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THE EFFECTS OF A PROGRAM OF VERTICAL JUMPING ON THE MYOSIN ATPASE ACTIVITIES OF THE RAT SOLEUS

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ABSTRACT

THE EFFECTS OF HIGH POWER OUTPUT JUMPING
ON THE MYOSIN ATPASE ACTIVITIES OF THE RAT SOLEUS

By

Michael Robert Ball

The purpose of this investigation was to determine the effects of a high power output jumping program on the myosin ATPase activities of the surgically overloaded rat soleus.

Twenty animals were brought into the laboratory and randomly assigned to three treatment groups: a sedentary-control group; a sedentary-surgery group; and an exercise-surgery group. Treatments were administered to the exercise-surgery group five days per week under controlled environmental conditions. All animals were provided with food and water ad libitum.

All animals were sacrificed four months after they were received by the laboratory. Body weight and soleus weight were obtained for each animal at the beginning and end of the four-month period. Due to unexpected complications the final sample size consisted of seventeen animals.

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This study is dedicated, with special appreciation, to my major advisor Dr. Kwok-Wai Ho. I would also like to thank Dr. William Heusner and Dr. Wayne VanHuss for their thoughtful assistance throughout my graduate career.

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CHAPTER I

THE PROBLEM

A large number of studies have been conducted in recent years to determine the mutability of skeletal muscle fiber types. The usual procedure is to place the muscle under various experimental conditions and subsequently to determine if any changes in energy metabolism and/or contractile characteristics can be induced. These experimental procedures have included denervation (6, 26), cross-innervation (8, 17), electrical stimulation (44, 61, 67), surgical overload (5, 37, 66) and various exercise routines (1, 28, 32, 47). As could be expected, the results of many of these studies have been contradictory. Therefore, continued research is needed to further understand the adaptability of skeletal muscle to various stimuli, including selected regimens of well-defined physical activity.

Statement of the Problem

The purpose of this study was to determine the effects of a three-month program of vertical jumping on myosin ATPase in the soleus muscle of the male albino rat. Vertical jumping is an activity which requires a high power output. The soleus is a predominantly slow-twitch muscle in the rat.

Importance of the Problem

At least two major types of fibers, "fast-twitch" and "slow-twitch", exist in the skeletal muscles of all classes of vertebrates. The so-called "fast-twitch" muscle fibers have a faster speed of contraction than do the "slow-twitch" fibers. From a biochemical point of view, the contractile speed of a muscle is determined by the ability of myosin ATPase to catalyze the breakdown of ATP which allows the cross-bridges formed between actin and myosin to shorten the sarcomere (7, 11). The intrinsic contractile speed and other metabolic factors, such as the glycolytic and/or oxidative capacities of the various fiber types, determine which fibers are selectively recruited under various exercise conditions. In general, fast-twitch fibers are used primarily when high degrees of muscle tension are generated; slow-twitch fibers are recruited preferentially during lower intensity muscle contractions.

Accumulating evidence has shown that low-intensity, chronic types of stimulation, including endurance exercise, can change the properties of myosin ATPase within fast-twitch muscle fibers (35, 53, 66). This results in an increase in the percentage of slow-twitch fibers within the muscle. However, changes in fiber composition from slow-twitch to fast-twitch, when either exercise or other experimental conditions have been applied, have not been as successful (15, 67, 69, 73).

Based upon the foregoing information, the present study was conducted to determine if the myosin ATPase activities in a slow muscle of adult rats could be changed by a program of vertical jumping.

Research Plan

The rats used in this study were divided into three groups. In the sedentary-control group surgery was performed to remove the soleus muscle from either the right or the left leg of each animal. No surgery was performed on the contralateral leg. Consequently, any change in fiber profile between the initially removed soleus and the soleus in the other leg represents the effect of aging. In the sedentary-surgery group the achilles tendon attachments of the gastrocnemius and plantaris muscles were cut on either the right or the left leg. In the opposite leg, the achilles tendon attachment of the gastrocnemius again was cut and the soleus was removed. Any change in fiber composition between the initially removed soleus and the soleus in the other leg would represent the combined effects of aging and surgical procedures. The animals in the exercise-surgery group underwent the same surgery as the animals in the sedentary-surgery group. In addition, each animal was subjected to a progressive program of vertical jumping. Any change in fiber composition in the group would represent the combined effects of aging, surgery, and exercise.

Jumping was chosen as the form of exercise to be used in this investigation because the resistance to movement occurs primarily at the beginning of the jump and is not continuous as is the case in activities such as running or weightlifting. The nature of jumping, therefore, requires a high velocity of muscular contraction which is conducive to the selective recruitment of fast-twitch motor units.

Following the initial and final muscle removal, serial cross sections were cut from the soleus and stained for myosin ATPase (ph 4.3) and NADH. The stained sections then were analyzed to determine the percentages of slow-and fast-twitch muscle fibers.

<u>Limitations of the Study</u>

1. The results of this study can be applied only to adult male rats and to the unique conditions to which they were subjected.
2. The duration of the treatment period and the training program were selected arbitrarily and, therefore, were not necessarily optimal for obtaining significant results.
3. A more complete understanding of the training effects would have been obtained if contractile and metabolic properties of the muscle had been studied in addition to the histochemical method used.
4. The attempt to isolate the soleus by means of surgery was unsuccessful. Consequently the training effects on this muscle were greatly reduced.
5. A necessary and sufficient sample size was not obtained and this limited the power of the statistical analysis.

CHAPTER II

REVIEW OF RELATED LITERATURE

Electrical stimulation of skeletal muscle has been a successful means of producing fiber-type conversions. Both external and implantable electrodes have been used, with the former being more popular because of convenience. Many studies have demonstrated an increase in contraction time as well as decreased ATPase activity in fast-twitch fibers when they are stimulated at a frequency characteristic of slow-twitch fibers (44, 61, 62, 68). In a classic experiment, Salmons and Vrbova (67) observed that chronic, low-frequency stimulation of the lateral peroneal nerve causes a slowing of contraction and half-relaxation speed in the fast-twitch tibialis anterior and extensor digitorum longus muscles of the rabbit. The nerve was stimulated at a frequency of 10 Hz, which is thought to be the typical impulse pattern of slow motorneurons (22), for 24 hours a day during the entire eight-week experimental period.

There is evidence to indicate that alterations in contractile properties occur in several stages and are due to entirely different circumstances. The increase in time-to-peak tension and half-relaxation time during the first few weeks of low-frequency stimulation possibly is due to alterations in the sarcoplasmic reticulum affecting calcium release (38, 39, 55). The delayed changes are believed to be the result of changes in myosin composition. Additional evidence for a change in myosin isoform can be seen in the transcription process following chronic stimulation. Co-expression of fast- and slow-type myosin light chains has been reported following long-term, low-frequency stimulation of fast muscles. This change in myosin light chain composition is believed to be due to the presence of mRNA normally found only in slow-twitch muscle (63).

In studies where predominantly slow-twitch muscle is stimulated by frequencies found in fast motorneurons the results are not so obvious. Jones (50) observed a shortening of contraction time in the soleus muscle of rats less than two-weeks-old when this muscle was stimulated at frequencies of 25 or 40 Hz. The increased speed of contraction was not compared with ATPase activity because the stained fibers are not easily differentiated at this young stage.

Lomo and Westgaard (53) used histochemistry and isometric twitch contraction time to show that fiber types are interchangeable if a slow muscle is stimulated "phasically" and fast muscle is stimulated "tonically". They divided rats into two groups both of which had their sciatic nerves cut. In the first group, the soleus muscle was stimulated tetanically at 100 Hz for 0.5 seconds every 25 seconds. In the second group, the denervated soleus received stimuli of 10 Hz lasting 10 seconds and repeated every 50 seconds. After five weeks the animals stimulated at 100 Hz had a mean contraction time of one-half that of a normal (innervated) soleus while those stimulated at 10 Hz had mean contraction times almost identical to a normal soleus. The results were supported histochemically by an increase in ATPase staining intensity among the fibers (preincubated at ph 10.4) in the soleus muscle stimulated at 100 Hz.

Other studies seem to contradict these results. Salmons and Vrbova (67) and Brown et al (15) found no effect on contraction time if slow muscle was stimulated intermittently at 40 Hz. Clearly, more work is needed regarding the effects of intermittent, high-frequency stimulation of slow muscle to determine if it can be made "faster" and, if it can, what conditions must be met with regards to age, stimulation frequency, etc.

Cross-innervation is known to change contraction time (8, 16, 17, 21). A slow muscle innervated by a nerve from a fast muscle becomes faster, and a fast muscle innervated by a nerve to a slow muscle becomes slower. These changes are supported by alterations in myosin ATPase activity following cross-innervation (8, 17).

Chronic spinal cord transection is known to cause a regression toward more fast-twitch fibers in the soleus muscle of adult and immature animals (6, 26). Increases in fast-myosin isozyme also have been observed in fast-twitch muscles under similar conditions (27, 49). Comparable results are seen following immobilization of slow muscle (13, 54).

Roy et al (66) showed that both fast and slow plantar flexors in rats have an increased percentage of slow-twitch fibers after functional overload. The soleus or medial gastrocnemius were overloaded by surgical removal of their major synergists bilaterally. After 12-14 weeks, histochemical analysis of myosin ATPase following alkaline preincubation indicated an increased percentage of slow-twitch fibers. Other fast-to-slow fiber transitions are well documented following surgical overload (5, 37, 46, 65).

Much research has been conducted with humans regarding histochemical changes in muscle following training. Oxidative enzyme capacities are enhanced in both fast- and slow-twitch fibers after endurance training (1, 32, 43, 45). On the other hand, enhanced glycolytic capacity is known to occur following high-intensity, anaerobic training (28, 32). However, no significant changes in the percentage of slow and fast muscle fibers as identified histochemically have been found in response to endurance training (28, 32), weightlifting (73) or short-term anaerobic work (69). Thus, most investigators agree that while individual human muscle fibers can adapt metabolically to the load placed upon them, it is not possible, at least given the relatively short time span in which most studies are conducted, to change the contractile characteristics of a given fiber.

In contrast, one study showed a small amount of muscle fiber conversion. Jansson et al (47) reported a significant decrease in the percentage of slow-twitch fibers in long-distance runners following anaerobic training. Initially, the subjects were trained aerobically at about their anaerobic threshold level (70-80% VO2 max) by running approximately 110 km a week. The subjects remained on this training schedule for an average of 18 weeks. Subsequently, the subjects were trained with an interval program at 90-100% of their VO2 max for 7-13 weeks. The cumulative distance per week, which also included some distance running, was reduced to 71 km. Muscle biopsies were taken from the vastus lateralis after each training period. Muscle sections were stained for myosin ATPase activity. The percentage of slow-twitch fibers decreased from 69% following aerobic training to 52% following anaerobic training. The increase in fast-twitch fibers occurred primarily in an intermediate staining type. However, the results of this study must be considered carefully not only because of the small number of subjects (n=4) but also because of the interpretation of stained serial muscle sections used for classification of fiber types. Photographs of the sections printed in the article indicate that fibers from the vastus lateralis which appeared dark after staining for myosin ATPase (ph 4.3) are labeled as both slow-twitch and intermediate types but the latter were included with fast-twitch fibers when percentages were determined. Also, fast-twitch fibers were further categorized based on their oxidative capacity at this low preincubation ph. Experience in our laboratory has indicated that no determination of oxidative enzyme activities is possible after staining for myosin ATPase in an acid ph.

A number of studies have demonstrated the presence of intermediate type fibers in humans following endurance training (1, 34, 45). It has been hypothesized that fibers of an intermediate type are in transition from either fast-to-slow or vice versa. Studies in humans indicate that intermediate fibers contain both fast and slow myosin (12) and that their cross-sectional areas fall in between the size of fast-twitch and slow-twitch fibers (70). Also, ATPase intermediate fibers in the rat soleus have contraction times in between those of fast and slow fibers (51, 52). The results of these studies are encouraging but more research is needed to determine if a true exercise-induced fiber conversion is possible in humans or if the intermediate fibers are simply a by-product of nerve damage or other factors.

Animals subjected to endurance training show concomitant increases in the ability to oxidize carbohydrates and fatty acids (3, 9, 42, 56). Recent evidence suggests that contractile parameters also may be subject to change. Green et al (35) found a 17% increase in the percentage of histochemically defined slow-twitch fibers in the deep portion of the vastus lateralis of endurance-trained rats. During the first seven weeks of training the animals ran once per day on a motor driven treadmill at a constant speed of 27 meters per minute. Grade and run duration were progressively increased. During the final eight weeks of training, the animals ran in the morning and the evening. The animals began the twice-per-day exercise program by running for 15 minutes in the morning and 90 minutes in the evening. The running time for the morning session was increased by 3 minutes per day while the duration of the evening session was held constant. Grade continued to be progressively increased as it was during the initial seven weeks of training. By the end of the 15 weeks of training the animals were running for 210 minutes per day at 27 meters per minute and at a 15-degree grade.

Staudte et all (72) observed a decrease in isometric twitch contraction time in the soleus muscle of sprint-trained rats. The animals, 46 days of age at the initiation of the experiment, were trained seven days a week for three weeks. Each animal ran four times daily, twice in the morning and twice in the afternoon, with each run lasting 45 minutes. At least 60 seconds rest was allowed between runs. Speed and grade of the motor driven treadmill were increased gradually throughout the three-week training period. At the cessation of training the isometric twitch contraction time of the trained soleus muscles decreased 15% when compared to that of the control muscles.

Jaweed et al (48) demonstrated alterations in myosin ATPase activity in rats following weightlifting exercises of various intensities. The trained animals were divided into two groups. The "high-intensity, prolonged-duration" group climbed a 50^{o} inclined ladder (90 cm in length) a total of 50 times each day. The "high-intensity, short-duration" group climbed a 50^{o} inclined ladder (45 cm in length) a total of 25 times a day. Additional weight was added to each animal as the training proceeded to increase the work load. Each group trained four days a week for six weeks. The results of the study showed a 3.7% decrease in the percentage of slow-twitch fibers in the soleus of the "short duration" group but an 8.2% increase in the percentage of slow-twitch fibers in the "prolonged-duration" group.

Exner et al (30) reported a 20% decrease in contraction time of fast muscle in female rats trained isometrically for 25 consecutive days. The animals exercised by holding a static body position in a 60^{o} inclined tube with weights attached to their tails. The training consisted of three sessions, performed twice daily, 12 hours apart. Between each session the animals were allowed to rest for 30 minutes. No session lasted longer than five minutes. If an animal could hold itself in the tube for five minutes the amount of weight attached to its tail was increased in the next session.

Surprisingly, when slow muscle from these rats was analyzed, a 20% increase in contraction time was observed.

In addition, no change was observed in contraction time in slow or fast muscle in male rats trained identically for 35 consecutive days (31).

Bagby et al (2) found no significant effect on myosin ATPase activities in the rat gastrocnemius following sprint of endurance training. All animals in the exercise groups were trained in motor-driven wheels by progressively increasing the distance run during daily sessions (5 days/week). The endurance group improved until they were running continuously for one hour at 28.4 meters per minute during each session. The sprint group alternated 30-second sprints with 30-second rest periods. At the completion of training this group was sprinting 18 times per day at a speed of 80.4 meters per minute. The duration of training for both groups was 11 weeks.

Baldwin et al (4) trained rats by means of a program of treadmill running for durations of between 18 and 24 weeks. All animals were trained progressively for the first 12 weeks and then kept on a routine that consisted of two hours of continuous running at 1.2 miles per hour up a 15% grade, five days per week. No changes in myosin ATPase activity or in the total amount of actomyosin recovered from the vastus lateralis muscle occurred in response to training.

Barnard et al (10) exercised male guinea pigs on a program of treadmill running. The animals ran five days per week for a total of nine or eighteen weeks. The training was progressively increased each week through the first nine weeks then kept at this workload for the remainder of the experiment.

On Tuesday and Thursday of the first week the animals ran for ten minutes at 20.8 meters per minute, 2% grade. On Monday, Wednesday, and Friday of the first week they ran ten minutes of wind sprints with 30 seconds of running at 40 meters per minute alternated with 30-second rest periods. Each session was preceded by a five minute warm-up at 20.8 meters per minute, 0% grade, and concluded by a 15-minute run at 33.3 meters per minute, 0% grade.

During the 9th week the program consisted of a five-minute warm-up at 27.5 meters per minute, 0% grade. On Tuesday and Thursday the animals ran for 20 minutes at 40 meters per minute, 2% grade. On Monday, Wednesday and Friday the animals alternated 30 seconds of walking at 20.8 meters per minute, 0% grade, with 30 seconds of sprints at 49.3 meters per minute, 0% grade, for a total of 20 minutes. All sessions were concluded with a 15-minute run at 36.8 meters per minute, 2% grade.

There were no significant differences between control and trained gastrocnemius or plantaris muscles in the proportion of myosin ATPase dark- and light-staining fibers after nine or eighteen weeks of running.

Edgerton (24) found no changes in the percentages of slow and fast muscle fibers as identified histochemically in the soleus muscle of rats subjected to endurance exercise. The trained rats were divided into two groups: one group was forced to swim for one 30-minute period per day with a weight equal to 3% of body weight attached to the tip of the tail; the second group was forced to swim for two 30-minute periods per day with a weight equal to 4% of the body weight attached to the tail. In addition, the rats in this group were permitted to exercise at will in a revolving drum adjacent to their cages. A third group of rats served as sedentary controls. The trained rats exercised six days per week for a total of fifty-two days.

In a study involving non-human primates, no changes were found in the percentage of muscle fiber types in the soleus, plantaris or tibialis anterior muscles following a six-month training program (25). The adult galago senegalenius (lesser bushbaby) was trained to run upright on a treadmill by using a horizontal bar to support its fore-limbs. The animals learned to run in this position within five training sessions. After this the training load was progressively increased. At the end of the first month the animals ran for an average of 25 minutes at 30 meters per minute, 0% grade. During the last two months all animals were able to run 60 minutes at 43 meters per minute, 4% grade.

Roy et al (64) found no change in the percentage of fast- and slow-twitch fibers in the rectus femoris of rats subjected to a weightlifting program. The rats were trained to stand upright on their hind legs by the use of light stimulus followed by electic shock. Each rat performed sixteen lifts daily, five days per week, for eight weeks. The duration of each lift was approximately two seconds and a rest interval of 30 seconds was provided between lifts. The training load was increased gradually, and by the end of the study each exercised animal was capable of lifting 130% of its body weight.

Goneya et al (33) trained cats to move a bar with their right fore-limbs to receive a food reward. The weights attached to the bar were gradually increased during 19-46 weeks of training. At the conclusion of the experiment, serial sections from the non-exercised left and exercised right flexor carpi radialis muscle were processed for alkali- and acid-stable myosin ATPase activity.

The question of whether the intrinsic contractile characteristics of skeletal muscle can be altered by training alone has yet to be answered. At the present time it would appear most beneficial in terms of economy, efficiency, and accuracy to use animal models for this kind of research. Apparently, specific species-related muscle adaptations do result from exercise, so any attempt to completely correlate the results in animals with humans would be unwise. Rigorous variable control and extreme exercise conditions are necessities in this field of research and until these conditions are consistently met any "answers" should remain assumptions.

CHAPTER III

RESEARCH METHODS

Twenty male, albino rats (Sprague-Dawley strain) were received by the laboratory at 140 days of age. The rats were assigned randomly to one of the three treatment groups. Surgery was performed on all animals two days after their arrival. Following surgery the rats were housed in sedentary cages for thirty days to allow recovery prior to the initiation of training.

One of the rats died during surgery and myosin ATPase activity in the stained serial sections of two other rats could not be accurately determined. Consequently, data was obtained from 17 animals in this study.

Treatment Groups

The three treatment groups used in the investigation were as follows:

Sedentary-Control Group (SCG) --- These rats were housed in individual sedentary cages (24 cm long by 18 cm wide by 18 cm tall) for the duration of the experiment. From either the right or the left leg, the soleus was surgically removed two days after the animal arrived at the laboratory. No surgery was performed on the contralateral leg. Therefore, any change in fiber composition between the initially removed soleus and the soleus in the other leg should represent the effects of aging. Data were collected on four animals in this group.

Sedentary-Surgery Group (SSG) --- This group of rats also was housed in individual sedentary cages. During the surgery, the achilles tendon attachments of the gastrocnemius and plantaris were severed in either the right or left hindlimb. In the opposite leg the achilles tendon attachment of the gastrocnemius again was cut and the soleus was removed surgically. Therefore, any change in fiber composition between the initially removed soleus and the isolated soleus in the other leg should represent a combined effect of aging and surgical procedures. Data were collected on four animals in this group.

Exercise-Surgery Group (ESG) --- These animals were housed in identical sedentary cages and were subjected to the same surgical operations as the animals in the SSG group. In addition, each animal was trained to jump to its maximum height twenty times a day, five days a week, for three months. Therefore, any change in fiber composition between the initially removed soleus and the isolated soleus in the other leg should represent a combined effect of aging, surgical procedure, and training effects. Data were collected on nine animals in this group.

Treatment Procedures

All animals were given commercial block food and water ad libitum during the entire four-month experimental period.

Lighting (light-dark cycles of 12 hours light and 12 hours dark) and room temperature (21^{o}-23^{o}C) were controlled automatically.

A jumping chamber with an electric grid and landing platform was used for training (figure 1). Each exercise session was conducted in the evening during the animals dark cycle. The rats were allowed approximately three minutes rest between "good" jumps. A good jump was classified subjectively based on the rat's effort. Often two or three unsuccessful jumps preceded what was classified as a good jump. Only good jumps were counted. Under all circumstances the rat remained in the chamber until a good jump was made.

When a given rat could reach the landing platform on 15 out of 20 good jumps, the height was increased by one inch in the next training session. Verbal encouragement as well as electric shock was used to motivate the rats to jump. The training program is shown in detail in Table I.

Surgical Procedures

Prior to the initial surgery, each animal was anesthetized intra-peritoneally with approximately 1 cc. of a 1 percent pentobarbitol solution. A skin incision then was made to expose the lateral portion of the lower hindlimb. Following tendon detachment and/or soleus muscle removal as previously described, the limbs were sutured and the animals were returned to their cages. Sham operations were not performed since it has been determined that no difference between sham and normal muscles occurs with regards to the physiological properties being observed (5).

Sacrifice Procedures

Seventy-two hours after the final training session, each animal was weighed and then anesthetized with approximately 3 cc. of 1 percent pentobarbitol. Each animal's right or left soleus muscle was excised and weighed. If necessary, an additional injection of pentobarbitol was used to kill the animal.

Histochemical Procedures

Following both the initial and final muscle removal, a muscle block was obtained from the belly of the muscle and placed vertically on a metal block with 5 percent gum tragacanth. The metal block was placed in fluid 150 pentane that had been cooled with liquid nitrogen. Serial cross sections, 10 mm thick, then were cut from each muscle block using an Ames lab-tek cryostat maintained at -20°C. The serial cross sections were stained for myosin ATPase using an acid (ph 4.3) preincubation (36). Other sections were stained for nicotinamide dinucleotide diaphorase (NADH) (58). Permount was used as the mounting medium for myosin ATPase and glycerin jelly was used to mount those sections stained for NADH. Thirty-minute incubation times were used for all histochemical procedures.

Methods of Tissue Analysis

The histochemically stained serial cross sections from each muscle were projected with a microprojector in a darkroom. All clearly differentiable fibers were counted and classified. Initially, the myosin ATPase sections were analyzed to differentiate between fast- and slow-twitch fibers. Following this, the NADH-stained slides were used to determine the general oxidative enzyme capacities of the fast-twitch fibers in order to further sub-classify these fibers.

A number of fibers of intermediate staining were observed in some of the myosin ATPase stained sections. These fibers were classified as intermediate type.

Muscle fiber splitting was exhibited in some of the muscle sections. When this occurred, an estimation of the number of "normal-sized" fibers that would have existed in this area was made and added to the total fiber count of the muscle section.

The numbers of fast-twitch, slow-twitch, and intermediate fibers were obtained for each section. Following this, the percentage of each fiber type was calculated.

Statistical Procedures

A dependent sample t-test was used for statistical comparisons within each group. A one-way analysis of variance was used to compare the mean changes between groups. The 0.05 level was established for statistical significance.

CHAPTER IV

RESULTS AND DISCUSSION

The histochemical data and statistical results are presented in the first part of this chapter. The last part of the chapter consists of a discussion attempting to relate the histochemical data with physiological and morphological observations.

Histochemical Results

The total fiber counts and fiber type percentages before and after the four-month treatment periods are presented in Table I. The t-test and analysis of variance results are presented in Table II and Table III, respectively. As can be seen in Table II, no significant changes occurred in the percentages of muscle fiber types in any of the three treatment groups. The results of the analysis of variance indicate that the changes in the percentages of intermediate staining fibers in the three treatment groups are significantly different from each other. In an effort to determine the source of this significance, a number of planned comparison and multiple range tests were run. These tests did not reveal any further information regarding the data.

Despite the lack of statistical significance in the results, a number of interesting observations can be made. In all three treatment groups the percentage of slow-twitch fibers increased and the percentage of fast-twitch fibers decreased following the experimental period. In addition, though no fibers of an intermediate staining type were present in any of the animals at the beginning of the experiment, at least some animals in each treatment group had fibers of this type at the conclusion of the experimental period. Finally, fiber splitting, while not observed in any of the animals at the initiation of the study, was present in animals from each treatment group in the post-experimental histochemical analysis.

Discussion

As previously described, the surgical procedure used in this study in an attempt to isolate the soleus proved to be unsuccessful. At some unknown time following the initial surgery the severed tendons of the gastrocnemius and plantaris reattached to the posterior surface of the calcaneus. The observed appearance and size of these muscles at the conclusion of the experiment indicated that they were functional for a good portion of the treatment period.

An argument could be raised that if the entire ankle plantar flexor group was functional then little, if any, additional stress was placed on the soleus in response to training. However, electro-myographic data have shown that motor unit activity in the soleus, while probably generating only small degrees of tension, is nevertheless enhanced during vertical jumps of increasing height. It is possible that this greater muscle activity occurs in response to the animal needing additional postural support prior to takeoff.

The changes in the percentages of the fiber types in the soleus, though not significant, suggest that the muscle was chronically overloaded to some degree since similar shifts in fiber populations are seen following surgical overload (5, 37, 46, 65, 66). However, it must be noted that in treatment group three, where no surgery was performed to isolate the soleus, changes indicative of overload also were observed. These changes contradict previous work where the effects of aging on the muscle were reported to be a decreased percentage of slow-twitch fibers (23, 29). One possible explanation for this discrepancy may be that the animals in this study used the leg with the soleus intact to a greater degree than the leg with the soleus removed. Consequently, the increased activity of the non-operated leg could have provided sufficient stimulus to retard a slow-to-fast fiber conversion.

The presence of intermediate-staining fibers in the post-treatment muscles seems to indicate that some fibers were in the process of conversion. Although there was a significant between-group difference in the percentage of these fibers, no determination of exactly which groups were different from each other could be made. Despite the fact that the power of the statistical tests used allowed the significance to be detected, simple inspection of the final results indicates that no drastic changes occurred.

Fiber splitting has been known to occur in the muscles of some animals following physical stress (20, 24, 33, 40). The exact cause of this phenomenon is not known and further research is needed to determine the functional ability of the fibers following splitting (if indeed a complete longitudinal division is occurring). In this study fiber splitting was present in animals from each treatment group, though it was more evident in the rats subjected to intensive surgery. It is interesting to note that all of the splitting occurred in slow-twitch fibers. Previous data suggests that this is not always the case (21, 33, 40). Also, one study found evidence of both slow- and fast-twitch fibers splitting from one parent fiber (40). However, regardless of the type of fiber undergoing splitting, most activity of this kind seems to be limited to predominantly slow-twitch muscle. The degree of overload required to induce splitting, the pattern of innervation, the position of nuclei, and other questions related to the status of these fibers remains subject to speculation.

TABLE I. Muscle Fiber Composition Before and After Four-Month Treatment Period.

		BEFORE		AFTER		
Treatment Group	Animal Number	ST, %	FT, %	FT, %	ST, %	INT, %
Sedentary/ Control	1	1460,92.5	118,7.5	1646,93.3	119,6.7	0,0
	2	1115,81.1	260,19.9	1426,81.1	303,17.2	29,1.7
	3	750,90.6	78,9.4	1110,88.2	133,10.6	15,1.2
	4	1146,83.8	221,16.2	1753,91.9	119,6.2	35,1.8
Mean ±S.E.		1118±145,87.0±2.7	169±43, 13.3±2.7	1484±142,88.6±2.7	169±45, 10.2±2.2	20±5, 1.2±0.4
Sedentary/ Surgery	5	1217,91.1	119,8.9	1350,99.6	5,0.4	0,0
	6	1225,90.1	134,9.9	1487,90.1	88,5.3	75,4.6
	7	1740,82.4	371,17.6	1700,94.7	50,2.8	46,2.6
	8	1431,96.3	55,3.7	1378,92.0	120,8.0	0,0
Mean ±S.E.		1403±123,90.0±2.9	170±69, 10.0±2.9	1479±79, 94.2±2.1	66±2.5,4.1±1.6	30±18, 1.8±1.1
Exercise/ Surgery	9	1210,90.3	130,9.7	1624,87.1	240,12.9	0,0
	10	1581,95.1	82,4.9	1484,83.8	162,9.1	125,7.1
	11	1216,86.4	192,13.6	1585,91.1	117,6.7	38,2.2
	12	1334,92.5	108,7.5	960,84.2	176,15.4	4,0.4
	13	1782,87.8	248,12.2	1504,92.2	108,6.6	19,1.2
	14	1562,83.0	320,17.0	1112,89.5	117,9.4	14,1.1
	15	1742,88.5	227,11.5	1705,93.2	125,6.8	0,0
	16	478,85.4	82,14.6	2004,92.2	126,5.8	44,2.0
	17	1390,82.3	330,17.7	1584,93.8	105,6.2	0,0
Mean ±S.E.		1366±131,87.9±1.4	191±32,12.1±1.4	1507±103,89.7±1.3	142±15.,8.8±1.1	27±13, 1.6±0.8

ST and FT are slow and fast twitch muscle fibers, respectively, as defined histochemically using myosin ATPase (ph 4.3). INT are fibers of an intermediate staining intensity. An asterisk denotes a significant difference ($P<0.05$) between before and after percentages within treatment groups.

TABLE II. Summary of the results of student's t-test on the percentage of fiber types in the rat soleus before and after the 4 month treatment period.

Comparisons	Slow-twitch Fibers	Fast-twitch Fibers	Intermediate Fibers
Treatment Group I (before) vs. Treatment Group I (after)	N	N	N
Treatment Group II (before) vs. Treatment Group II (after)	N	N	N
Treatment Group III (before) vs. Treatment Group III (after)	N	N	N

N= Not significant
S= Significant difference at .05 level.

TABLE III. Summary of the results of analysis of variance on the changes in the mean percentages of fiber types in the rat soleus before and after the 4 month treatment period.

Comparisons	Slow-twitch Fibers	Fast-twitch Fibers	Intermediate Fibers
Treatment Group (I) vs. Treatment Group (II) vs. Treatment Group (III)	N	N	S

N= Not significant
S= Significant difference at .05 level.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The purpose of the present investigation was to determine the effect of jumping on the activity of myosin ATPase in the soleus muscle of male albino rats.

Twenty animals were brought into the laboratory and assigned randomly to one of three treatment groups. Due to complications final data were collected on only seventeen animals. In each animal one soleus muscle was removed, weighed and stained for myosin ATPase and NADH at the beginning and at the end of the four-month treatment period. Changes in fiber composition between the initially and finally removed soleus muscles represented the effects of aging, aging and surgery, and exercise with aging and surgery, respectively, in the three treatment groups. The animals in the exercise group were trained to jump to their maximum height twenty times a day, five days a week, for the final three months of the treatment period.

Visual images of the entire transverse section of the muscle were projected onto drawing paper with a microprojector. All discernable fibers in each section were counted and classified based on their staining intensity.

No significant changes occurred in the percentages of the three fiber types in any of the treatment groups. A significant difference in the change in the percentage of intermediate fibers between the three groups was present but the source of this significance could not be found. In addition, fiber splitting occurred in the slow-twitch fibers of animals from each of the three treatment groups.

<u>Conclusions</u>

The results of the study have led to the following conclusions.

1. The myosin ATPase activities in the soleus muscle of adult male albino rats are not significantly altered by the type of high- intensity exercise used in this study.
2. The number of intermediate (transitionary) fibers in the adult muscle seems to be related to the severity of the load to which the muscle is subjected.

<u>Recommendations</u>

1. In order to completely isolate the soleus muscle its major synergists should be removed.
2. To obtain a thorough understanding of the cellular alterations taking place, additional enzyme activities and isometric twitch contraction times should be determined.
3. Animals should be sacrificed periodically during the treatment period to ascertain the time course of any involved changes.

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APPENDIX

TABLE A-I. Body Weight and Soleus Weight Before and After Four Month Treatment Period.

Treatment Group	Animal Number	BEFORE Body Weight, g	BEFORE Soleus Weight, g	AFTER Body Weight, g	AFTER Soleus Weight, g
Sedentary/ Control	1	395	0.1506	489	0.2112
	2	364	0.1350	451	0.1647
	3	348	0.0800	439	0.1576
	4	343	0.1266	413	0.1532
	Mean I.S.E.	363 ± 12	0.1231 ± 0.015	448 ± 16	$0.1717 \pm .0134$
Sedentary/ Surgery	5	382	0.1006	459	0.1320
	6	361	0.1247	485	0.1876
	7	393	0.1396	484	0.1695
	8	407	0.1180	502	0.1799
	Mean I.S.E.	386 ± 10	$0.1207 \pm .0081$	483 ± 9	$0.1673 \pm .0123$
Exercise/ Surgery	9	370	0.0925	443	0.1681
	10	411	0.1325	476	0.2113
	11	345	0.1255	444	0.1973
	12	403	0.1043	467	0.1780
	13	365	0.1165	454	0.1658
	14	377	0.1784	460	0.1047
	15	367	0.1271	411	0.1644
	16	373	0.0733	443	0.1978
	17	362	0.1351	425	0.1804
	Mean I.S.E.	375 ± 7	0.1206 ± 0.010	447 ± 7	0.1742 ± 0.0102

TABLE A-II. THREE MONTH JUMPING ROUTINE FOR MALE ALBINO RATS.

WEEK	DAY	TOTAL TRAINING TIME	TOTAL JUMPS/ AVERAGE NO. SUCCESSFUL JUMPS	AVERAGE HEIGHT (inches) ATTAINED
	M	123 minutes	20/20	5.0
	TU	147	20/20	6.0
1	W	142	20/20	7.0
	TH	138	20/20	7.0
	F	119	20/20	7.0
	M	137	25/25	8.0
	TU	141	20/20	9.0
	W	140	20/20	10.0
2	TH	98	15/15	10.0
	F	111	20/20	10.0
	SA	153	20/20	10.0
	SU	90	20/20	10.0
	M	114	20/20	11.0
	TU	132	20/20	12.0
3	TH	155	35/35	13.0
	F	119	20/20	13.0
	SA	127	20/20	13.6
	SU	120	20/20	13.6
	M	116	20/19.5	14.6
	TU	132	20/19.2	14.6
4	W	108	20/18.6	14.6
	TH	82	10/7.2	15.6
	F	113	20/17.6	15.6
	SA	124	20/18.0	15.6
	M	157	20/19.5	16.6
	TU	116	20/18.6	17.6
5	W	113	20/17.4	17.6
	TH	106	20/18.0	17.6
	F	134	20/17.5	17.6
	M	112	20/16.2	18.0
	TU	107	20/15.4	18.2
6	W	94	20/14.0	19.7
	TH	138	20/13.7	19.7
	F	100	20/14.2	19.7
	SA	94	20/14.6	19.7

TABLE A-II (cont'd.)

WEEK	DAY	TOTAL TRAINING TIME	TOTAL JUMPS/ AVERAGE NO. SUCCESSFUL JUMPS	AVERAGE HEIGHT (inches) ATTAINED
	M	116	20/11.6	20.0
	TU	121	15/7.2	20.0
7	W	111	15/8.4	20.0
	TH	192	30/14.8	20.0
	SA	123	20/16.1	19.5
	M	132	20/14.7	19.0
	W	114	20/14.7	19.0
8	TH	72	10/5.2	19.4
	F	139	20/14.2	19.6
	SA	201	20/16.6	18.7
	M	117	20/11.0	20.0
9	TU	130	20/9.3	20.4
	F	147	20/8.7	21.3
	SA	110	20/9.0	19.8
	M	109	20/12.9	21.1
	TU	124	20/13.6	21.0
10	W	133	20/14.0	21.0
	TH	119	20/12.0	20.6
	F	117	20/12.7	20.6
	SA	127	20/12.5	19.8
	M	109	20/13.3	19.4
	TU	121	20/11.7	20.3
11	W	126	20/14.6	20.7
	TH	140	20/13.0	21.0
	F	107	15/13.1	21.0
	M	119	20/8.4	22.1
	TU	91	20/7.6	22.3
12	W	137	20/9.7	21.4
	TH	42	20/10.2	21.2
	SU	107	20/15.8	19.3
	M	93	15/4.7	20.0
13	W	115	10/9.2	19.6
	F	38	5/5.0	14.3