PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **158**, 288–291 (1978)

Skeletal Muscle Fiber Size and Capillarity[1] (4990)

A. H. SILLAU[2] AND NATALIO BANCHERO

Department of Physiology, University of Colorado School of Medicine, Denver, Colorado 80262

Muscle capillarity is not adequately defined by either capillary density or by capillary to fiber ratio alone (1). Because capillarity is an important determinant of O_2 delivery and CO_2 removal at the tissue level, we have studied it in the muscles of different animals to determine the normal arrangement of capillaries around the fibers and perhaps, more importantly, to understand the functional significance of the distribution of capillaries as the girth of the fibers increases. Our results indicate that capillary to fiber ratio is a linear function of fiber cross sectional area. The data presented here suggest that muscle "regulates" the maximal volume of tissue served by one capillary.

Materials and methods. The solei muscles of 24 guinea pigs (bw between 200 and 637 g), six rabbits (bw 3.9–4.5 kg) and six cats (bw 1.26–3.37 kg) and the medial head of the gastrocnemius of the same guinea pigs were surgically removed under sodium-pentobarbital anesthesia. Also, the sternothyroid muscle of 24 dogs (bw 3.5–27.0 kg) was studied. The sternothyroid was selected because being small complete cross sections could be cut and because previous work in our laboratory has shown it to be a muscle with fibers arranged mostly in a parallel fashion (2). Dogs have no soleus muscle. Immediately after removal the muscles were placed in a moist gauze until weighed. They were cut transversely at the widest point of the belly and quickly frozen in isopentane cooled with liquid N_2 to about −130°C. The samples were then stored in liquid N_2 until sectioning in a cryostat.

The myosin ATPase technique (pH 9.4) after preincubation at a pH of 4.5 for 5 min was used to identify fiber types. Brooke and Kaiser (3) have shown that at a pH of 4.5 the inhibition of the ATPase is partial and the fibers usually show a color pattern opposite to the one observed when no preincubation is used. The DPNH tetrazolium reductase method was used to ascertain the oxidative capacity of each type of fiber in serial muscle sections (3, 4).

In the guinea pig gastrocnemius the fibers with the highest oxidative capacity, as judged by the DPNH tetrazolium reductase, were the ones with the lowest ATPase activity after preincubation at a pH of 4.5. We call these red fibers. The white fibers had the lowest oxidative activity and intermediate ATPase activity. These two types are fast twitch fibers (5). Intermediate fibers had very high ATPase activity and appeared black. These are slow twitch (5). As shown by Dubowitz and Brooke (4) it is easier to distinguish differences in color intensity of the three-fiber types after preincubation at a pH of 4.5 than in the conventional technique with no acid preincubation.

Several photomicrographs were obtained from each tissue section to assure maximal coverage. The actual area covered by each photomicrograph was 289,144 μm^2. The photomicrographs were then projected on a screen, at a known magnification, to estimate by the differential point counting method, the average cross sectional area of the fibers (6). The fibers that were cut by the perimeter were counted on two sides of the rectangle (frame) while they were not counted on the other two. The absolute numbers of fibers of each type was also counted at this time. The same ATPase technique following preincubation in an acid medium (pH 3.8–4.0) was used to visualize capillaries in muscle cross sections (7). In each muscle, 30 different microscopic fields, selected at random over the areas where fiber composition was analyzed, were used to count capillaries under oil immersion (1000 ×). The fields counted had

---

[1] This work was supported by IVITA, San Marcos University, and Research Grant No. HL 18145 from the National Heart, Lung and Blood Institute.

[2] Dr. A. H. Sillau was recipient of a fellowship award from the Rockefeller Foundation, Present address: IVITA, Universidad de San Marcos, P.O. Box 4270, Lima, Peru.

0037-9727/78/1582-0288$01.00/0

actual tissue total areas between 543,000 and 804,000 μm^2. These differences resulted from the use of different microscopes. These counts were averaged and the values for capillary density were expressed as capillaries per mm^2. With this information, capillary density to fiber density ratios were obtained.

Results and discussion. Three fiber types were identified in most of the medial head of the guinea pig gastrocnemius. The means ± 1 SD for these fibers were: red 44.5% ± 5.0, white 31.2% ± 6.7 and intermediate 22.6% ± 4.7 in the central portion of the medial head. In the sternothyroid of the dog only two fiber types were evident with the myosin ATPase after preincubation at pH of 4.5. The dark fibers (42.0% ± 5.5) showed the greatest oxidative activity as judged by the DPNH tetrazolium reductase. Light fibers, with the lowest oxidative activity, were more numerous averaging 58.0% ± 5.6. The solei we studied had only one fiber type, the intermediate (4).

Postnatal growth of skeletal muscle occurs mainly as a consequence of an increase in the girth of the fibers (8). Hence, in a given animal species, the weight of the muscle increases mainly because of an increase in the cross sectional area of the fibers with a smaller increase in length (9). In our animals the relationship between muscle weight and fiber cross sectional area were statistically significant for the guinea pig and the dog, the two species in which a wide range of body weights existed (Table I). In the other two species the changes in these variables were qualitatively the same but were not statistically significant because of the smaller number of animals and the smaller range in body weights.

When the fiber girth increases, the capillaries surrounding the fibers are pushed apart. In the guinea pig and rabbit, capillary density remained unchanged despite changes in fiber cross sectional area (Table II). In both the dog and the cat, however, capillary density decreased with increasing fiber cross sectional area (Table II). Nevertheless, in all four species the absolute number of capillaries around the fiber increased to compensate for a larger cross sectional area. When capillary to fiber ratios were plotted against fiber cross sectional area, a linear increase in C:F was found in all cases (Fig. 1). The magnitude of the increase in C:F is given by the slope of the regression lines. However, the effect of growth on capillary density appeared to be related to both the radius of the cross sectional area of the fiber and the change in C:F. For example, the increase in C:F in both dogs and cats as the fibers increased in girth, even though significant, was apparently not sufficient to maintain a constant capillary density which decreased with increasing fiber girth.

When data from the solei, with homogeneous composition, were combined, the correlation between fiber cross sectional area and C:F was excellent (Fig. 2). The C:F varied tenfold from 0.37 to 3.85 as the fiber cross sectional area increased from 600 μm^2 to 7500 μm^2. The intercept of this regression equation for this relationship was not significantly different than zero. This, therefore, suggests that the area of the cross section

TABLE I. RELATIONSHIPS BETWEEN MUSCLE WEIGHT AND FIBER CROSS SECTIONAL AREA.[a]

Animal	Muscle	N	Equation	*r*	*P*
Guinea pig	Gastrocnemius	23	FCSA = 587.1 MW + 825	0.78	<0.01
Guinea pig	Soleus	17	FCSA = 7896.0 MW + 270	0.89	<0.01
Dog	Sternothyroid	23	FCSA = 659.9 MW + 1423	0.72	<0.01

[a] FCSA = fiber cross sectional area (μm^2); MW = muscle weight (g).

TABLE II. RELATIONSHIP BETWEEN CAPILLARY DENSITY AND FIBER CROSS SECTIONAL AREA.[a]

Animal	Muscle	N	Equation	*r*	*P*
Guinea pig	Gastrocnemius	17	CD = −0.033 FCSA + 775	−0.14	>0.05
Guinea pig	Soleus	23	CD = −0.045 FCSA + 664	−0.34	>0.05
Dog	Sternothyroid	23	CD = −0.099 FCSA + 1124	−0.62	<0.01
Cat	Soleus	6	CD = −0.130 FCSA + 1045	−0.92	<0.01
Rabbit	Soleus	6	CD = −0.017 FCSA + 620	−0.60	>0.05

[a] CD = capillary density (cap/mm^2) FCSA = fiber cross sectional area (μm^2).

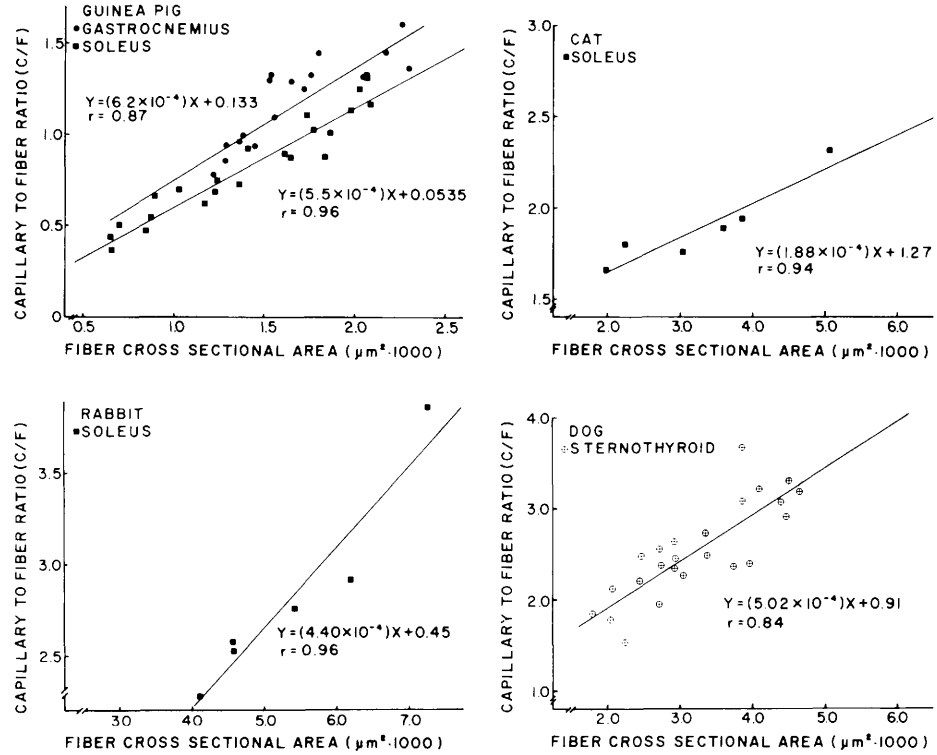

FIG. 1. Relationship between fiber cross sectional area and C:F in various muscles of four different animal species.

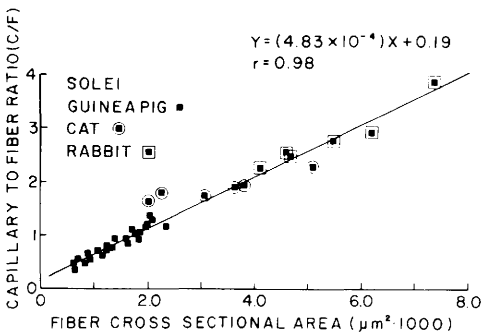

FIG. 2. Relationship between fiber cross sectional area and C:F in the solei of guinea pigs, cats and rabbits.

served by a capillary in a muscle like the soleus, is a constant, irrespective of fiber size or species. This means that if the length of the capillary is unchanged the volume of tissue served by a capillary is a constant. Furthermore, it could be speculated that the cross section of large fibers are probably elongated. This is precisely the microscopic image in the rabbit, the species with the largest fibers. Loats *et al.* (1) have reported a relative increase in the number of capillaries with a sharing factor of two as the cross sectional area of the fibers increases. Our data show that more capillaries are found around large muscle fibers indicating that a functional relationship between these variables may exist. That C:F values are higher in muscles with large fibers is evident in published data despite the widely varying values for C:F (10). C:F values were also related to the particular fiber composition of the skeletal muscle under examination. In the guinea pig, for instance, at a given fiber cross sectional area, the C:F is about 20% higher in the gastrocnemius than in the soleus (Fig. 1). This is because red fibers are surrounded by more capillaries than either white or intermediate fibers (12, 13). However, some of the variability in C:F values reported in the literature is due to methodology. It is generally accepted that filling of capillaries with foreign substances can result in variable degrees of underfilling (10, 11).

Values of C:F or capillary density alone provide no information on fiber size or on maximal distance the O_2 molecules have to diffuse to reach the innermost portions of the muscle fiber. Loats *et al.* (1) have used a system of circles to estimate the capacity for O_2 transport of the capillary system in skeletal muscle.

The cross sectional area and the volume of tissue perfused by one capillary appears to be an indicator of the capacity of the muscle to maintain adequate levels of oxygenation. The fiber cross sectional area plays an important role in O_2 transport, because O_2, transported by diffusion, must reach the innermost portion of the muscle fiber. In theory, at least, at very large fiber cross sectional areas, oxygenation may be hindered regardless of the number of capillaries around the fibers unless considerable changes in the shape of the cross section do occur.

Summary. The ATPase technique following preincubation in an acid medium (pH 3.8–4.0) was used to visualize capillaries. After preincubation at a pH of 4.5, in combination with the DPNH tetrazolium reductase, it was used to identify fiber types. A positive correlation has been demonstrated between fiber cross sectional area and C:F, indicating that large fibers are surrounded by more capillaries. The increase in fiber size may or may not be associated with changes in capillary density.

---

1. Loats, J. T., Sillau, A. H., and Banchero, N., Adv. Exp. Med. Biol. Oxygen Transport Tissue III. Edited by I. A. Silver, M. Erecińska and H. I. Bicher, Plenum Publishing Co. p. 41–48, (1978).
2. Banchero, N., Proc. Soc. Exp. Biol. Med. **148,** 435–439 (1975).
3. Brooke, M. H., and Kaiser, K. K., Arch. Neurol. **23,** 369 (1970).
4. Dubowitz, V., and Brooke, M. H., *in* Muscle Biopsy: A Modern Approach. P. 475. W. D. Saunders Co. (1973).
5. Barnard, R. J., Edgerton, V. R., Furukawa, T., and Peter, J. B., Amer. J. Physiol. **220,** 410 (1971).
6. Weibel, E. R., Kistler, G. S., and Scherle, F. W., J. Cell Biol. **30,** 23 (1966).
7. Sillau, A. H., and Banchero, N., Pflügers Arch. **369,** 269 (1977).
8. Goldspink, G. *in* Physiology and Biochemistry of Muscle as Food. (E. J. Briskey, R. G. Cassens, and B. B. Marsh, eds.). pp. 521, University Wisconsin, Press. Madison. (1970).
9. Faulkner, J. A., Maxwell, L. C., Brook, D. A., and Lieberman, D. A., Amer. J. Physiol. **221,** 291 (1971).
10. Plyley, M. J., and Groom, A. C., Amer. J. Physiol. **228,** 1376 (1975).
11. Willner, L. A. and Groom, A. C., Fed. Proceed. **36,** 325 (1977).
12. Mai, J. V., Edgerton, V. R., and Barnard, R. J., Experientia **26,** 1222 (1970).
13. Romanul, F. C. A., Arch Neurol. **12,** 497 (1965).

Received June 7, 1977. P.S.E.B.M. 1978, Vol. 158.