastomoses, forming a mesh. The length and height of each wave were $45.5\mu\text{m}$ and $11.9\mu\text{m}$, respectively. Only the length was longer than that of the shortened one.

The capillary pattern in the shortened tibialis anterior muscle (Fig. 9) was straighter than that of the Vastus intermedius muscle. The length, height and diameter of each wave were $39.3\mu\text{m}$, $12.8\mu\text{m}$ and $7.3\mu\text{m}$, respectively. There were significant differences in the length and height between the shortened soleus and tibialis anterior muscles. The length of the tibialis anterior muscle was much longer than that of the soleus muscle and the height was shorter than that of the soleus muscle but there was no significant difference in diameter.

Discussion

The first studies on the capillary vessels in the skeletal muscles were made by Ranvier (11) and Spalteholz (12). Spalteholz described that the branches of the dog and human newborn in the majority of cases run parallel to the muscle fibers and are connected by numerous anastomoses. Eriksson and Myrhage (13) measured the lengths and diameters of the different vascular segments in the tenuissimus muscle of cats with a vital microscope. There was wide variation in these variables with time and among capillaries. However, only the superficial muscles of some laboratory animals can be studied, under parapsychological conditions. Most previous studies of microvascular geometry have made use of histochemical techniques to identify the capillaries (2, 6) or of filling the vasculature with India ink (4, 5) or silicone rubber (3, 7). These methods, however, are restricted to two dimensions.

Appell (14) reported that both capillary patterns, straight as well as undulating capillaries were observed within the same muscle, namely the gastrocnemius muscle of the Japanese Waltzing Mouse. There are convincing differences in the fiber composition of this muscle, because in one head white fibers predominate, which are surrounded by straight capillaries, whereas in the other head of the same muscle red fibers predominate, which are surrounded mostly by undulating capillaries. The functional significance of such a tortuous capillary pattern is probably a means of enlarging the capillary surface. This may facilitate the exchange of oxygen from the blood to the muscular tissue and of metabolites in the opposite direction.

Gaudio *et al.* (15) tried to clarify the differences between fast-twitch muscles such as the tibialis anterior and the pectineus muscle and slow-twitch muscles such as the soleus muscle using a microcorrosion cast technique to study the microvascular bed of skeletal muscles in the rat. They found astonishing differences between capillary networks in the soleus and the tibialis anterior muscles, based on peculiar characteristic of the capillary network in the soleus. The soleus capillary network differs from the tibialis anterior network not only in terms of the degree of tortuosity of the vessels or the frequencies of transverse interconnections, but also the pattern of the architecture. The peculiar characteristic that makes the soleus microvascularization denser is the presence of capillaries which at both ends are connected with the same capillary, having the function of a collateral circle.

Gaudio *et al.* (16) said that different problems made the study of skeletal muscle microcirculation interesting and all of them must be taken into consideration. First, not all the muscles show variability in terms of metabolism, even within the same muscle group. Second, work load varies among different activities. Third, microcirculation is influenced or regulated differently by the nervous system and by local factors. Fourth, microcirculation is influenced by the variation of the muscle belly morphology and by the heightening of interstitial pressure during contraction.

The soleus muscle is a slow-twitch, mainly oxidative muscle and its capillary architecture is more advantageous for blood supply. Pannarale *et al.* (17) observed that there is a permanent endogenous difference in microcirculation between oxidative muscles such as the soleus, and glycolytic muscles such as the tibialis anterior muscle, which are determined by muscle fiber metabolism irrespective of extension and shortening of the muscle belly.

In most studies, the degree of tortuosity of the capillary vessels was not measured. They only divided them into two groups based on their appearance. The first to measure the degree of the tortuosity was Ito (10); he recognized it as a wave and measured each length, height and diameter. The observation were only used for the rat soleus muscle. In previous studies, the degree of the tortuosity was not compared to fiber type composition.

In this study, the capillary architecture of the soleus muscle is tortuous and the length of its wave is of the short pattern. The capillary architecture of the tibialis anterior muscle is straight and its wave is significantly longer than that of the soleus muscle. The vastus inter-

medius muscle shows the intermediate pattern. It becomes straighter as it contains progressively more Type 2 fibers. In most of the studies, the soleus muscle is selected as representative of Type 1-dominant muscle fibers and the tibialis anterior muscle of Type 2-dominant fibers (16, 17).

Our results revealed that the vastus intermedius muscle is Type 2-dominant. We chose this muscle because its position in the deep layer of the hind limb yields finer and easier casts than the muscles in the superficial layer. The tibialis anterior muscle in the superficial layer is easily damaged, especially when rinsing in distilled water. The vastus intermedius is Type 2-dominant, but it contains more Type 1 fibers than the tibialis anterior muscle. As such, it is useful to compare the fiber composition of the other muscle groups.

Our findings that the capillary architecture of the skeletal muscles extended or shortened in conjunction with similar changes of the skeletal muscle is consistent with other reports (15, 16). Gaudio *et al.* (18) reported that the three-dimensional arrangement of pericytes at the level of the capillary bifurcations underlines their role in red cell flow regulation. However, if the mechanical linkage of the pericytes to the endothelium and their contractibility is taken into account, it seems likely that these perivascular cells may have additional roles. Gaudio *et al.* (19) reported that their strong connection with the endothelium and their resistance against stretching imply that they provide mechanical support for the vessel wall.

Skeletal muscles shortened maximally when the sclerotic tissue was injected in its vascular bed in the present study. Capillary architecture of the soleus muscle fully shortened was quite different from that of the vastus intermedius and the tibialis anterior muscle. Mathieu-Costello *et al.* (20), however, contended that capillary tortuosity, as seen in microcorrosion casts, was not due to different fiber metabolism but was a consequence of fiber shortening when various rat skeletal muscles (soleus, gastrocnemius, and gracilis) were examined at lengths ranging from full shortening to full extension. The height of the capillary architecture of the soleus and the vastus intermedius, the tibialis anterior muscle showed remarkable differences. Not only the degree of the tortuosity but also the heights of the capillaries are thought to be characteristic.

As to the capillary diameter, Potter and Groom (21) have used modified Batson No. 17 anatomical casting compound to prepare the casts of the microvasculature of

the heart and skeletal muscles in anesthetized rats. In casts from the left ventricle, the capillary network appeared to parallel the arrangement of the muscle fibers, but showed many capillary loops and anastomoses. In skeletal muscles (gastrocnemius and gracilis) held at full extension *in situ*, the casts showed long straight capillaries with fewer branches than in the heart. In shortened skeletal muscle, the capillaries exhibited an undulatory configuration. The mean values for capillaries in these three tissues did not differ significantly. When the capillary bed is fully distended, the smallest capillaries, $2.3\mu\text{m}$ or less and amounting to 1–2 % of the total number, must be channels for plasma flow alone. Canham *et al.* (22) reported that the lowest range of capillary diameter was at the limits of deformation of the circulating erythrocyte and 95 % of the erythrocytes could pass through a channel $2.9\mu\text{m}$ in diameter. A small number of the capillaries measured were narrower than this limit. The range of the diameter in our study was from 3.1 to $13.2\mu\text{m}$. There was wide variation among capillaries and some channels for plasma flow. Appell (14) said that the mean diameter of the capillaries in Type 1-dominant muscles is larger than in Type 2-dominant muscles. They thought that the differences in diameter were related to the differential blood flow. However, we did not find a marked difference in the capillary diameters between the soleus, vastus intermedius and the tibialis anterior muscles in this study. We thought differential blood flow was a result of the tortuosity of the capillaries.

In conclusion, the soleus muscle in the hind limb of the rat shows type 1 dominance, and its tortuous capillary pattern is thought to be advantageous for blood supply. The vastus intermedius and the tibialis anterior muscles show Type 2 dominance, and their capillary pattern is straight; however, the latter contains more type 2 fibers and its capillary pattern is straighter than the former. Thus, capillary architecture appears to reflect fiber metabolism. Capillary architecture elongates or shortens as the skeletal muscles extends or flexes.

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