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thesis entitled

THE EFFECTS OF SPRINT TRAINING ON FAST- AND SLOW-TWITCH
FIBERS IN ISOLATED RAT SOLEUS MUSCLE

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Health Education,
Counseling Psychology,
and Human Performance

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Date November 1988

THE EFFECTS OF SPRINT TRAINING ON FAST- AND SLOW-TWITCH FIBERS IN ISOLATED RAT SOLEUS MUSCLE

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

School of Health Education, Counseling Psychology,
and Human Performance

1988

ABSTRACT

THE EFFECTS OF SPRINT TRAINING ON FAST- AND SLOW-TWITCH FIBERS IN ISOLATED RAT SOLEUS MUSCLE

By

Madge Irene Haven

This study was undertaken to evaluate the effects of sprint training on the composition of fast- and slow-twitch fibers in surgically isolated rat soleus muscle. Male Sprague-Dawley albino rats were assigned randomly to three treatment groups consisting of surgery + sprint, surgery + sedentary, and sedentary. The last two treatment groups were later combined into one group. The sprint-running program consisted of 38 training sessions in controlled-running wheels. The intensity of the program from training period 24 until 38 had a work:rest ratio of 1:4 and speed of 82 m/min. Sprint animals were sacrificed 2 days after the last training period. Before and after fiber counts of all animals revealed no differences between treatment groups at the .05 level of significance.

To JIKERI, my three sons, who will
always be my password to success.

ACKNOWLEDGMENTS

I would like to thank my mom and dad for making it possible for me to be so happy and learn so much. I would also like to thank Dr. W. W. Heusner, who always treated me like "the little engine that could."

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LIST OF VARIABLES, NOMENCLATURE, AND THEIR ABBREVIATIONS

mATPase	Myosin adenosine triphosphatase
TFIBF	Total fiber before
TFIAF	Total fiber after
FTBF	Fast twitch before
FTAF	Fast twitch after
STBF	Slow twitch before
STAF	Slow twitch after
SWTAPBW	Soleus weight after as percentage of body weight
PFTBF	Percentage fast twitch before
PSTBF	Percentage slow twitch before
PFTAF	Percentage fast twitch after
PSTAF	Percentage slow twitch after
PGSFT	Percentage gain score for fast twitch
PGSST	Percentage gain score for slow twitch
PSF	Percentage of shock-free time
AVPSF	Average of percentage of shock-free time
PEM	Percentage of expected meters run
AVPEM	Average of percentage of expected meters run
Treatment I	Surgery + sprint
Treatment II	Surgery + sedentary
Treatment III	Sedentary
Treat IIp	Treatment II + Treatment III
FG	Fast-glycolytic
FOG	Fast-oxidative-glycolytic
SO	Slow-oxidative
FT	Fast twitch
ST	Slow twitch
IPSP	Inhibitory post synaptic potential
EPSP	Excitatory post synaptic potential

CHAPTER I

THE PROBLEM

Almost half of the mass of the human body is skeletal muscle; therefore, experiments that add to the knowledge of the function and response of the muscle system are important. In order to comprehend the whole, individual parts must be analyzed. The roles of the muscle cell in creating force and adapting to functional demands have been studied in this investigation. Comprehension of normal activity sometimes is better understood in juxtaposition with abnormal activity, so the research question centered on how a fiber may be made to change.

Currently, fiber-type change has been seen in experiments using methods of cross-innervation, electrical stimulation, tenotomy, cordotomy, and endurance exercise with or without ablation. However, there has been little research dealing with sprint activity and its effects on muscle fibers in vivo. By using maturing rat soleus muscle exposed to sprint training, a situation was created that allowed for the observation of possible mutability of fiber type in response to a changed functional demand.

> Only when integrative factors controlling tissue interactions are known will it be possible to bridge the gap between studies of development at the level of the whole organism and at the

> molecular level and then trace in detail the pathway from the action of the gene to the final expression of its phenotype. (Villee, 1966)

Need for the Study

This study was undertaken to explore the consequences of imposing sprint activity on the isolated rat soleus, a predominantly slow-twitch muscle, as evidenced by myosin adenosine triphosphatase (mATPase) activity. Because of the interdependence of all parts of the muscular system, any one part can be a limiting factor on the functioning of the entire system. Therefore, discovering how various components react to demands on the system provides a means for evaluating the malleability of the system (Weibel, 1985). Knowledge of the degree and type of malleability of the mammalian motor system's response to specific training methods may help eventually in understanding the ever-increasing performance records of highly trained athletes (Howald, 1985).

Plasticity of muscle fiber has been an evolutionary "perk" for mammals. Knowing more about this phenomenon could help to explain the mechanisms of adaptation to environmental demands (Guth & Yellin, 1971). Review of the literature revealed that there are few studies that have evaluated muscular adaptation to short-term, high-intensity work (Edgerton, 1978; Syrovy, Gutmann, & Melichna, 1972), and one study that did reported that fiber transformation as demonstrated by mATPase activity could not happen (Burke & Edgerton, 1975). Two studies using prolonged endurance exercise or electrical stimulation of fast fibers showed that skeletal muscle can undergo significant alterations in fundamental contractile properties that are neurally determined (Fitts & Holloszy, 1977) and that skeletal muscle can respond adaptively to imposed endurance activity (Salmons & Vrbova, 1967). These results were encouragement to see if sprint regimens can produce similar, but reversed, alterations in slow-twitch fibers. Finally, the physiological properties of a muscle cell are known to be determined by the characteristics of their proteins, so knowledge of factors controlling protein synthesis and degradation can add to science's armamentarium for research.

Purpose of the Study

The purpose of this research was to determine whether or not fiber-type composition, as measured by Ca^{++}-activated mATPase activity, can be changed significantly in the adult rat soleus muscle as a result of imposing sprint exercise on the maturing animal. Sprint activity in a controlled-running wheel (CRW) (Wells & Heusner, 1971) plus surgery composed Treatment I. Treatment II consisted of surgery and confinement, and Treatment III involved confinement alone.

Counts were made of the total number of fibers. Percentages of fast- and slow-twitch fibers before and after the treatments and percentage changes during the treatments were the dependent variables. CRW percentage shock-free time (PSF) and percentage of expected meters run (PEM) were additional dependent variables for the animals in Treatment Group I.

Research Plan

Twenty male rats were assigned randomly to one of three treatment groups. In Treatment Group I, 10 rats underwent a procedure that isolated the soleus in one leg by surgical ablation of synergists and removed only the soleus in the other leg. These rats, after four weeks rest, ran sprints in the CRW for a total of 38 training periods. The animals of Treatment Group II received the same surgery but were sedentary for the duration of the investigation period. In Treatment Group III, the animals were sedentary for the duration of the study. A contralateral soleus removed at the beginning of the study served as a control for the soleus removed at the end of the study. Before and after values were recorded for all animals on the total number of fibers, the total number of slow-twitch fibers, and the total number of fast-twitch fibers. The animals of Treatment Group I also were evaluated for PSF and PEM, which were generated from data obtained during the running program. Statistical procedures for determining central tendency, variability, and normality were performed on the data. Standard tests of significance were used to compare mean values between treatment groups as well as the before and after data within groups.

Rationale for the Research Plan

The results of previous studies concerning fiber responses to various experimental treatments formed the basis of the rationale for this research plan. Contractile, ultrastructural, and biological properties of embryonic and adult mammalian muscles are not predetermined but may be altered by functional demands (Vrbova, 1980; Vrbova, Navarrete, & Lowrie, 1985). This process of matching structure with function has occurred throughout evolution, but it can occur within an individual animal's lifetime as a result of experimentation (Goldspink, 1985). It appears that properties that change in response to normal functional demands do so early in experimental situations and that properties that are resistant to change under normal functional demands do so only after being exposed to intense experimental conditions of long duration (Salmons & Henriksson, 1981). Two factors are fundamental to exercise conditions: activity patterns and neural regulation. Skeletal muscle, despite its high degree of specialization, is very responsive to the amount and type of work it performs and thus acquires physiological and biochemical characteristics that are best suited to its functional needs (Guth, 1968; Salmons & Henriksson, 1981). Activity is a major factor in determining muscle fiber phenotype (Pette, 1985) even in the absence of neurotrophic factors (Lømo & Westgaard, 1974), but these factors cannot be ignored. Muscle properties are not immutable after birth, and the tonic or phasic pattern of the nerve on these properties exerts an influence throughout life (Guth, 1968; Vrbova, Gordon, & Jones, 1978).

Special characteristics of the rat soleus muscle had a role in determining the approach that was followed in this study. Between 80 and 84% of the total number of muscle fibers in the rat soleus are slow oxidative (SO) (Ariano, Armstrong, & Edgerton, 1973;

Syrovy, Gutmann, & Melichna, 1972). This homogeneity, combined with the fact that the fibers run parallel to the long axis of the muscle, makes the rat soleus a good choice for experimental studies that involve histochemical sectioning (Parsons, Reidy, Shepard, & Gollnick, 1982). In addition, the adult fiber population of the rat soleus is established by 140 days (Ho et al., 1983) and is enzymatically very responsive to sprint training (Saubert, Armstrong, Shepard, & Gollnick, 1973). In regard to the intensity of the sprint training program selected, Saubert et al. (1973) established that for the Sprague-Dawley rat a running speed of 80.5 m/min is equivalent to 160% of max $\dot{V}O_2$ and imposes heavy demands on the anaerobic system with energy being derived mainly from phosphagen depletion and anaerobic glycolysis. According to Howald (1985), a training program such as this is what is necessary to transform fiber types at the level of the molecular structure of myosin. With this in mind, the activity of the enzyme mATPase was chosen as a criterion because it is so closely associated with fiber speed (Bernard et al., 1971; Kugelberg, 1976), because mATPase is a highly conserved protein that appears genetically invariant, and because major differences between fast- and slow-twitch fibers focus on their myosin isoforms (Weibel, 1985). All of these factors were considered during the formulation of the research plan for this investigation.

Significance

Many times in research, the treatments used introduce situations that could never happen in vivo, and observers not directly involved question the legitimacy of the means and, therefore, the relevancy of the ends. The significance of the study addresses this issue, for it is through analyzing the abnormal that the normal may be more fully understood. From a practical standpoint, if this research and its macro-approach could determine whether or not fiber-type conversion can take place, the justification for a micro-approach to determine the mechanisms of such conversions would be established. It is these general reasons plus the specific arguments mentioned under Need for the Study that constitute the ultimate relevance of this investigation.

Limitations of the Research Plan

1. The results of this study cannot be generalized to mammals other than rats.

2. The shock stimulus used in Training Group I may have had a hormonal effect, such as changing blood levels of thyroxin or the catecholamines, which in turn may have had an effect on fiber-type development.

3. The histochemical methods are qualitative rather than quantitative and evaluate only one parameter.

4. Three of the rats died during surgery.

5. Even though great care was taken to remove as much of the gastrocnemius and plantaris as possible and yet not impinge on the

tibial nerve, one or both muscles reattached in some cases, thereby reducing the demands on the soleus.

6. The probability of making a Type II statistical error was set at .10; however, sample sizes necessary and sufficient to meet these criteria were not obtained due to prohibitive animal costs. Therefore, in some cases failure to detect significant differences may be the result of the small samples used, and final conclusions may have to await further data.

Muscle: L., musculus, a little mouse (which jumps the way muscles twitch).

CHAPTER II

RELATED LITERATURE

The hypothesis of fiber-type mutability was tested in this investigation by the response of normal, adult-rat soleus fibers, as measured by Ca^{++}-activated myosin adenosine triphosphatase activity, to a treatment of sprint exercise imposed on isolated rat soleus muscle of maturing animals. However, for communication to be effective, the complexities and confusion concerning muscle fibers and their characteristics must be addressed. This chapter discusses these issues by defining terms, analyzing the components of fiber type, establishing the parameters of mutability, and recognizing the relationship between gene expression and the proteins that define a muscle fiber.

Definitions

If there is anything uniform in the area of fiber-type research, it is the lack of uniformity in the definition of terms. Because little communication can evolve from the discussion of complex ideas if there is misunderstanding about the premises from which these ideas are derived, some basic physiological concepts will be defined and then be considered axiomatic for the rest of the thesis.

Motor Unit

An α-motoneuron and the skeletal-muscle fibers innervated by it constitute a motor unit. In mammals each muscle fiber is innervated by a single α-motoneuron twig, and under normal conditions all fibers in the motor unit are activated by the action potential of their α-motoneuron. The fibers in a motor unit are scattered throughout a muscle, creating overlap between fibers of as many as 20 to 50 motor units, yet each motoneuron and its concomitant fibers are thought of as a single entity (Burke & Edgerton, 1975). The aggregate fibers of an individual motor unit are metabolically and histochemically homogeneous, with physical and metabolic properties matched to tasks required of them by their motoneuron (Burke, Levine, & Tsairis, 1973; Pette, 1985; Vrbova & Tessa, 1978).

Size Principle

The orderly recruitment of motoneurons, whereby small neurons supplying fewer number of fibers discharge first and large neurons supplying a larger number of fibers discharge later as force of contraction increases, constitutes Henneman's Size Principle (1985). The development of muscle tension is graded both in force production and energy expenditure as the motor units with fewer fibers and the most fatigue resistance are recruited first (Burke & Edgerton, 1975; O'Brien & Vrbova, 1980).

Excitation-Contraction

Control of the contractile state as delineated by Peachy (1985) is assumed.

Vertebrate Sarcomere Homogeneity

A sarcomere is of the same basic design in all vertebrates, and all sarcomeres are equal in length (Huxley, 1985; Weibel, 1985). Within the sarcomere, myosin filaments are of equal length as are actin filaments (Perry, 1985). These lengths do not change upon contraction. Characteristics such as lateral spacing of A filaments, units of repeat for myosin heads, and axial repeat of cross-bridges are uniform also (Huxley, 1985). The fact that actin and myosin proteins and the structures they form are highly conserved leads to the assumption that their interaction follows the same structural and chemical pathway in all skeletal muscles. Due to this homogeneity of vertebrate sarcomere characteristics, each individual cross-bridge cycling generates the same tension over the same working stroke yielding the same amount of work (Huxley, 1985). Potential force production per vertebrate sarcomere may be equal, but potential power production is not, for the enzyme patterns of slow and fast myosin differ and, therefore, the speed of cross-bridge cycling differs.

Myosin Molecule Structure

The native molecule of myosin has two heavy chains and four light chains. (See Figure 1.) The light chains are separated into two classes, of which only one can be phosphorylated (Brown, Salmons, & Whalens, 1983).

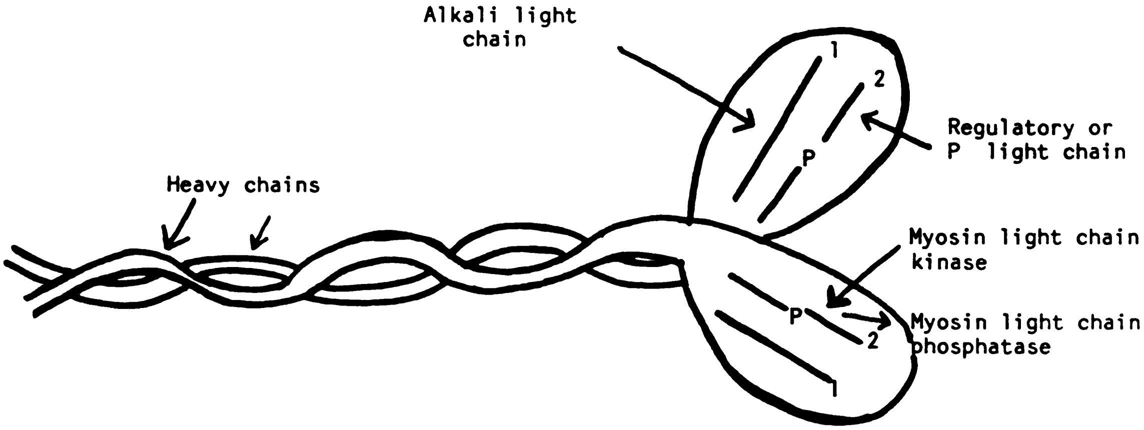

Figure 1.--Myosin molecule. From "Properties of the muscle proteins--A comparative approach" by S. V. Perry, 1985, Journal of Experimental Biology, 115.

Myosin ATPase

The speed of cross-bridge cycling is determined by the kinetics of the enzyme myosin adenosine triphosphatase (mATPase). The difference in mATPase activity is commensurate with the difference in the rate of ATP use during the contractile process and also appears to be the rate-limiting step in speed of muscle shortening (Baldwin, Winder, & Holloszy, 1974). Burke and Edgerton (1975) did point out, however, that the release and uptake of Ca^{++} in the sarcoplasmic reticulum may contribute to the control of speed of shortening and relaxation also. Because the activity of mATPase is correlated with the contraction time of muscle and because mATPase measured biochemically correlates with histochemical evaluations of mATPase (Barnard, Edgerton, Furukawa, & Peter, 1971), this enzyme plays a pivotal role in the elucidation of fiber type with regard to twitch characteristics.

Plasticity

The ability of muscle to adapt to short-term changes such as seasonal levels of activity plus long-term changes such as increase in body weight is of survival value to the animal (Salmons, 1980). This wide-ranging flexibility to adjust to developmental and functional demands is called plasticity (Perry, 1985) and is regulated through control of cellular systems independent of each other, i.e., metabolic and myosin-related properties (Pette, 1984) in both the young and the adult animal (Vrbova & Tessa, 1978). Of all tissues, skeletal muscle is probably the most adaptable (Goldspink, 1985), thus allowing unlimited potential for modulation of physiological properties (Gauthier & Lowey, 1979).

Fiber Type

Crucial to the understanding of this research is a clear and shared understanding of fiber type, both of muscle in general and the soleus of the rat in particular.

Nomenclature

Contractile proteins, existing in isoforms such as myosin heavy chains, myosin light chains, actin, troponin, and tropomyosin, can be criteria for evaluating the different characteristics of muscle fibers (Goldspink, 1985). More common is the use of twitch, metabolic, and fatigue characteristics to define a fiber type and name it. Color is used as a criterion also, but this characteristic has contributed greatly to nomenclature confusion in fiber-type research. The confusion stems from the interpretation of the words "red" and "intermediate." Red has been used to denote fibers that share that color but not oxidative or ATPase characteristics (Baldwin, Klinkerfuss, Terjung, & Mole, 1972). Intermediate has been used to denote fibers that are intermediate in terms of fatigue resistance and mitochondrial content but with fast contraction times (Gauthier & Lowey, 1979). It also has been used to denote fibers that are intermediate in oxidative capacity and low in ATPase activity but with slow contraction times (Baldwin et al., 1972). Although it lacks precision, the system of labeling fibers based on contractile and metabolic characteristics (Peter, Barnard, Edgerton, Gillespie, & Stempel, 1972) seems to be the most capable of conveying standardized information: FG = fast-twitch, glycolytic; FOG = fast-twitch, oxidative-glycolytic; SO = slow-twitch, oxidative. Because this study involves the histochemical evaluation of mATPase, only one characteristic is revealed--twitch. Therefore, in this study, fibers will be called either fast twitch (FT) or slow twitch (ST).

Developmental Characteristics

In the four stages of fiber-type development of a rat (embryonic, neonatal, maturing, and then adult), the effects on the fiber of the maturing activity patterns and the maturing nervous system are intertwined (Salmons & Henriksson, 1981). During embryonic development, the muscle cells and the motoneurons develop independently of each other (Vrbova, Navarrete, & Lowrie, 1985), with synthesis of embryonic myosin being determined by limb position or functional demand (Dhoot, 1985). In the neonate, a second type of developmental myosin, neonatal, predominates (Hoffman et al., 1985; Whalen, 1985), with all muscle fibers having slow contraction times even though they have a predominance of myosin light chains and ATPase activities characteristic of fast muscle. This suggests that the speed of contraction at this stage of development is in concert with other components of the muscle fiber rather than the type of contractile proteins present (O'Brien & Vrbova, 1980). During this neonatal period, polyneuronal innervation predominates for about 2 weeks (O'Brien & Vrbova, 1980; Vrbova et al., 1978), and the early activity patterns of these motoneurons are different from adult patterns. During this polyneuronal innervation period, the contractile and biochemical characteristics of individual muscles and their fibers are poorly differentiated (Vrbova et al., 1978). The elimination of polyinnervation in the maturing animal coincides with increased activity of the animal, decreased difference in hind-limb activity patterns between young and adult animals (O'Brien &

Vrbova, 1980; Vrbova et al., 1978), maturation of the nervous system (Salmons & Henriksson, 1981), differentiation of fibers into fast and slow categories based on contraction time (Guth, 1968), and the development of the adult forms of fast and slow myosin (Whalen, 1985). The switch to adult myosin forms is nerve independent for fast forms and nerve dependent for slow forms (Hoffman et al., 1985; Salmons & Henriksson, 1981) and does not involve the recapitulation of the two developmental forms of myosin (Hoffman et al., 1985).

The speed of contraction of the rat neonatal soleus muscle is very slow; then for 2 to 3 weeks the speed increases slightly until it conforms to the adult pattern of slow contraction times. This prolongation of contraction time and change to the adult slow myosin form may be a developmental adaptation to the soleus's antigravity function. Rate of growth of the muscle, body size, and maturation of the neuromuscular system affect the time it takes for these changes to take place (Gutmann, Melichna, & Syrovy, 1973). The neural role in fiber-type development is represented in Figure 2. It appears that a functional nervous system is necessary for differentiation and maintenance and that fibers become fast by default when the nervous system is nonfunctional.

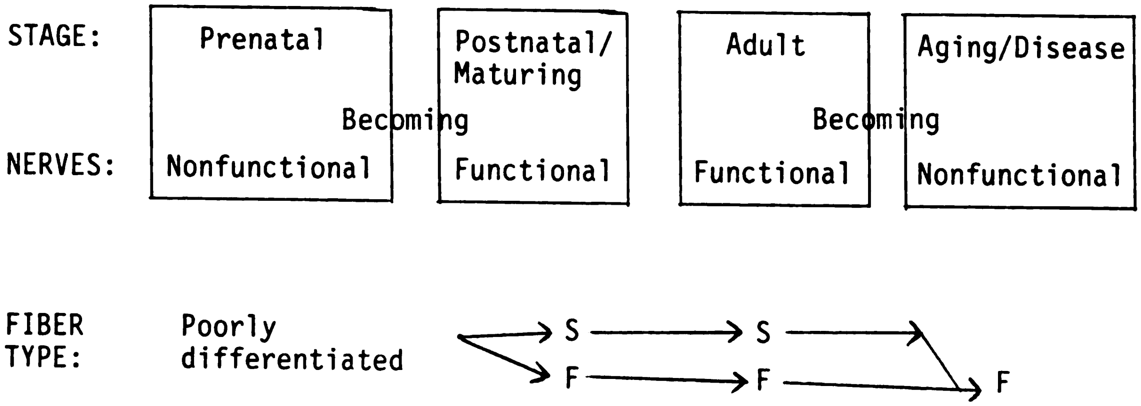

Figure 2.--Schematic representation of neural role in fiber development.

Similarities and Differences

This section is presented to help demonstrate the inadequacy of the present nomenclature to deal with such a complex area. The chart is divided into dichotomies based on twitch for simplicity of reference, but it should be remembered that many of these characteristics form continua, and one continuum may or may not covary with another. The section also may be used as a reference throughout this thesis.

Fast Twitch	Slow Twitch
Greater covalent modification of myosin P light chain, therefore faster contractile response	Lesser covalent modification of myosin P light chain, therefore slower contractile response
Actin identical to slow Fast C protein	Actin identical to fast Slow C protein
(Perry, 1985)	
Motor end plate widespread, long, smooth	Motor end plate more tightly packed, shorter, with alternating swellings and constrictions
More acetylcholinesterase	Less acetylcholinesterase
(Burke & Edgerton, 1975)	
Fibrils regularly arranged	Fibrils irregularly arranged
Poor in myoglobin, fat	Rich in myoglobin, fat
Located in peripheral of muscle	Located in interior of muscle
(Goss, 1978)	
In response to 1 month of jumping with young rats, protein synthesis rate increased while protein degradation decreased slightly; equals a net gain in protein metabolism (extensor longus)	In response to 1 month of jumping with young rats, protein synthesis rate greatly decreased and degradation slightly increased; equals a net loss in protein metabolism (soleus)
(Goldspink, 1985)	
Fast muscle activated by uptake of 2 Ca^{++} per troponin molecule	Slow muscle activated by uptake of 1 Ca^{++} per troponin molecule
(Perry, 1985)	
T-system extensive	T-system half as extensive
Terminal cisternae more extensive	Terminal less extensive
Sarcomplasmic reticulum same as slow	Sarcomplasmic reticulum same as fast
(Eisenberg, 1985)	

Rate of firing of motoneurons to fast fiber is extremely variable	Rate of firing of motoneurons to slow fiber varies little
Changes contractile properties in response to previous activity	No great change in contractile properties in response to previous activity
Increase in force generated by increased firing rate of motoneuron	Increase in force generated by increased recruitment

(Vrbova et al., 1985)

Even though there is conservation of basic fiber ultrastructure and its organization as stated earlier, there is great variation and adaptation in the fiber's fine molecular and biochemical details (Huxley, 1985), as this chart illustrates.

Adult-Rat Soleus

The soleus of the adult rat was once considered to be composed entirely of homogeneous "red" fibers, but now it is known that those "red" fibers are really a heterogeneous distribution of FOG and SO fibers (Stein & Padykula, 1962), with these fibers equaling about 96% of the total population (Baldwin et al., 1972). The rat soleus is a postural muscle and functions in locomotion.

Mutability

The fact that the concept of fiber type has so many facets adds to the complication of discussing mutability. In general, however, it can be said that a muscle can keep its general characteristics but still react in a plastic manner to normal or induced stimuli for change. This plasticity or mutability results in phenotypic fiber-type change, probably mediated through altered gene expression (Jolesz & Sreter, 1981). The type and extent of change depend on the continuous or discrete nature of each of a fiber's characteristics and the intensity, duration, and modality of the stimulus for change.

What Changes, When, and Why

Great care must be used when discussing mutability of fiber types. A specific parameter must be isolated and analyzed, its normative values established, the methods for attempting to induce change documented, whether or not change takes place ascertained, the time course of change recorded, and the question of causation addressed. Fiber-type mutability really should be thought of in terms of continuous rather than discrete values because fibers, rather than mutating, actually shift values of one or more characteristics in response to a stimulus for change. In addition, the covariance of other parameters in the cell must not be ignored.

The subject of mutability must be related to time scale of change and degree of change. Shifts in characteristics within a fiber range from hours and days (membrane systems) to days and weeks (contractile proteins and metabolic systems (Eisenberg, 1985). Time also is a critical factor when evaluating mutability (Vrbova, Gordon, & Jones, 1978) because differing rates of transformation are related to turnover rates of the cellular systems involved (Pette, 1984). Myosin's resistance to change is a result of its slow turnover rate (Edgerton et al., 1969). Time is a consideration when a cellular system is being evaluated for degree of change, as a single muscle fiber can demonstrate both slow and fast myosins coexisting transiently. Therefore, when evaluating a fiber for its response to a treatment, knowledge of the characteristic being used as a criterion is essential so that enough time will have elapsed to see a full degree of change (Kugelberg, 1980; Pette & Schnez, 1977).

The neural role in fiber definition of characteristics was demonstrated in a now-classic cross-innervation experiment of Buller, Eccles, and Eccles (1960), which resulted in dramatic shifts in fiber characteristics. It is of interest that this study forms the foundation of the nerve-fiber concept, and yet the transformations fell short of complete change from fast to slow and slow to fast. These alterations were brought about via changes both in pattern of impulses (quality) and total number of impulses (quantity) (Edgerton, Martin, Bodine, & Ray, 1985). Some of the changes that are brought about by cross-innervation involve twitch, tetany, force-velocity ratios, metabolism, enzyme activity patterns, thick and thin filaments, sarcoplasmic reticulum, and Ca^{++} sequestration and release (Close, 1965; Vrbova, Gordon, Jones, 1978). A specific cellular reaction to cross-innervation is the appearance of a new, qualitatively different mATPase--the result of de novo synthesis rather than a change in its degradation rate (Samaha, Guth, & Albers, 1970).

Also demonstrating the role of neural influence on fiber characteristics are experiments using different frequencies and patterns of electrical stimulation. Initially these studies were conducted using tonic 10 Hz stimulation of fast muscle and phasic 100 Hz stimulation of slow muscle. As in cross-innervation, shifts were seen in twitch, metabolism, sarcoplasmic reticulum ability to bind Ca^{++}, enzyme kinetics, vascularization, and mATPase activity (Salmons, 1980; Vrbova, Gordon, & Jones, 1978). Interestingly, in a study by Sreter, Pinter, Jolesz, and Mabuchi (1982), a pattern of 60 Hz for 2.5s duration every 10s over 5 weeks produced a fast to slow shift as measured by mATPase activity. This gives credence to the concept that fiber transformation depends more on total activity than on pattern of activity. Since no signs of regeneration of fiber de novo were seen, the authors suggested reprograming of existing fibers mediated the change (Sreter et al., 1982).

Experiments to affect fiber characteristics using tenotomy have been used to evaluate what happens when the nerve is left intact but the muscle is no longer able to respond to the stretch reflex, i.e., altered activity pattern but unaltered neural influence. Vrbova (1980) used a rabbit soleus, which is extremely sensitive to stretch, and found that the contractile properties shifted toward those of a fast muscle.

While the effect of nerve on fiber mutability is important, it cannot be separated from activity patterns that the muscle undergoes. The tenotomy experiment of Vrbova is an excellent example of this syngerism of mechanisms. It appears that fibers adapt to activity patterns by covalent modification of their light chain myosin (Perry,1985), which demonstrates that there is a link between mechanical activity of a muscle and protein synthesis. This observation, and the fact that denervated muscle will become fast or slow depending on the pattern of activity imposed on it (Lømo, Westgaard, & Engebretsen, 1980), support the concept that not all control mechanisms are directly programmed by the presumptive myoblast's DNA. Some control is generated through changing activity patterns of the tissue (Goldspink, 1980).

There are many characteristics that can shift in a fiber in response to treatment, and the degrees to which they shift can vary. The duration, modality, and intensity of the treatments can vary also, so it is most important to specify exactly the parameter of a fiber's characteristic that is to be evaluated and the conditions under which it is to be placed. Only then should pronouncements about mutability be made.

Gene Expression

The muscle's great sensitivity to the environment may be mediated via genetic control (Guth, 1968). Changes, both in adulthood and during maturation, give an animal's muscle evolutionary advantages. The changes can be detected through evaluation of the different forms of proteins the muscle produces, which are themselves regulated by gene expression (Eisenberg, 1985). Normal growth and development involves an orderly progression from embryonic to adult forms of protein, whereas drastic changes in functional demands would probably result in nonoptimal, inefficient use of existing contractile proteins. In both cases, gene expression would function to then produce the best-suited protein for executing the changing demands (Eisenberg, 1985). The mechanism whereby the proper isoforms of myofibrillar proteins would be produced is complex and probably related to the activity pattern of the muscle and trophic influences of vertebrate neurons (Guth, Samaha, & Albers, 1970). It may be that in some cases individual genes control production of an individual isoform, while in other cases the final isoform is determined by how the RNA is processed (Perry, 1985). There is evidence that the activity pattern of the animal's muscle may suppress genetic production of the no-longer-functional protein, perhaps by increasing its degradation rate, while enhancing genetic production of a more appropriate form of protein (Eisenberg, 1985). The messengers from the external to the internal environment appear to be the motoneurons. All the treatments of muscles that have been mentioned in Chapter II have been evaluated in terms of quality shifts in proteins. Pattern of activity or neural influence or both have affected the muscle. Because protein synthesis is a result of gene action (Samaha, Guth, & Albers, 1970), it is reasonable to assume a message-messenger relationship.

According to Perry (1985), both fast and slow fibers have the capacity for producing all skeletal-muscle isoforms of the myofibrillar proteins. Therefore, since fibers are characterized by the specific patterns of their myosin light chains (Heilig & Pette, 1983), and since genetic control of myosin synthesis involves at least 13 genes for heavy chains and complex sequencing of mRNA to produce light chains (Perry, 1985), complex mechanisms of coordinated control of gene expression must be present inside the muscle cell to ensure that the function of the muscle and its myofibrillar protein match (Perry, 1985). Myosin isoforms are believed to be members of a large, highly conserved, multi-gene family, but how the nerve activity and the activity pattern of the muscle communicate with the nucleus so that there is a genetic response is unknown (Whalen, 1985). However, the effects of motoneuron activity and functional demands on fibers are measured by evaluating a fiber's proteins. The nucleus controls the synthesis and degradation of these proteins. Therefore, it is not unreasonable to look for a causal relationship.

CHAPTER III

RESEARCH METHODS

This study was conducted to determine the response of maturing mammalian slow-twitch fibers to enforced sprint training. Rats randomly were assigned to treatments that: (a) isolated one soleus muscle by surgical ablation of all synergists and imposed sprint running--Group I, (b) isolated one soleus and confined the animals to sedentary cages--Group II, and (c) confined the animals to sedentary cages--Group III. All animals had one soleus removed at the beginning of the treatment period and one soleus removed at the conclusion of the treatment period and so served as their own controls. Before and after fiber-type-count percentages constituted the dependent variables for all animals, while the percentage of shock-free time (SHF) and the percentage of expected meters run (PSF) were additional dependent variables that were used to evaluate the quality of the training responses of the animals in Treatment Group I.

Sampling Procedures

Twenty normal male albino rats of the Sprague-Dawley strain were obtained at 56 days of age. They were assigned randomly to treatment groups and given two days to adjust to their cages. On day 58, surgery was performed, and on day 84 the controlled running wheel (CRW) sprint treatment began.

Research Design

In this study the dependent variables were evaluated on the basis of which of the three treatments the animals received. The animals served as their own controls, which provided inter- and intra-animal comparisons. This was especially important because the percentage of fast-twitch fibers decreases in the rat soleus with aging (Goldspink & Ward, 1978). Two days after the conclusion of the 38 training sessions, the animals in Treatment I were sacrificed. The animals in Treatments II and III were sacrificed subsequently in random order but with no set schedule.

Treatments

The rats arrived in the laboratory at 56 days of age and were given two days to adjust to cage conditions. On day 58, surgery was performed. Treatment I consisted of removing the soleus muscle from one randomly selected hind leg while leaving the gastrocnemius and plantaris intact. The soleus in the other hind leg was left intact but isolated by ablation of approximately 75% of the gastrocnemius and plantaris muscles. Care was taken to leave the tibial nerve intact. After four weeks of recovery, these animals were subjected to a sprint-training program (see Appendix A) in controlled running wheels (CRW) (Wells & Heusner, 1971). The rats learned to run as a result of an avoidance-response operant conditioning that used a low-intensity controlled-shock current as a stimulus. The animals in Treatment Group II underwent the same surgery as did the animals in Treatment Group I, but instead of exercise they were confined to sedentary cages for the duration of the experiment. The animals in Treatment Group III received no surgery other than ablation of one soleus. They also were confined to sedentary cages for the length of the experiment.

Training Procedures

The animals' training consisted of alternated work and rest intervals with a specified number of such intervals equaling one bout of exercise. A brake that functioned during the rest intervals prevented spontaneous activity. A training period consisted of several exercise bouts interspersed with 5-min rest periods. The training periods were conducted for two days and discontinued for one day until there were 38 training periods.

The start of each work interval was signaled to the animal by a light turning on above the wheel. If the animal did not reach a preset running speed within the specified acceleration period, the light did not go out and a shock was applied through the running surface. The shock and light were discontinued as soon as the animal accelerated to the preset speed. There was no penalty for running faster than the preset speed. The light and shock procedure was repeated any time during an exercise bout when the animal failed to maintain the preset speed.

The CRW displayed values for total meters run (TMR) and cumulative duration of shock (CDS). These performance data provided bases for computations of percentage of expected meters run (PEM) and percentage of shock-free time (PSF), which were the dependent variables used to evaluate the training responses of the animals in Treatment Group I. The values of PEM and PSF were obtained as follows:

PEM = 100 (total meters run/total expected meters)

PSF = 100 - 100 (cumulative duration of shock/total work time)

Training Program

In this study, all exercise programs were administered during the wakeful period. Body weights were recorded before each training period, and the rats were assigned to the same CRW each exercise period. The criteria for the sprint program were varied to maintain an increasingly challenging program but one that fell within the physiological ability of a rat to complete (see Appendix A). The sprint criteria were in agreement with the fact that $\geq$60 m/min was considered by Armstrong and Laughlin (1985) to be a speed at which FG fibers are known to be recruited. By the end of the training period, the rats were expected to run at a velocity of approximately 82 m/min. Their ability to maintain the preset program varied (see Appendix B).

Animal Care

All animals were housed in individual sedentary cages (24 cm x 18 cm x 18 cm) with access to food (Wayne Laboratory Blox) and water ad libitum. The rats were maintained on a circadian schedule of 12 hours of light alternated with 12 hours of darkness by an automatic timer that kept the lights in the animal quarters on from midnight until noon. The ambient temperature was maintained at approximately 72 degrees Fahrenheit, and the cages were cleaned regularly.

Sacrifice Procedures

Final body weight was recorded for each animal immediately prior to sacrifice via an intraperitonal injection (4 mg/100 g body weight) of a 6.48% sodium pentobarbital solution. The hind limb then was skinned and the soleus muscle was dissected and removed. After the muscle was weighed, it was immediately frozen in isopentane precooled (-170 degrees Celsius) with liquid nitrogen. Serial cross sections (10 μm) taken from the middle of each muscle belly were cut on a microtome in a cryostat maintained at -20 degrees Celsius.

Histochemical Methods and Tissue Analysis

The tissue sections were assayed for myosin adenosine triphosphatase activity according to the method of Padykula and Herman (1955) as modified by Guth and Samaha (1969) and then mounted on glass slides. Images of the sections were projected onto paper by a Prado Universal projecting microscope. All available fibers in a transverse midportion section of muscle were classified to avoid sampling errors (Ho, Heusner, Van Huss, & Van Huss, 1983; Ianuzzo, Gollnick, & Armstrong, 1976). Total fiber counts consisted of the number of fibers that stained dark plus the number of fibers that took no stain and appeared white. If there was any color at all, that fiber was determined to be "dark." Stains of serial sections at a pH of 4.3 were used to assign each fiber to a specific fiber type (Edgerton et al., 1985). (For results see Appendix C.)

Statistical Analyses

Measures of normality were calculated for each of the dependent variables. These computations revealed that parametric methods of statistical analysis were appropriate.

Means and variances were obtained. Homogeneity of variances was tested with the usual F-ratio test.

Subsequently, t-tests (or Cochran-Cox t-tests when the assumption of equal variances was not met) were used to determine if the values from Treatment Groups II and III could be pooled as a Treatment Group IIp. In all cases the data could be pooled. This creation of a combined or pooled control Group IIp provided a more powerful evaluation of the isolated effects of the exercise regimen.

Finally, standard t-tests were used to compare Treatment Groups I and IIp.

The .05 significance level was used for all analyses. The probability of making a Type II statistical error was set at .10. Transformations were used to obtain the additional dependent variables: Soleus Weight After as a Percentage of Body Weight (SWTAPBW), Percentage of Fast Twitch Before (PFTBF), Percentage of Slow Twitch Before (PSTBF), Percentage of Fast Twitch After (PFTAF), Percentage of Slow Twitch After (PSTAF), Percentage Gain Score for

Fast Twitch (PGSFT), Percentage Gain Score for Slow Twitch (PGSST), Average Percentage Shock-Free Time (AVPSF), and Average Percentage Expected Meters Run (AVPEM).

CHAPTER IV

RESULTS AND DISCUSSION

This chapter presents the results of the training procedure and then of the histochemical observations. The discussion covers three areas: trends suggested by the data, statistical rationales employed, and reconsideration of the research question.

Training Results

A criterion of 75% of PEM (percentage of expected meters run) and of PSF (percentage of shock-free time) was set as the minimum acceptable performance level. From test period 24 to test period 38 the animals had mean values for PEM of 84% and for PSF of 83% (see Appendix A). These training periods correspond to the most intense sprint protocol of the entire training program, i.e., a work:rest ratio of 1:4 at a speed of 82 m/sec.

Histochemical Results

Based upon the staining procedure for mATPase, preincubated at 4.3, there were no significant differences between Group I and Group IIp in the percentages of fast-twitch fibers after or slow-twitch fibers after.

Discussion

Even though statistical significance was not established in this study, many observations were generated. These observations concern trends suggested by the data, statistical rationales, and reconsideration of the research question.

Trends Suggested by Data

The results of this study show that adult-rat soleus fiber-type composition, as measured by Ca^{++}-activated mATPase activity, cannot be changed significantly as a result of imposing sprint exercise on the isolated rat soleus of a maturing animal. There were no significant differences in the percentages of slow-twitch fibers or fast-twitch fibers between treatment groups.

Appendix D contains fiber count information. The significant difference in Total Fibers Before (TFIBF) between treatment groups probably is due to the use of two different shipment groups. There is no significant between-group difference in Total Fibers After (TFIAF) or any of the other percentage fiber count or gain score categories. While the differences in Soleus Weight Before (SWTBF) and Body Weight Before (BWTBF) are significant, Soleus Weight After as a Percentage of Body Weight After (SWTAFPBW) is not (Appendix E). The significance of SWTBF and BWTBF may be due to the different shipments used. There are no significant differences in any other category. However, in the important category of Percentage of Fast-Twitch Fibers After (PFTAF), Treat IIp had a mean value of 3.9% as compared to a Treat I mean value of 1.2% ($p = .052$). The data from three animals are missing (Appendix F), and the before and after total fiber counts are unusual (Appendix C). Treat IIp animals are numbered 11 through 19 and were sedentary. The etiology of this unexpected datum is discussed within Reconsideration of Research Question.

Statistical Rationales

The statistical analysis concerning the assumption of a normal distribution generated a G_3 skewness value for FTAF in Treatment I that was significant, but it was determined that this violation would have minimal effect on the robustness of the following t-test and that the probability of a Type I error would remain at $\alpha = .05$ (Glass & Stanley, 1970).

Reconsideration of the Research Question

The research question of "How can a fiber be made to change?" is much too limiting a view and inhibits intellectual and scientific speculation. The research question should be been "How do fibers adapt to changing demands?" This question is not limiting and fosters innovative a posteriori research. The more effects observed, the greater the ability to determine the cause and to understand the phenomenon.

In light of this, several aspects of the current study need discussion. The first of these is mATPase activity as determined histochemically. A caveat against overinterpretation of the results is necessary (Burke et al., 1973; Burke & Tsairis, 1974). There are many reasons for this caution. A study by Hintz, Lowry, Kaiser, McKee, and Lowry (1980) demonstrated that rabbit soleus fibers that had been designated as FT or ST because of their reaction to mATPase stains in actuality contained almost identically low levels of lactate dehydrogenase and almost identically high levels of malate dehydrogenase. Burke and Edgerton (1975) found with cat and guinea pig medial gastrocnemius muscle that motor units identified as identical via mATPase staining actually exhibited twitch contraction times varying over a twofold range. ATPase activity of mitochondria, sarcoplasmic reticulum, and tubular systems may be demonstrated also (Burke & Edgerton, 1975; Guth, 1973; Guth & Yellin, 1971). Mechanics of the actual staining procedure seem to limit conclusions also. Jablecki and Kaufman (1973) believed that the specific activity of mATPase can vary as much as 15% in different preparations from the same muscle. This is credited to differences in the extent of mATPase inactivation when the muscle is being removed. Spurway (1980) believes that there are interfering reagents and structures that remain ineluctably present in the histochemical section. The possibility of coexistence of both fast and slow mATPase within the same fiber (Edgerton et al., 1969), plus the fact that enzyme activity levels can vary as much among as within muscles, adds even more weight to the caveat.

Also in need of discussion is the intensity and duration of the stimulus necessary to affect fiber characteristics. There is agreement that the change in functional demand must be radical and sustained for muscle to manifest its full capacity for adaptation

(Roy, Gilliam, Taylor, & Heusner, 1983; Salmons, 1980; Vrbova, 1978, 1980). The duration of stimulus must be long because mATPase activity is slow to change due to the fact that much of the normal muscle activity (postural and reflex in the case of the soleus) is still present (Vrbova, 1980; Vrbova et al., 1978) and also because of the extended protein turnover rate of mATPase. Saubert et al. (1973) suggested that duration may be more important than intensity in eliciting an adaptive response. When exercise is used as a stimulus to change, its duration must be compared to the duration of the rest of the activity the muscle performs. Not only is the work:rest ratio within the exercise program to be considered, but also the work:rest ratio of the rest of the 24 hours (Salmons & Henriksson, 19871). This study may not have been of sufficient duration to detect a change. Also, even though the regime was sprint modality, it may have constituted too little of the total day's regime.

The results need to be evaluated in relation to certain problems engendered by the study. Electric shock can elicit biochemical, respiratory, and cardiovascular responses (Salmons & Henriksson, 1981), and these were not controlled in the animals of Treat IIp. In addition, thyroxin is a stress hormone that has numerous effects on body systems, including contraction times, speed of Ca^{++} uptake, and relative number of FT fibers (Jolesz & Sreter, 1981). Also, there existed a lack of consistency in rating fibers either dark or light regardless of the position in the muscle of the section being evaluated. In some of the animals, the gastrocnemius and the plantaris regenerated and then attached to the bone even though well over half the length of muscle had been surgically removed before the treatment was imposed. The degree of this regeneration added uncertainty to the results because the degree the soleus was being "assisted" by such regeneration could not be ascertained.

The question of how fibers adapt to changing demands of necessity involves consideration of the neural relationship to muscle. No matter what the intensity or duration of the functional demand, it is doubtful that it can override the strong reaction of the soleus to the stretch reflex, which becomes an even stronger stimulus when synergists are ablated (Burke, Tsairis, Levine, Zajac, & Engel, 1973; Guth, 1969; Sreter et al., 1982; Vrbova et al., 1978). In addition, increased strength of ST fiber contraction is accomplished through increased recruitment of motor units, whereas increased strength of FT fiber contraction is achieved by increasing the firing rate (Jolesz & Sreter, 1981). Therefore, a pertinent question is: Can the nerve to the slow soleus make the adaptation to a faster firing rate when sprint training is imposed?

Growth and development of a normal rat soleus impose a "treatment" in that increasing leg length and body weight increase the need for ST fibers, as does the soleus's increasing function as a postural muscle (Kugelberg, 1980). In addition, Goldspink (1985) believed that the ratios of fibers change during growth and usually favor an increase in the already predominant fiber type. When ablation of synergists is imposed, there results an additional increase of ST fibers, an increase in contraction time, a decrease in mATPase activity, and an increase in oxidative enzymes (Gutmann & Hajek, 1971; Ianuzzo et al., 1976; Kugelberg, 1976; Salmons & Henriksson, 1981). All of these factors that foster ST development were present in this study and were in juxtaposition to the increased phasic activity of a sprint program, which results in the increase in synthesis of contractile proteins as well as increased speed and activity of mATPase (Gutmann & Hajek, 1971). In some respects, therefore, there existed a "push-pull" relationship that was not being controlled in the protocol.

Throughout this study, fibers have been dichotomized into FT and ST because the main thrust of this research was not nomenclature revision but rather mATPase activity. It must be remembered, then, that fibers possess many characteristics that exist in different quantities and qualities along various continua. These characteristics and their gradations of values are what should be used to describe a fiber. Therefore, there should be a nomenclature revision whereby fibers are not "named" but rather are "described."

Finally, the results should be viewed in light of how change is mediated. At present, the bridge between the external world and the internal world of the muscle cell is unknown, but there are two hypotheses that may address this issue:

1. Since tonic, endurance treatments stimulate ST fibers to increase their aerobic ability and since phasic, sprint treatments stimulate FT fibers to increase their anaerobic ability, this may

mean that the "bridge" mediating these effects is the relative hypoxic state of the cell. When tonic, endurance demands are imposed on the muscle, the perfusion of blood may not be interrupted because the force of contraction is not great enough. On the other hand, when phasic, sprint demands are imposed, just when oxygen is needed most, the force of contraction may interrupt its supply. The stimulus for fiber adaptation may be the relative oxygenation of the muscle cell.

2. If it is accepted that tonic nerve activity has a specific pattern of inhibitory and excitatory stimulus (IPSP and EPSP) and if it is accepted that phasic nerve activity has a specific pattern also, then perhaps the "bridge" may be a scrambling of the pattern when the functional demand changes, resulting in a phenomenon known as silent inhibition (Poggio & Koch, 1987). Since the motoneuron plays such an important role in differentiation and maintenance of fiber characteristics, looking for the mediation of fiber adaptability in the nerve pattern of IPSP's and EPSP's seems prudent.

Looking at fiber types in light of the research question "How do fibers adapt to changing demands?" rather than "How can a fiber be made to change?" sets the stage for a continuum theory of fiber-type characteristics existing in a dynamic equilibrium. Up to now, fiber classification, while making verbal communication relatively uninhibited, has encapsulated communication. "Change" should read "shift" because a fiber's characteristics such as contraction time, intensity of mATPase staining, succinic dehydrogenase activity, fiber size, properties of mechanical twitch, output of force, and resistance to fatigue (Eisenberg, 1985), as well as other contractile, histochemical, and biochemical properties (Pette, 1984), exist on various continua. Fibers undergo continual alteration in response to varying functional demands. Histochemically determined fiber types are but one frame of a time-lapse recording of a muscle's survival. The continuum theory is based on the observation that continuously varying demands can produce continuous variation of parameters and yet these parameters may or may not covary with each other (Salmons, 1980).

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The purpose of this study was to evaluate the effect of a sprint training program on the fiber-type composition of maturing-rat soleus muscle.

Animals were assigned randomly to either Treatment I (surgery + sprint), Treatment II (surgery + sedentary), or Treatment III (sedentary). Treatment II and Treatment III were later combined into a single group, Treatment IIp. Thirty-eight training sessions were initiated on Day 84 for Treatment I. Before and after fast-twitch and slow-twitch fiber percentages were calculated on each soleus from all treatments after histochemical staining at a preincubation of pH 4.3. No significant differences were found between groups.

Conclusions

Data from this study can be generalized only to adult male Sprague-Dawley rats and are specific to the training protocol using controlled-running wheels. No significant differences were found between treatment groups in regard to fiber-type composition of the soleus.

Recommendations

1. The number of animals in each treatment group should have been greater.

2. All animals should have been weighed 38 times and sacrificed at the same age so that body weights, soleus weights, and fiber-gain scores could have been compared.

3. Some control animals should have been shocked for an amount of time similar to the shock times of the animals in Treatment I.

4. The nomenclature of fibers should be changed to reflect the ideas of plasticity and of fiber-characteristic continua.

5. The theory that mammals have a constant number of muscle fibers from birth on should be re-evaluated.

6. The theories of relative muscle cell hypoxia and varying patterns of Inhibitory Post Synaptic Potential (IPSP) and Excitatory Post Synaptic Potential (EPSP) and their role as possible mediators of muscle cell change should be investigated.

APPENDICES

APPENDIX A

TRAINING PROGRAM

Table 1.--Training program.

Day of Training	Acceleration Time (sec.)	Work Time (min.:sec.)	Rest Time (min.:sec.)	Repetitions/Bout	No. of Bouts	Time Between Bouts (min.)	Shock (ma)	Run Speed (m/min.)	Total Time of Program (min.: sec.)	Total Expected Revolutions (TER)	Total Work Time (sec.) (TWT)
1	3.0	0:10	0:20	30	3	5:0	1.2	36	54:00	450	900
2	2.0	0:10	0:20	30	3	5:0	1.2	36	54:00	450	900
3	1.5	0:10	0:20	30	3	5:0	1.2	36	54:00	450	900
4	1.5	0:10	0:20	30	3	5:0	1.2	45	54:00	563	900
5	1.5	0:15	0:30	20	3	5:0	1.2	45	53:30	563	900
6	1.5	0:15	0:30	20	3	5:0	1.2	45	53:30	563	900
7	1.5	0:15	0:30	20	3	5:0	1.2	54	53:30	675	900
8	1.5	0:15	0:30	20	3	5:0	1.2	54	53:30	675	900
9	1.5	0:15	0:30	20	3	5:0	1.2	54	53:30	675	900
10	1.5	0:15	0:30	20	3	5:0	1.2	54	53:30	675	900
11	1.5	0:15	0:30	20	3	5:0	1.2	54	53:30	675	900
12	1.5	0:15	0:40	20	3	5:0	1.2	63	63:00	788	900
13	1.5	0:15	0:45	20	3	5:0	1.2	63	67:45	788	900
14	1.5	0:15	0:45	20	3	5:0	1.2	63	67:45	788	900
15	1.5	0:15	0:60	15	3	5:0	1.2	63	67:45	591	675
16	1.5	0:15	0:60	15	3	5:0	1.2	63	63:15	591	675
17	1.5	0:15	0:60	15	3	5:0	1.2	63	63:15	591	675
18	1.5	0:15	0:60	15	3	5:0	1.2	72	63:15	675	675
19	1.5	0:15	0:60	15	3	5:0	1.2	72	63:15	675	675
20	1.5	0:15	0:60	15	3	5:0	1.2	72	63:15	675	675
21	1.5	0:15	0:60	15	3	5:0	1.2	72	63:15	675	675
22	1.5	0:15	0:60	15	3	5:0	1.2	72	63:15	675	675
23	1.5	0:15	0:60	15	3	5:0	1.2	72	63:15	675	675
24	1.5	0:15	0:60	15	3	5:0	1.2	72	63:15	760	675
25	1.5	0:15	0:60	15	3	5:0	1.2	72	63:15	760	675
26	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
27	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
28	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
29	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
30	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
31	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
32	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
33	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
34	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
35	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
36	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
37	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675
38	1.5	0:15	0:60	15	3	5:0	1.2	82	63:15	760	675

APPENDIX B

PERCENTAGE OF SHOCK-FREE TIME (PSF)

PERCENTAGE OF EXPECTED METERS RUN (PEM)

Table 2.--PSF/PEM: Treatment I.

Day of Training	Animal Number 2	3	4	5	6	7	8	9
1	96/93	93/72	95/79	90/72	90/76	91/86	90/84	85/56
2	99/107	94/90	97/109	92/106	98/125	97/109	95/92	77/47
3	94/115	97/116	98/142	92/122	93/145	97/124	96/125	92107
4	93/114	91/129	98/143	89/120	89/136	93/145	97/133	88/104
5	88/124	97/142	95/146	93/136	93/144	95/159	95/133	93/128
6	94/101	88/124	98/133	83/94	95/129	93/120	97/112	92/103
7	96/95	86/116	95/113	82/94	92/116	93/107	95/110	89/89
8	91/91	75/82	94/113	82/98	90/121	92/114	95/116	84/77
9	92/94	98/97	94/119	89/94	87/108	88/107	94/103	85/85
10	92/89	82/97	92/112	94/113	88/109	81/106	97/114	79/81
11	96/105	82/79	87/96	91/97	85/108	74/86	93/109	80/83
12	95/98	88/98	88/86	91/95	79/81	75/82	88/95	86/74
13	91/90	74/79	93/104	90/94	66/69	75/75	94/124	78/77
14	90/94	80/91	93/106	91/106	82/97	77/75	92/107	81/79
15	90/101	83/105	91/105	91/92	69/69	72/72	93/115	68/66
16	95/115	73/89	92/104	90/98	66/72	68/71	92/117	75/70
17	94/112	87/101	92/108	94/116	68/71	67/69	96/122	76/78
18	93/107	76/93	92/101	88/99	64/68	60/62	72/92	76/74
19	92/105	88/113	96/98	82/97	76/86	85/79	93/107	84/98
20	89/97	75/97	94/104	90/96	77/95	86/78	98/127	75/75
21	86/98	95/116	94/94	93/98	82/101	84/84	95/119	80/85
22	88/115	94/119	95/99	93/100	84/95	79/74	95/103	77/85
23	82/99	85/103	92/89	93/95	81/91	82/72	90/101	93/79
24	82/93	92/108	94/91	89/89	88/88	73/66	92/103	88/77
25	83/98	75/85	88/79	91/89	76/81	46/51	89/102	34/65
26	81/89	77/85	83/75	92/89	89/84	54/51	87/101	55/57
27	85/100	73/85	91/89	93/94	80/82	55/61	89/99	66/67
28	86/109	69/79	93/92	89/89	82/93	63/65	94/103	68/70
29	82/107	68/72	89/84	93/89	80/77	72/73	88/99	68/68
30	74/90	74/78	69/83	69/64	92/84	84/81	90/100	67/66
31	84/97	69/73	80/72	95/89	77/68	68/64	88/102	74/74
32	86/94	92/100	91/83	94/88	94/88	82/79	81/81	93/96
33	90/97	68/75	81/77	92/90	80/78	84/87	94/98	73/73
34	86/97	70/76	85/79	92/89	79/79	71/75	95/99	71/71
35	88/98	76/82	82/75	96/94	88/76	71/74	94/103	63/64
36	86/99	93/106	88/80	93/91	77/79	85/78	85/108	72/71
37	85/93	64/77	82/78	90/89	89/76	89/87	96/103	84/84
38	90/97	73/74	81/75	92/86	79/78	84/80	95/120	64/64

APPENDIX C

ANIMAL DATA

Table 3.--Animal data.

Before/After Values	Animal Number/Treatment																
	2	3	4	5	6	7	8	9	11	12[a]	13[a]	14[a]	15[a]	16	17[a]	18	19
	I	I	I	I	I	I	I	I	II	II	II	II	II	III	III	III	III
Body wt. (gm)	237 320	236 363	207 374	237 382	234 358	240 351	239 325	229 393	221 444	260 478	253 530	243 475	250 472	235 381	250 487	241 419	246 425
Soleus wt. (gm)	.0907 .1706	.0876 .1637	.0803 .1701	.0982 .2652	.0765 .1656	.0759 .1501	.0874 .1856	.0806 .1743	.0804 .2332	.1050 .2413	.0919 .2200	.0962 .1822	.0970 .3167	.0211 .1303	.1130 .1736	.1013 .1421	.0863 .1170
Total fibers	2141 2032	1823 2012	2526 946	2476 1176	2135 1670	1832 1892	1694 1817	2764 2023	1617 1582	1801 1967	1751 4014	2194 b	1938 b	1706 1921	1925 b	1575 1497	1928 1248
Fast-twitch fibers	88 8	260 133	188 13	262 6	40 9	369 0	236 1	107 0	51 53	125 22	306 13	97 b	149 b	236 192	254 b	206 222	178 69
Slow-twitch fibers	2053 2024	1563 1878	2338 933	2214 1170	2095 1661	1463 1892	1458 1816	1757 2023	1566 1529	1801 1945	1445 4001	2097 b	1789 b	1470 1829	1671 b	1370 1275	1750 1177

[a] Older animals.

[b] Missing data.

APPENDIX D

FIBER COUNTS: TREAT I AND TREAT IIb

Table 4.--Fiber counts.

	No. of Cases	Mean
Total Fiber Before		
Treat I	8	2174
Treat IIp	9	1826*
Total Fiber After		
Treat I	8	1696
Treat IIp	6	2038
Fast Twitch Before		
Treat I	8	194
Treat IIp	9	178
Fast Twitch After		
Treat I	8	21
Treat IIp	6	95
Slow Twitch Before		
Treat I	8	1868
Treat IIp	9	1662
Slow Twitch After		
Treat I	8	1675
Treat IIp	6	1958
Total Fiber Gain		
Treat I	8	-478
Treat IIp	6	308
Fast Twitch Gain		
Treat I	8	-173
Treat IIp	6	- 88
Slow Twitch Gain		
Treat I	8	-193
Treat IIp	6	393

*Significant at p = .05.

APPENDIX E

STATISTICAL DATA: TREAT I AND TREAT IIb

Table 5.--Mean values.

	Treat I	Treat IIp
Soleus Weight Before (g)	0.0846	0.0947
Body Weight Before (g)	311.6	244.3
Soleus Weight After as a Percentage of Body Weight After	0.0498	0.0424
Percentage of Fast-Twitch Fibers Before	9.54	9.89
Percentage of Slow-Twitch Fibers Before	86.5	90.6
Percentage of Fast-Twitch Fibers After	1.2	5.9
Percentage of Slow-Twitch Fibers After	98.8	95.0
Percentage Gain Score for Fast-Twitch Fiber	-8.4	-4.8
Percentage Gain Score for Slow-Twitch Fiber	12.4	4.4
Average Percent Shock Free	85.3	--
Average Percent Expected Meters Run	94.0	--

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