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Effect of Maturation on Capillary Density, Fiber Size and Composition in Rat Skeletal Muscle[1] (39694)

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In most mammals, postnatal growth of skeletal muscle appears to be due to a hypertrophy of existing fibers rather than to hyperplasia (1). This means that the fibers gain in length and in girth, but their number does not increase with maturation. Because in skeletal muscle the capillaries run parallel around the fibers these vessels are pushed apart by the fibers as they grow. If the number of capillaries per muscle fiber remains constant, the increase in body weight should result in a decrease in capillary density, i.e., the number of capillaries per mm^2. Differences in capillary density associated with body weight have been recognized by some investigators (2, 3), but overlooked by others when interpreting their experimental results (4).

We have studied capillary density and muscle fiber size and composition in three muscles of the rat in order to quantitate the changes that occur with maturation.

Materials and Methods. Forty-two female Sprague–Dawley derived rats[3] weighing between 99 and 291 g were studied in Denver, at an altitude of 1610 m above sea level. The animals were kept in plastic cages at a temperature of 23 ± 1° and given water and food (Purina chow) *ad lib.* The rats were weighed just before the experiments.

Three muscles were selected for study: the soleus, the tibialis anterior and the gastrocnemius (medial head). These muscles were dissected out after the animals had been anesthetized with sodium pentobarbital (30 mg/kg) administered intraperitoneally. The rats were sacrificed with a pentobarbital overdose. Immediately after dissection the muscles were weighed on a Mettler electronic scale (Model P1200). The gastrocnemius and the plantaris were weighed together. The medial head was then separated. The muscles were cut transversely at the widest point of the belly, where the cross section is usually the largest, and frozen in isopentane cooled with liquid N_2 at a temperature of −130°, while attached to a cork using tragacanth gum. We found no difference in weight in samples weighed before and after freezing. Samples were stored in a freezer at a temperature of −70° before slicing at a thickness of 15–20 μm in a cryostat (Type HR, Slee International, Inc., Pennsauken, N.J.) at −20°.

The calcium method of Padykula and Herman (5) demonstrates the adenosinetriphosphatase (ATPase) activity in tissue sections. Tissues with active ATPase systems are incubated in a medium containing ATP and a calcium salt. The calcium phosphate that forms is then made visible by cobalt chloride and ammonium sulfide producing insoluble dark cobalt sulfide which color intensity is proportional to the enzyme activity. The ATPase systems in skeletal muscle exhibit different pH lability which permits the identification of fiber types (6). We have used the ATPase method with preincubation at a pH of 4.4 and 9.4. In the rat the red fibers are those with the highest oxidative capacity and highest oxidative enzyme activity, the white fibers have the lowest oxidative capacity and the intermediate fibers are those with medium oxidative capacity (7). In all three muscles the strongest ATPase activity, after preincubation at a pH of 4.4, indicated by an intense black color, was seen in the intermediate fibers, while the lowest ATPase activity, indicated by a light sepia was seen in the red fibers. White fibers exhibited intermediate ATPase activity in the tibialis anterior and the gastrocnemius. A fourth type of fiber, possessing high oxidative capacity, shown

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by high DPNH tetrazolium reductase activity, but with intermediate ATPase activity after preincubation at a pH of 4.4 can occasionally be recognized in the soleus (6). We have included this fourth fiber type with the red fibers when reporting composition in this muscle.

In the gastrocnemius and tibialis anterior, photomicrographs were taken, using a Leitz Orthomat system, of areas where three fiber types were readily discernible. In the soleus, two photographic fields usually covered the whole cross section of the muscle. These photomicrographs were then projected, at a known magnification, on a screen to estimate, by stereology using the differential point-counting method, the area occupied by the different types of fibers (8). The absolute number of fibers of each type per unit area was also counted. With this information the average muscle fiber cross sectional area was calculated. The average number of fibers counted per animal was 1071 in the soleus, 2325 in the gastrocnemius and 2921 in the tibialis anterior. With these large samples the use of sterology was justified.

The Gomori technic for alkaline phosphatase (9) using β-glycerophosphate in barbital buffer at a pH of 9.2 and the ATPase reaction, after preincubation at a pH of 3.8 were used to identify capillaries. These two technics permit the visualization of capillaries by reacting with the capillary wall. No differences in capillary counts were found when these two methods were employed on serial cross section slices of the same muscle from the same animal. In each muscle, 30 different microscopic fields, selected at random over the areas where fiber composition was studied, were used to count capillaries under oil immersion (1000X). Each field had an area of 0.0268 mm^2 (Fig. 1). These direct counts were then averaged and the values for capillary density were expressed as capillaries per mm^2. Capillary to fiber ratios for each muscle were calculated from the values for capillary and fiber densities.

Results. The weight of the three muscles increased linearly with body weight as seen in Fig. 2. The correlation between these variables was strong. On the average, the weight of the gastrocnemius, tibialis anterior and soleus increased by 272, 229, and 206%, respectively with increases in body weight from 100 to 300 g. Figure 3 shows that the average cross sectional area of the fibers, at a given body weight, was different in the three muscles. It was the largest in the soleus, the smallest in the tibialis anterior, and intermediate in the gastrocnemius. In each muscle the cross sectional area of the

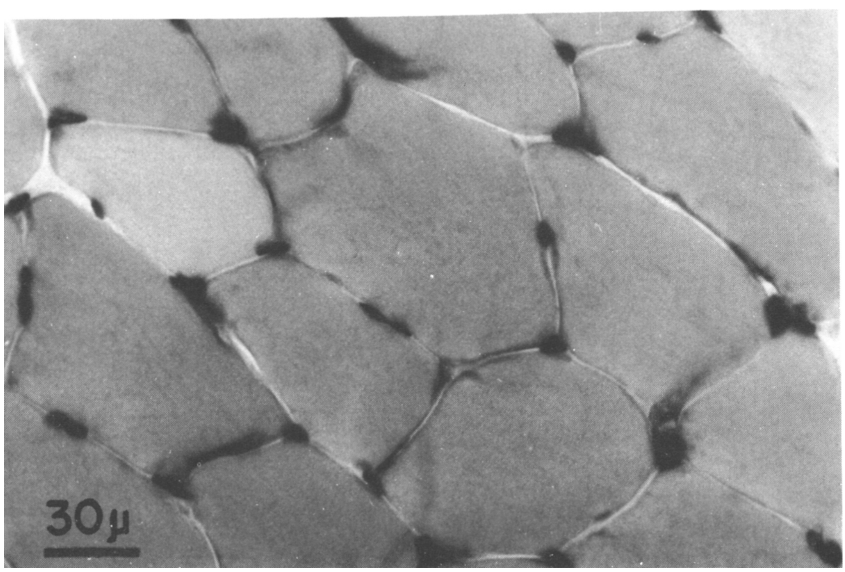

FIG. 1. Photomicrograph of cross section of rat soleus processed for ATPase after preincubation at a pH of 3.8. Capillaries are stained in black.

fibers was linearly related to the body weight of the rats. The correlation between these two variables was also strong. The increase in cross sectional area of the fibers over the range from 100 to 300 g body weight was less in the tibialis anterior than in the other two muscles.

As illustrated in Fig. 4, at a given body weight, the capillary density was significantly higher in the tibialis anterior than in the gastrocnemius and the soleus. This was mainly the consequence of the size of the cross sectional area of the fibers in each muscle. In all three muscles capillary density decreased with increasing body weight due to the gain in cross sectional area. However, the magnitude of the decrease in capillary density could not be entirely accounted for by the changes in area. In fact, in the tibialis anterior, the magnitude of the decrease was less than expected because of a concomitant increase in the capillary to fiber ratio (C:F) from 1.39 to 1.79, with increasing body weight (Table I). This increase in C:F appears to be associated with the increase in the relative population of red fibers (Fig. 5). Conversely, in the soleus and the gastrocnemius, the magnitude of the decrease in capillary density was larger than expected on the basis of changes in cross sectional area. In the soleus this was partly

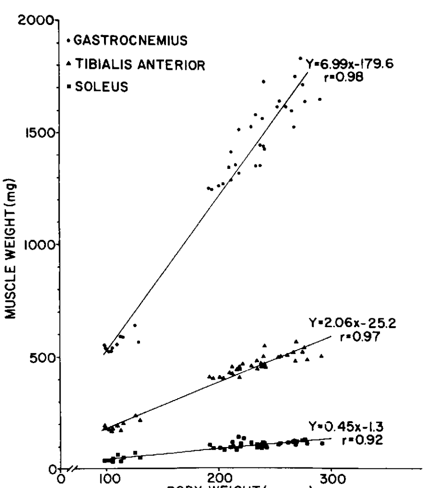

FIG. 2. Relationship between body weight and weight of three muscles of the rat. The gastrocnemius includes the plantaris.

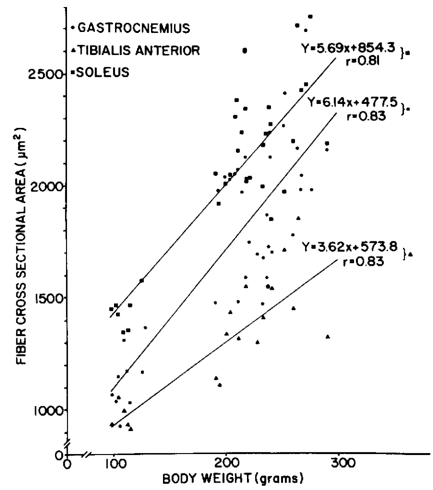

FIG. 3. Relationship between body weight and average cross sectional area of the fibers in three muscles of the rat. These three lines are statistically different; furthermore, the slope of the tibialis anterior line is different from the other two.

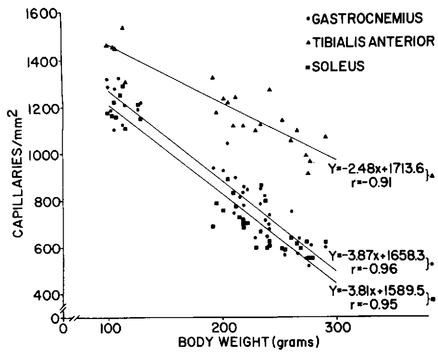

FIG. 4. Relationship between body weight and capillary density in three muscles of the rat. The three lines are statistically different. The slope of the line of the tibialis anterior is different than the other two.

TABLE I. RELATIONSHIPS BETWEEN C:F RATIO AND BODY WEIGHT (GRAMS) FOR THE TIBIALIS ANTERIOR AND SOLEUS MUSCLES OF RATS.

Muscle	Equation	*r*	*P*
Tibialis anterior	C:F = 0.001946 BW + 1.20	0.54	<0.05
Soleus	C:F = −0.001697 BW + 1.93	−0.48	<0.01

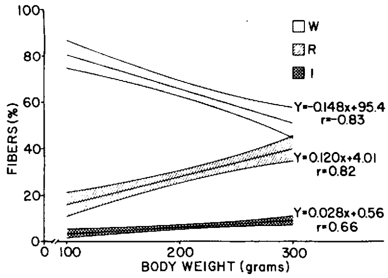

FIG. 5. Average muscle fiber composition in the tibialis anterior in rats of different body sizes. The 95% confidence intervals are shown. A decrease in white fiber population (W) and an increase in red fiber population (R) occurred with maturation ($P < 0.001$). The intermediate fiber population (I) changed only slightly but it was also significant ($P < 0.005$).

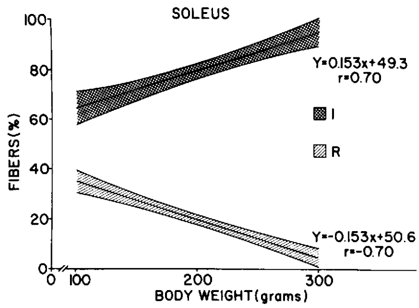

FIG. 6. Average fiber composition and 95% confidence intervals in the soleus. Significant changes in intermediate (I) and red (R) fiber populations were observed with maturation ($P < 0.001$).

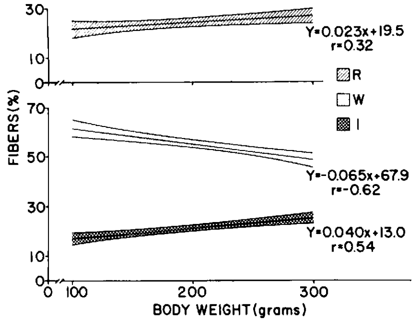

FIG. 7. Average fiber composition and 95% confidence intervals in the medial head of the gastrocnemius. The red fiber population (R) did not change with increasing body weight (upper panel) ($P > 0.05$). However, white (W) and intermediate (I) fibers changed significantly with maturation ($P < 0.001$) (lower panel).

due to a decrease in the C:F ratio from 1.76 to 1.42 (Table I), but in the gastrocnemius, the changes in C:F were statistically insignificant. The changes in C:F ratios with increasing body weight were accompanied by changes in fiber composition in the soleus (Fig. 6) while the gastrocnemius exhibited only moderate changes but with less scatter (Fig. 7).

Discussion. It is known that fixation of skeletal muscle with formalin leads to a reduction of approximately 34–56% in cross sectional area (4, 10). We have used frozen muscle samples to measure fiber size and capillary density which minimizes the error due to shrinkage (11) and renders more accurate data. Our capillary density values and fiber dimensions are, therefore, not comparable to most data published in the literature unless the shrinkage factor is known.

The rats we studied weighed between 99 and 291 g, which corresponds to ages of about 35–100 days according to growth charts provided by Simonsen Labs., Inc. This is the period when rats grow rapidly. After 110 days of age these rats grow at a much slower rate. Sprague–Dawley rats from different laboratories have different growth patterns. Our findings, therefore, apply to a specific age and weight group. Rats of these sizes are commonly used in the laboratory.

We have shown that, in young rats, growth causes a predictable increase in muscle weight, mainly due to an increase in the girth of the muscle fibers which is accompanied by a change in fiber composition. Due mainly to this increase in cross sectional area of the fibers capillary density decreases. Valdivia recognized that capillary counts in skeletal muscle of guinea pigs vary with body weight but he did not provide a quantitative estimate of these differences (2). We believe the variability in capillary density with body weight explains in part the wide discrepancies found in the literature (12). Furthermore, we found considerable differences in capillary density, fiber size and composition between muscles of the same animal, an observation previously made by others (11, 13). This probably reflects differences in muscle function.

Our findings strongly suggest that body weight must be considered when capillary density and muscle fiber size and composition are studied in rats and others rodents as well (2, 3, 11, 13). For example, Heroux and St. Pierre (4) concluded that exposure of rats to cold (6°) for 4 weeks produces an increase in capillary density from 454 to 594 cap/mm² in the soleus and from 563 to 745 cap/mm² in the medial head of the gastrocnemius. However, the rats exposed to a temperature of 6° were smaller than the rats kept at 30°, as indicated by the weight of the individual muscles, and this accounts for most of the differences in capillary density and fiber size they measured. They also found higher capillary density associated with a higher C:F ratio in the inner mass of the lateral head of the gastrocnemius of rats at 6°, an area that we did not study. It is possible that this higher C:F ratio represents the visualization of previously "empty" vessels because their technic stains the red blood cells and not the vessel wall. In addition, the C:F ratios we calculated from their data on capillary and fiber densities for the gastrocnemius are lower than the values reported. Heroux and St. Pierre (4) estimated the shrinkage factor of their histological preparations and corrected their values by this factor. Their values for capillary density and fiber cross sectional area for the soleus and the medial head of the gastrocnemius are in close agreement with our values in rats of similar sizes. They did not study the tibialis anterior.

More recently Cassin *et al.* (3) concluded that severe hypoxia (6 wk of exposure to 6100 m) results in opening of preexisting capillaries in rat skeletal muscle. In our view this conclusion is not supported by their data on C:F ratios. The histochemical technic they used stains the vessel wall and no interference on the functional state of the capillaries can be drawn from such an experiment. The body weight difference between the control and the experimental rats was significant and appears to be responsible for the difference in capillary density between the two groups, while C:F ratios, which should be indicative of changes in the absolute number of capillaries, were essentially the same (3).

Faulkner and coworkers (13) reported a decrease in total red fiber population and area with increasing body weight in the plantaris of guinea pigs and suggested that this change represents a decrease in the capacity for O_2 metabolism in older animals. We found a similar decrease in red fiber population in the soleus. For each muscle our data on changes in fiber composition with maturation parallels our findings on C:F ratios. Differences between individual muscles were found. Increases in the percentage of red fibers, that is, in fibers with greater numbers of capillaries around them (7, 11, 14), as seen in the tibialis anterior were accompanied by increases in the C:F ratio, whereas decreases in the percentage of red fibers, as in the soleus, were accompanied by a decrease in the C:F ratio. The explanation for these changes in fiber composition is not yet clear but they probably reflect differences in the physiology of these muscles with age. The fact that changes in fiber composition of skeletal muscle occur with maturation, independently of experimentally imposed conditions, must be born in mind by those interested in quantitating histochemical and structural changes in muscle.

Summary. Capillary density, fiber size and composition, were quantitated by histochemical methods in the tibialis anterior, soleus and gastrocnemius (medial head) of rats of different body weights. The capillary density was different in these muscles and it decreased with increasing body weight in a linear fashion due to a concomitant increase in the average cross sectional area of the fibers. This observation suggests that part of the variability in capillary density found in the literature is due to differences in body size. A change in muscle fiber composition with maturation was also observed. These were more marked in the tibialis anterior and in the soleus than in the gastrocnemius.

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