FIBER SIZE AND CAPILLARY TO FIBER RATIOS IN SELECTED MUSCLES OF EXERCISED RATS

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ABSTRACT

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By

Linda Jane Frome

One hundred and seventy-six male rats (Sprague-Dawley), 72 days of age, were used in this experiment. The animals were randomly assigned to one of seven treatment groups: Sedentary Control (CON); Voluntary Running (VOL); short-duration, high speed endurance running (SHT); medium-duration, moderate-speed endurance running (MED); long-duration, low intensity swimming (SWM). Treatments were administered once a day, Monday through Friday. All animals had access to food and water ad libitum. Animals from each group were sacrificed after zero, eight, and twelve weeks of training.

Animals were sacrificed under anesthesia by intraperitoneal injection of pentobarbital sodium. Pelikan ink was injected into the vascular system for capillary per muscle fiber calculations. The triceps surae and plantaris muscles were removed as a unit, and flash frozen in an isopentane-liquid nitrogen system.

Fresh-frozen, distal-proximal serial sections, were cut at 10 microns using a rotary microtome-cryostat.

A group of 30 adjacent muscle fibers was selected for study in each of three predetermined areas of the gastrocnemius, plantaris and soleus muscles. From the hematoxylin and eosin sections, each fiber was traced carefully using a microprojector at 200 magnifications. The cross-sectional area of each fiber was measured by polar planimetry. The same 30 fibers were identified on the PAS sections, and the number of ink-filled capillaries surrounding each fiber was recorded.

The most prominent features of the study were those attributed to duration affects. Over the twelve week period of the experiment, muscle fibers increased in size and this was interpreted as a growth affect. No statistically significant changes were detected in fiber sizes in relation to the various exercise treatments. Capillary to fiber ratios were not altered with respect to either duration or treatment affects.

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INTRODUCTION

The recent trends toward enhanced physical performance in everyday life have renewed concern and interest in the study of bodily systems. This concern and interest has led to the inspection of the characteristics unique to each system and of the characteristic interrelationships between systems. Skeletal muscle and its vascular supply are two examples of systems studied in this manner. For normal subsistence, skeletal muscle must obtain an adequate supply of oxygen and nutritive materials via its connections with the vascular system.

Parallel investigations of the anatomical, physiological and biochemical aspects of skeletal muscle and its vascular supply have been carried out. Due to the broad literature base as well as numerous and varied experimental conditions, such as limited exercise regimens and a variety of animal species and muscles used, a consideration of the muscular-vascular relationship has been difficult.

To provide a stronger base for future studies, the present investigation is concerned with the examination of adaptive response of the muscular-vascular relationships

under the conditions of seven chronic exercise regimens with duration ranges of zero to twelve weeks. Of prime consideration is the response of skeletal muscle fibers with regard to size and capillary to fiber ratios in the triceps surae and plantaris muscles of the rat.

REVIEW OF LITERATURE

Muscle Fiber Sizes

From investigations of the development of skeletal muscle fibers, it has been established that there is little differentiation embryonically or at birth (Halban, 1894; Buller et al., 1960; Rowe and Goldspink, 1969). In the newborn kitten all fibers of the gastrocnemius muscle were 100 square microns in cross-sectional area. In the soleus muscle from the same animals, fifty percent of the fibers measured 100 square microns and those remaining were 220 square microns (Denny-Brown, 1929). He also noted a uniform size at birth but added that "the size of skeletal muscle fibers is directly related to function" so that "with development of activity more differentiation is seen." Martin et al. (1932) did a comparative study of the gracilis muscle of the puppy and dog, noting the larger size of fibers in the full grown dog. They also commented on the "greater variation in (the) size of fibers in this muscle from a puppy than in the muscles obtained from full grown animals." Rowe and Goldspink (1969) stated that continued differentiation is a function of work load per fiber and that "post-natal increase in

in size and weight of muscles was brought about by an increase in the size of the constituent fibers, the fiber number remaining constant."

George and Naik's studies (1957, 1959) of different adult species of birds, showed red fibers to be smaller than white fibers. Henneman and Olson (1965) found that the smaller fibers, being resistant to fatigue, were specially adapted for long, sustained activity, while the broad fibers, which fatigue quickly, were utilized in quick, faster action. Henneman and Olson determined the size of red fibers as 900-2200u^2 and white fibers as 2100-3300u^2 in the cat gastrocnemius. In the soleus muscle the sizes ranged from 2800-3700u^2, while white fibers were larger. He added that red fibers were uniform, whereas white fibers were variable, in size and shape. As stated by Carrow et al. (1967) Dellasanta observed the red fibers of the rat gastrocnemius to be 1353u^2, but white fibers were 2652u^2. In contrast, "Stoel (1925) counted nearly three times as many white as red per square millimeter area."

Investigations have also determined the effect of activity on whole muscle size. After running dogs on an exercise wheel, Morpukgo (1897) examined the sartorius muscle and observed a 53-55% enlargement in muscle size due to an increase in sarcoplasm. Steinhaus (1933) stated that chronic exercise produced hypertrophy, "due

entirely to a true hypertrophy of individual fibers and not to the appearance of new fibers." Holmes and Rasch (1958) studied the same muscle in exercised rats and noted "the number of myofibrils per fiber in exercised animals was more variable than in controls and the pattern of distribution suggested increases in the number of myofibrils per fiber at the ends of the muscle." Steinhaus (1933) remarked that Siebert had seen hypertrophy in the gastrocnemius of exercised rats and determined it to be "a function of the speed rather than duration of running." Work by Rakusan and by Kleeberger also cited by Steinhaus supports the hypertrophy concept in exercised muscle. However Rakusan found only 15% hypertrophy in the pectoralis major muscle of pigeons, while Kleeberger reported a 90% increase in the cross sectional area of electrically stimulated rabbit ear muscle.

In 1962, Van Linge likewise encountered hypertrophy (71-92%) in relocated exercised, rat plantaris muscle, but he also noted some hyperplasia in the same animals. Rowe and Goldspink (1968) observed longitudinal splitting and budding. And stated that "under conditions of extreme work load the number of fibers may increase." In their experiments of surgically induced hypertrophy in mice, they also commented on the 56% increase in cross sectional area of the soleus after partial removal, and the 75% increase in cross sectional area after total

incapacitation of the gastrocnemius: "The effect of increased work load caused a proportion of the fibers to undergo further hypertrophy." Hypertrophy of the muscle as a whole, therefore, was due to an increase in the size of some fibers but not a gradual increase in the size of all fibers."

Tomanek (1970), working with the plantaris muscle, observed what he termed compensatory hypertrophy associated with increase in myofibrillar protein in response to high resistance-low repetitive activity. In contrast, Edgerton (1970) found necrotic, angular and split fibers in the soleus muscle but not in the gastrocnemius nor plantaris muscles of sedentary, moderate and heavily exercised rats. He further noted that the total number of fibers resulting from split fibers was greatest in the heavily exercised group, less in the moderate exercise group, and less still in the sedentary group.

Other investigators have observed the individual red and white fiber adaptation to exercise. Carrow (1969), studied the effects of three exercise regimens, sedentary, voluntary and swimming, on the red and white fiber populations of the rat gastrocnemius. Although significant differences in both red and white fiber sizes between sedentary and voluntary, and sedentary and swim, were detected, the red fiber size increased more than the white. Man-i (1970) disagrees with these findings. In

his study of exercised rat tibialis anterior, he noted an 11% hypertrophy of red fibers, but a 21% hypertrophy of white fibers, adding that there were no transformation of fiber types as a result of training.

Muscle Vascularity

For a comprehensive understanding of the capillary to fiber ratios in muscle, some basic points should be established concerning the circulatory pattern to red and white muscles. In 1956, Smith and Giovacchini examined the vascular patterns of red and white muscles of the domestic rabbit's thigh and leg. In red muscles they discovered that numerous vessels entered the muscle, as "11-16 separate small arteries," while in white muscles there were only one or two main arteries of supply. The main arteries of the white muscles entered unbranched and only after entering did they branch to supply the various parts of the muscle. Smith and Giovacchini cited Bloomfield and agreed with his findings in the human gastrocnemius and soleus muscles. "In the gastrocnemius muscle, vessels are commonly derived from a single main artery which split into branches to enter the upper end of the muscle and then descend throughout its entire length. In the soleus, there are at least five separate vessels entering the muscle separately and in succession."

Lee (1958) elaborated on red and white muscle vascularity while working with the domestic rabbit. Like Krogh (1918), Lee found sac-like dilations at the arteriole-capillary branching points in red muscle only where the branches come off at a wide angle. But, Lee also discovered these sac-like dilations at the site of capillary-venule joinings. In white muscle, he found no dilations at the arteriole-capillary branching points. Regarding the course of capillaries and arterioles, Lee commented on the tortuous pattern in red muscle, whereas in white muscle the patterning was simple and straight, with few branches. From this picture he contended that "the alternating branching manner of arteries in red muscle is obviously the best way for distributing more capillaries to a limited area. On the other hand, the repeating nature of branching in white muscle supplies a wider area with few capillaries."

Lee was not the first researcher to encounter a higher number of capillaries associated with red muscle. Stoel (1925) and Duyff and Bauman (1927), established a capillary per fiber ratio of 1.2 and 2.3 for red muscle, and 0.7 and 1.3-1.5 for white muscle, of the rabbit. Working with the rabbit leg and thigh muscles, Smith and Giovacchini (1956) likewise observed a higher capillary per fiber ratio in red compared to white muscle. From a physiological viewpoint they noted that "those muscles

which cannot function without a supply of oxygen apparently are equipped with a greater capillary bed." While actually analyzing the relationship of body size to capillary density, Schmidt-Nielson and Pennycuik (1961) provided values for comparing the capillary per fiber ratio of red and white muscle in several species and also supported the greater vascularity of red muscle.

Henneman and Olson (1965) were concerned with the red and white muscle vascularity in inactive muscles. In the medial portion of the gastrocnemius, they found more capillaries around the red or C fibers, while less were associated with the white or A fibers. In the soleus muscle they observed only an intermediate type, or B fiber. Reis et al. (1967) substantiated these findings, and observed that the main blood flow in the soleus was 2.6 times that of the gastrocnemius. Paff (1930) extended the study of red and white fiber vascularity while working on a comparative metabolic study of the rat, guinea pig and cat. He summarized his findings by stating that there was an "inverse proportion between intensity of metabolism and (the) area of active tissue supplied by a single capillary," thus "the greater metabolic demand of muscle tissue, (the) more capillaries will supply that tissue." These findings were further substantiated by Romanul (1965) in his study of fresh rat, rabbit and human muscle capillarity. He agreed that "the difference between the

number of capillaries around individual muscle fibers correlate(s) with the oxidative metabolic activity of the fibers" but he extended this idea by stating that "it is apparent that the capillary supply of the individual muscle fibers is intimately related to their energy metabolism," that is muscle fibers with low oxidative metabolic activity derive their contraction energy from anaerobic glycolysis which is stored as glycogen and metabolized without the use of oxygen. Consequently, these self-sufficient fibers depend on their blood supply only for the removal of their contraction by-products and hence are found to be provided with few capillaries. The opposite was determined to be true for fibers of high oxidative activity.

While some researchers were interested in the blood supply to resting muscle, others were concerned with what would be the adaptive change, if any, in the vascularity of exercised or stimulated muscles. In experiments with the guinea pig and frog, Krogh (1918, 1919) noted a 4-9 fold increase in the number of capillaries open to blood flow during muscle contraction produced by nerve stimulation. From his observation, he theorized that the number of open capillaries was a function of the intensity of muscle metabolism. Data on dog gracilis muscle by Martin et al. (1932), agreed with an increase in capillarity but they only found a two-fold increase upon obturator nerve

stimulation. In a similar study Kjellmer (1964) revealed his agreement with Krogh's data on the numerical increase in blood flow, and also with Krogh's theory of metabolic influence.

Instead of studying the general blood flow to active muscle, Hermanson (1971) examined the capillary per fiber ratio under the conditions of activity. From his observations on trained and untrained human quadriceps, he discovered the ratio to be 49.9% lower in untrained muscles, and deduced that "well trained muscle consumes more oxygen per unit of muscle than untrained." By contrast, Rakusan (1971), worked with the pectoralis major muscle of flying and restricted pigeons, and found that the number of capillaries and the number of fibers per square millimeter both decreased in active muscle. But the decreases were proportional so that the capillary per fiber ratios remained unchanged.

Carrow (1967) extended the circulatory investigations by analyzing the response of the capillary per fiber ratios in red and white fiber populations of the gastrocnemius muscle of rats under the influence of three different exercise regimens. This analysis revealed a greater vascularity of the red portion in all groups, his data on the capillary per fiber ratio agreeing with that of Schmidt-Nielson and Pennycuik (1961) but with a comparison of the exercise groups to the sedentary group,

there was a greater percentage increase in capillarity of the white area. He summarized his findings by stating that "red (vascularity) does not have to increase as much to meet metabolic demands." Mai's findings (1970) disagree and after examination of the capillary per fiber ratios of the red and white fiber populations of the medial gastrocnemius, and the intermediate fiber populations of the soleus in the guinea pig, he stated that there was a greater difference between controls and treadmill runners in the red portion.

METHODS AND MATERIALS

Animals

One hundred and seventy-six, 72-day old male, albino rats (Sprague-Dawley strain)[1] were utilized. Throughout the experiment, all animals received water, and were fed a commercial animal diet,[2] ad libitum. To insure a constant environment, a daily routine of handling, humidity and temperature control, and cage cleaning was maintained.

According to Wells and Heusner (1971), rats respond best to forced exercise between four hours before and four hours after the lights are turned off in the animal living quarters. In adherence to this, the lights were automatically sequenced to be turned off between 1:00 p.m. and 1:00 a.m. Thus the animals were trained during their normal active period.

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[1]Received from Hormone Assay Laboratory, Chicago, Illinois.

[2]Wayne Laboratory-Blox, Allied Mills, Incorporated, Chicago, Illinois.

Treatment Groups

As soon as the animals were received in the laboratory, they were randomly assigned to one of seven treatment groups. Before treatment began, a 12-day acclimatization period was allowed to permit adjustment to the laboratory environment.

During the acclimatization and treatment periods, the control animals were housed in individual sedentary cages. During the same time period, the animals designated for training programs were housed in individual voluntary activity cages which were standard sedentary cages equipped with freely revolving activity wheels to which the animals had free access. Once training began the experimental animals, were also housed in individual sedentary cages.

The following seven treatment groups were employed.

Control (CON)

The animals assigned to the control group received no special treatment and were maintained in their individual sedentary cages throughout the entire experiment.

Voluntary (VOL)

The voluntary animals received no special treatment. Recording of their activity in the individual free-activity cages was noted daily from an attached revolution counter.

Short (SHT)

The animals in this treatment group were exposed to short-duration, high-speed endurance training intervals in controlled-running wheels. The first day of the program consisted of completion of three bouts of exercise with five minutes of inactivity between bouts. Each bout consisted of forty repetitions of ten seconds of work alternated with ten seconds of rest time. The intensity of training was progressively increased until the 37th day, after which the animals were expected to complete 8 exercise bouts with 2.5 minutes of inactivity between bouts. Each bout then consisted of 6 repetitions of 10 seconds of running, at 5.5 ft/sec, with 40 seconds of inactivity between.

Medium (MED)

The animals belonging to the medium treatment group were subjected to a medium duration, moderate speed endurance program of interval training in the controlled-running wheels. The requirements of the first day of training was the same as the SHT group. The program was then gradually increased until the 37th day, after which the animals were expected to complete 5 bouts of exercise with 5 minutes of inactivity between each bout. Each bout consisted of 8 repetitions of 30 seconds of running at 4.0 ft/sec, alternated with 30 seconds of inactivity.

Long (LON)

The long-duration running group was subjected to a long-duration, low-speed endurance program of continuous running in the controlled-running wheels. The first day of the program was again the same as the SHT and MED groups. After a progressive increase of the program up to the 37th day, the animals were expected to run at the speed of 2.0 ft/sec in four 12.5 minute bouts of continuous exercise, with 2.5 minutes of inactivity between each bout.

Electrical-Stimulus Control (ESC)

The animals of the ESC group were permanently paired with the animals of the short duration running group. Only the short duration animals were paired because they received slightly more shock than the other running groups. During the SHT group's treatment, the paired ESC animals were housed in cages attached to their pairs controlled-running wheel. Each ESC animal received the same light stimulus and electrical shock as its SHT partner.

Swimming (SWM)

These animals swam in individual cylindrical tanks during their training periods. On the first day of training, they were expected to swim for 30 minutes with no weight attached. The expected swimming time and percent

of weight attached to their tail was gradually increased until the second day of the eighth week. On the last four days of the eighth week, they were expected to swim for 60 minutes with the attached weight equal to 3 percent of their body weight.

Durations

In order to establish a more complete conception of the chronic treatment effects, each treatment group was divided into zero, four, eight and twelve week durations. Timing of these durations began after the onset of treatment and terminated with the sacrifice of the animal. The treatments of SHT, MED, LOW and SWM for the twelve week duration, was a maintenance of the respective programs 37th day levels until sacrifice.

Treatment Procedures

Initiation of treatment began after a 12-day acclimatization period, at which time the animals were 85 days old. The animals assigned to the zero week control group were immediately sacrificed at the end of the acclimatization period. The experimental treatments were carried out on a regular schedule, Monday through Friday, from 12:30 p.m. to 5:30 p.m., once a day in the Human Energy Research Laboratory, at Michigan State University, East Lansing, Michigan. Animals of the SHT, MED, LON and ESC groups were weighed before and after each training

period. The SWM group animals were only weighed before training, in order to maintain dry weights as a constant for all treatments.

Daily records were kept of the total revolutions run by each voluntary-activity animal by reading from a revolution counter attached to their individual cages. Controlled-running wheel results were recorded daily for each animal of the SHT, MED, LON, and ESC groups.

The apparatus referred to as the controlled-running wheel has been described as

> . . . a unique animal-powered wheel which is capable of inducing small laboratory animals to participate in highly specific programs of controlled, reproducible exercise. The CRW can be used to train groups of animals simultaneously and yet allows each animal to respond separately to the exercise program expected of his group. The CRW is programmed to operate and collect individual data automatically (Wells and Heusner, 1971).

The motivation to run was through avoidance reaction to a low-intensity controlled shock current which was automatically terminated when the animal attained the specified speed. A light above the wheel indicated the beginning of each exercise period and remained on for a predetermined time, the acceleration period. If by the end of the acceleration period, a specified speed was not attained, the light was turned off and a controlled shock current was applied through the grid running surface of the wheel. As soon as the specified speed was obtained, the shock was discontinued. If the animal attained a

specified speed by the end of the acceleration period, the light was turned off and no shock was applied. If the animal's speed decreased below the specified speed during the running period, the light and shock sequence was repeated. If the animal's speed was equal to or greater than the specified acceleration speed during the running period, no shock was given. Initially all animals ran in response to the shock stimulus. However, by the third 40 minute learning period, most animals ran in response to the light stimulus. In order to prevent any spontaneous activity during the rest periods, the wheels were automatically braked, while during work periods the wheels were freely moveable.

A result unit attached to the frame of each controlled-running wheel, automatically recorded the total number of revolutions run and cumulative duration of shock received by the SHT, MED, and LON groups, while the ESC used the SHT values. A value for the total expected revolutions, or the total number of revolutions the animal would have run if he were to continue at exactly specified speed, was determined. An index of the work performed relative to the work expected or percent expected revolutions was calculated from the total revolutions run and the total expected revolutions. From total work time and cumulative shock duration, the percent shock free time was determined to represent the percent of total work

time during which the animal was able to avoid shock by running at a speed equal to or greater than the specified acceleration speed.

For the SWM group, a value for percent expected swim time was calculated from the expected swim time and the recorded value of the swim time completed for each exercise period.

Sacrifice Procedures

Animals selected for sacrifice received their last treatment on Friday and were sacrificed the following Monday, with the exception of the first sacrifice which consisted of the zero-week controls. Besides the criteria of good health, a performance criterion of 75% expected revolutions and 75 percent shock free time was established for the SHT, MED and LON groups. The performance criterion for the SWM group was 100 percent expected swim time. Each sacrifice involved one CON animal plus pairs of animals from either VOL-MED-LON or SWM-SHT-ESC trios. The treatment programs were designed so that groups for sacrifice according to durations were consistent with the sacrifice schedule. The final sample involved 94 animals.

After weighing, each animal received an intraperitoneal injection of 0.26ml of 6.48% sodium pentobarbital solution. As soon as the animal was anesthetized, a transverse laparotomy was performed, exposing the inferior vena cava. Following syringe-barrel exchange,

4ml of 1% heparinized Pelikan ink[1] was injected for capillarization determinations of the hind limb muscles. After three minutes of *in vivo* ink circulation the right hind limb was skinned. The gastrocnemius, soleus and plantaris muscles were exposed and removed as one unit. The unit was covered with talcum powder and immersed for approximately one minute in 2-methylbutane (isopentane), which had been precooled (-140 to -185°C) by liquid nitrogen. The frozen muscle unit was then placed in precooled metal 35mm film containers and stored in a cryostat (-20°C).

Histological and Histochemical Procedures

After two to three hours in the cryostat, a block of tissue, approximately 7mm long, was cut from the bellies of the right gastrocnemius, plantaris, and soleus muscles. The use of a "sandwich" block (Figure 1) ensures identical freezing, cutting, incubation, fixation, and mounting of tissues from the three muscles of each animal. The blocks were mounted on cryostat chucks using 5% gum tragacanth. Eight, distalproximal serial cross sections were cut at 10 microns on a rotary microtome-cryostat. Sections were mounted on a cover glass and allowed to air-dry for one hour. Each slide was coded by animal number.

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[1]Obtained from John Henschel and Company, Inc., Farmington, Long Island, New York.

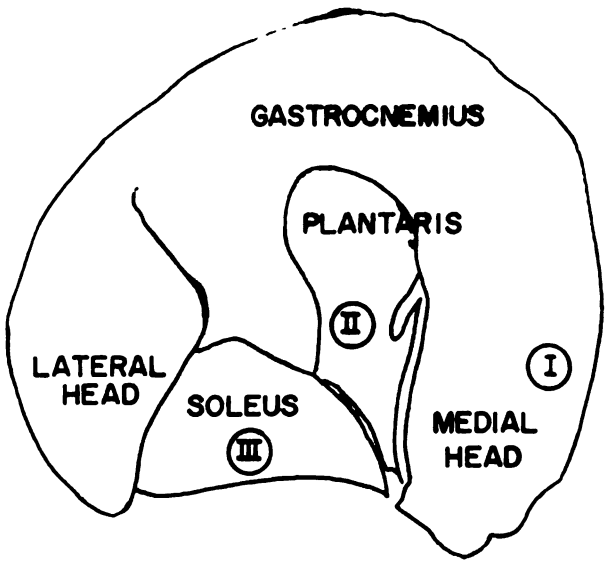

Fig. 1.--Diagram of a typical cross section of a "sandwich" block of the gastrocnemius, plantaris and soleus muscles. The three areas chosen for study are shown as numbered circles.

The present study is part of a much larger effort. Although eight histochemical procedures corresponding to the eight serial sections are noted below, only the hematoxylin and eosin and glycogen localization (PAS) methods were utilized for this report.

1. Succinic dehydrogenase (SDH) activity demonstrated using NBT [2, 2'-di-p-nitrophenyl-5, 5'-diphenyl-3, 3'- (3,3'-dimethoxy-4, 4'-biphenylene) ditetrazolium chloride] as described by Barka and Anderson (4).

2. Amylophosphorylase (phosphorylase) activity was demonstrated by the method of Takeuchi (142) modified by using hydroxymethyl aminomethane (tromethamine) buffer at a pH of 7.4.

3. Membraneous adenosin triphosphatase (ATPase) localization was investigated using the technique described by Wachstein and Meisel (148), at a pH of 7.2.

4. Glycogen localization was determined from fresh-frozen sections by the periodic acid-Schiff reaction (PAS) method (139).

5. Lipid localization was determined from fresh-frozen sections by the use of Sudan balck B (97).

6. Harris' alum Hematoxylin and Eosin (H & E) applied to fresh-frozen sections was used to identify basic morphologic characteristics (97).

7. Gomori's Trichrome (68) was used to demonstrate quantity of collagen and to augment basic morphological data.

Fiber Sizes and Capillary to Fiber Ratios

A group of 30 adjacent muscle fibers was selected for study in each of three predetermined areas (Figure 1) of the gastrocnemius (Area I), plantaris (Area II), and soleus (Area III) muscles. The fiber groups were chosen as subjective representations of the fibers of the respective areas. From the hematoxylin and eosin sections, the fibers of each area were numbered from 1 to 30. Each fiber was traced carefully using a microprojector at 200 magnifications. The cross-sectional area of each fiber was then measured by polar planimetry. The serial sectioning technique made it possible to identify the same 30 fibers on the PAS sections. The number of ink-filled capillaries surrounding each fiber was recorded. Individual fiber sizes and their associated capillary numbers were utilized in the statistical procedures outlined below.

Statistical Procedures

The data were analyzed by treatment group, durations and areas by computer (CDC 3600). Mean values were calculated and analyzed by a two-way fixed effects analysis of variance (ANOVA) model. The probability of committing a type I error (a) was set at 0.05 for the two-way ANOVA. The probability of committing a type II error (b) was set at 0.25.

RESULTS

The fiber size and capillary to fiber ratio results for each of the three areas are presented in tabular form (Tables 1-6). The analysis of variance and post hoc Scheffe comparisons are presented in the discussion of each area. In the analysis of variance, the significance level (9) was held at .05, but in the Scheffe analyses due to the rigorousness of the test, the significance level was set at .10.

Area 1

The fiber size results are presented in Table 1 and the capillary to fiber ratio results are given in Table 2. This area from the medial head of the gastrocnemius muscle is mixed in fiber type with a predominance of "white" fibers. The F value was significant ($P < .05$) and the Scheffe comparisons across durations for all groups except SWM were significant ($P < .10$) for fiber size. However, none of the duration down treatment Scheffe comparisons was significant. These results indicate no significant treatment effects attributable to the various training programs. There was s significant increase in fiber size which would be attributable to

Table 1.--Fiber Sizes: Area 1 (mixed but predominantly white).

Group	Weeks (area in square micra) 0	4	8	12	Percent Change 0-8	0-12	Scheffe Comparisons Across Durations*
CON	3846.9	5384.4	5218.8	5743.8	26	33	S
VOL	3675.0	4234.4	5046.9	4184.4	27	12	S
SHT	3887.5	4043.7	4968.7	4909.4	12	21	S
MED	3728.1	4509.4	4453.1	4306.2	16	13	S
LON	3790.6	3987.5	4200.0	4840.6	10	22	S
ESC	3818.7	4353.1	5215.6	5053.1	27	24	S
SWM	4881.3	2646.9	8959.4	3753.1	46	-23	N

*N, P > .10; S, P < .10.

Table 2.--Capillary/Fiber Ratio: Area 1 (mixed, predominantly white).

Group	Weeks (area in square micra) 0	4	8	12	Percent Change 0-8	0-12	Scheffe Comparisons Across Durations*
CON	2.48	3.19	2.70	2.51	8	1	N
VOL	3.16	2.80	2.69	3.21	-15	2	N
SHT	2.81	2.63	2.33	2.61	-17	-7	N
MED	2.36	2.59	2.13	2.64	-10	11	N
LON	2.26	2.64	2.26	3.03	0	25	S
ESC	2.75	3.26	2.90	2.71	5	-1	N
SWM	2.64	6.61	6.18	3.76	57	30	N

*N, P > .11, S, P < .10.

Table 3.--Fiber Sizes: Area 2 (dark, intermediate and light fibers).

Group	Weeks (area in square micra) 0	4	8	12	Percent Change 0-8	0-12	Scheffe Comparisons Across Durations*
CON	2806.2	3356.2	3443.7	3575.0	19	22	S
VOL	2800.0	3578.1	3437.5	3646.9	19	23	S
SHT	2562.5	2881.2	3325.0	3737.5	23	23	S
MED	2853.1	3006.2	3218.7	3181.2	11	10	S
LON	2637.5	3006.2	3050.0	3124.9	14	16	S
ESC	2662.5	3162.5	3493.8	3746.9	24	29	S
SWM	2250.0	2806.3	3568.8	2940.6	37	23	N

*N, P > .10; S, P < .10.

Table 4.--Capillary/Fiber Ratio: Area 2 (dark, intermediate and light fibers).

Group	Weeks (area in square micra) 0	4	8	12	Percent Change 0-8	0-12	Scheffe Comparisons Across Duration
CON	4.43	4.58	4.39	4.85	-1	-9	N
VOL	4.10	4.58	4.83	5.09	15	19	N
SHT	4.73	4.74	4.31	5.15	-10	8	N
MED	4.91	4.28	4.93	4.88	0	0	N
LON	4.44	4.40	4.49	4.46	1	0	N
ESC	4.53	4.78	4.66	4.50	3	-1	N
SWM	5.78	7.31	7.66	4.06	25	-30	N

Table 5.--Fiber Sizes: Area 3 (dark and intermediate fibers).

Group	Weeks (area in square micra) 0	4	8	12	Percent Change 0-8	0-12	Scheffe Comparisons Across Durations*
CON	3871.9	4896.9	4678.1	5159.4	17	25	S
VOL	3450.0	4371.9	5112.5	4859.4	33	29	S
SHT	3903.1	3718.7	4481.2	4846.9	13	19	S
MED	3650.0	3762.5	4328.1	4053.1	16	10	S
LON	3275.0	3809.4	3984.4	4293.7	18	24	S
ESC	3612.5	3856.2	4393.7	4881.2	18	26	S
SWM	3875.0	4962.5	5659.4	6250.0	32	38	N

*N, P > .10; S, P < .10.

Table 6.--Capillary/Fiber Ratio: Area 3 (dark and intermediate fibers).

Group	Weeks (area in square micra) 0	4	8	12	Percent Change 0-8	0-12	Scheffe Comparisons Across Durations
CON	5.66	5.96	5.18	4.93	-9	-15	N
VOL	5.66	5.55	5.31	5.89	-7	4	N
SHT	6.16	6.29	5.12	5.50	-20	-12	N
MED	5.69	5.46	5.61	4.89	-1	-16	N
LON	5.01	4.84	6.20	5.09	19	2	N
ESC	5.80	5.41	5.53	5.74	-5	-1	N
SWM	8.45	7.00	10.33	4.99	18	-41	N

growth and which appears to be independent of the treatment. No logical explanation can be made of the SWM results as they were highly variable. This is likely due to the inability to control swimming adequately as a treatment.

The capillary to fiber ratio results yield a significant F value but on post hoc examination only the LON group showed a significant duration effect. The treatments were not significantly different. The greatest relative change occurred in the SWM group but the variability in response was large. In the LON group in which the training is well controlled the increase in capillary to fiber ratio may have meaning. However, since the treatment results were not significantly different, interpretations should be reserved.

Area 2

Three fiber populations, dark, intermediate and light are present in this the plantaris muscle. The fiber size results are presented in Table 3 and the capillary to fiber ratio results are given in Table 4. This area from the plantaris muscle is quite heterogeneous in regard to enzyme activities in individual muscle fibers. It has a relatively high percentage of dark and intermediate type fibers in addition to a small percentage of low activity fibers. The F value was significant ($P < .05$) and the Scheffe comparisons across durations for all groups except

SWM were significant ($P < .10$) for fiber size. However, none of the durations down treatment Scheffe comparisons was significant. These results indicate no significant treatment effects attributable to the various training regimens. There was a significant increase in fiber size which would be attributable to growth but this appears to be independent of treatment.

Capillary to fiber ratio did not provide a significant F value across durations. Likewise, statistical significance was not detected relative to treatments. A large relative change was noted in the SWM group however, the variability was also great. Since the treatment and duration results were not significantly different, interpretations should be reserved.

Area 3

This area from the soleus muscle contains both dark and intermediate type fibers with a predominance of the latter. Fiber size and capillary to fiber ratio results are provided in Tables 5 and 6 respectively. The F value was significant ($P < .05$) and the Scheffe comparisons across durations for all groups except SWM were significant ($P < .10$) for fiber size. However, none of the duration down treatment Scheffe comparisons was significant. These results indicate no significant treatment effects attributable to the various training programs. There was a significant increase in fiber size which

would logically be attributable to growth and appears not to be associated with the type of treatment employed.

Again, capillary to fiber ratio results did not provide a significant F value across durations. In addition, statistical significance was not detected when the various experimental treatments were considered. The greatest relative change was seen in the SWM group at twelve weeks, however, variability was also large. Since the treatment and duration results were not significantly different, interpretation in light of this variability would be hazardous.

DISCUSSION

Discussion of the results of this experiment are complicated by the fact that several muscle fiber types are represented in each of the three areas selected for study. In order to simplify consideration of these differences, each area will be discussed separately.

Area I

The medial head of the gastrocnemius muscle contains at least three muscle fiber types. White fibers predominate but intermediate and red fibers are also present.

The significant increase in fiber sizes between zero and twelve weeks was expected. However, similar increases in both exercised (except the swimming group) and control animals was not expected. These results indicate that size increase was strictly an aging phenomena and not attributable to the exercise regimens. This is in conflict with the results of others who have reported significant changes in fiber size with exercise (Carrow et al., 1967; Gordon, 1967; Gordon et al., 1967). The absence of a fiber size increase in the SWM group commensurate with age or activity was totally unaccounted for

and was not in agreement with previous findings from this laboratory (Carrow et al., 1967). Individual muscle fiber types were not considered as separate entities in this study. It might be thought that such attention would reveal meaningful results. That is, if one fiber type were greatly affected in one direction and the others oppositely or not at all there would be an evening affect and no change would appear. This could not be the case however, since all fiber types have been shown to increase in size to some extent with various exercise regimens (Carrow et al., 1967; Gordon, 1967; Gordon et al., 1967; Mai et al., 1970).

Capillary to fiber ratio data was equally confusing. The absence of changes with respect to aging (duration) is in line with the results of growth studies (Goldspink, 1970). However, the significant increase shown by the LON group is unaccountable. The lack of significant treatment affects may be grounds for future work since similar affects have been reported (Mai et al., 1970) in the guinea pig. However, most investigators report increasing capillary to fiber ratios with exercise (Valdivia, 1958; Carrow et al., 1967; Gordon, 1967). Additional confusion is added to the picture since few investigators have used the same animal under identical exercise conditions.

Area 2

The plantaris muscle also has a heterogeneous population of fiber types. Specifically, it has a relatively high percentage of high and intermediate-activity fibers in addition to low-activity fibers (Edgerton et al., 1967).

The fact that fiber size increases were detected across durations was expected and indicated a growth affect. These results were statistically significant for all exercise groups except SWM and again this finding does not coincide with that of other investigators. A possible explanation might be found in the fact that exercise variability is so great in the swimming group.

The plantaris muscle reacted similarly to the gastrocnemius by showing no change in fiber size with the various treatment applied.

Capillary to fiber ratio alterations were not found and while this is in line with the findings of others (Groom and Plyley, 1973) it does not conform to the results of most investigators (Valdivia, 1958; Carrow et al., 1967; Mai et al., 1970). It would seem that these results should be "guarded" since Carrow has shown an increase in fiber size and capillary to fiber ratios in both red and white fibers with exercise. Furthermore, Mai et al. (1970) and Edgerton et al. (1972) showed a

preferential change in red fibers and their blood vessel counterparts after exercise.

Area 3

The soleus muscle in the rat contains only two fiber types (Edgerton et al., 1967). The red type constitutes about eighty percent of the muscle while intermediate type fibers account for the remaining portion.

The statistical analysis revealed information identical to that for the plantaris muscle. While it has been noted that these results are at odds with those normally seen under these conditions it is interesting that our findings were consistent for two muscles containing overriding populations of red and intermediate fiber types. These results were true for both fiber sizes and capillary to fiber ratios.

The results of this study are difficult to discuss in view of the fact that fiber size alterations were not seen in relation to the various exercise programs. This situation is difficult to interpret since all published results have shown changes in fiber sizes regardless of the type of exercise utilized. The increase in fiber sizes with time can be accounted for as a normal growth factor.

The only conclusion that can be reached which might account for the essentially negative results obtained here is related to the animal population

utilized. The animal strain normally used in this laboratory was not available for this portion of the work. Therefore, a different strain was used. The complicating factor here proved to be one of obesity and general lack of exercise by the animals.

Under these conditions it would be reasonable to assume that fiber sizes would not be changed from those of control animals. Capillary numbers have been shown to change in line with changes in their muscle fiber sizes with activity. Since the muscle fibers were apparently not influenced sufficiently to create size changes it would be reasonable to expect a similar reaction with respect to their blood supply.

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