EXERCISE-INDUCED BIOCHEMICAL ALTERATIONS IN DIFFERENT TYPES OF SKELETAL MUSCLE

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
ROBERT CHARLES HICKSON
1974

This is to certify that the

thesis entitled

Exercise-Induced Biochemical Alterations

in Different Types of Skeletal Muscle

presented by

Robert Charles Hickson

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physical Education

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Date Sept. 27, 1974

O-7639

ABSTRACT

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By

Robert Charles Hickson

The purpose of this study was to determine the effects of aerobic and anaerobic programs of endurance running on selected enzymatic activities in the left plantaris, the left soleus, and the "white" area of the left vastus lateralis muscles of the adult male albino rat. The training regimens were the Controlled Running Wheel programs previously reported from this laboratory. Biochemical determinations of the muscle homogenates were made on the levels of activity of phosphoglucomutase, an enzyme of glycogenolysis; phosphoglucoisomerase, an enzyme of glycolysis, lactate dehydrogenase, an enzyme concerned with anaerobic metabolism of glycolytically-formed pyruvate; and fumarase, a mitochondrial enzyme of the tricarboxylic acid cycle. Enzyme activity ratios also were investigated in each of the fiber types.

Eighty-four normal, male, albino rats of the Sprague-Dawley strain were randomly assigned to three treatment groups. The treatment groups were a sedentary-control group (CON); a high intensity,

short-duration running group (SHT); and a low-intensity, long-duration running group (LON). The animals were provided food and water ad libitum.

Initiation of treatments for all animals began at 84 days of age. Performance criteria were used as the basis of animal selection for subsequent investigation. Animals were sacrificed 72 hours after their last training period. Biochemical analyses were performed before the initiation of treatments (0-wk) and after eight (8-wk) and sixteen (16-wk) weeks of exercise. The final sample consisted of 36 animals with 4 animals in each treatment-duration subgroup.

The results did not reflect a differentiation of metabolic activity between the SHT and LON groups. This observation was supported by the large number of significant overall training (TRAIN) effects found at 16 wks and by the fact that there were no significant LON vs SHT contrasts at that time.

Decreases in glycogenolytic capacity as measured by phosphoglucomutase and increases in tricarboxylic acid cycle activity as measured by fumarase, in all muscles of the TRAIN group at 16 wks, suggest an exercise-induced inverse relationship between levels of these enzymes. This result is opposite to that expected with maturation. In addition, decreases in enzyme ratios of variable metabolic organization in all muscles, along with a decrease in the lactate dehydrogenase activity of the "white" vastus lateralis muscle, imply an increased dependence upon oxidative metabolism by the TRAIN group.

Alterations in some of the constant proportion enzyme ratios occurred as a result of either maturation or training. The variable enzyme ratio data support the validity of histochemical fiber-typing methods.

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By

Robert Charles Hickson

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Health, Physical Education, and Recreation

1974

DEDICATION

To

William W. Heusner

ACKNOWLEDGMENTS

Sincere thanks to Dr. William W. Heusner and Dr. Wayne D. Van Huss for making my four years at Michigan State an unsurpassed experience.

Special appreciation is extended to Dr. John E. Wilson for his enthusiasm, scholarly input, and direction toward the completion of this study; and for the use of his laboratory.

Gratitude is extended to Dr. Loran Bieber for his meaningful assistance on the doctoral committee.

The following people have contributed many ways throughout this study: JoAnn LaFay, David Anderson, Phil Felgner, Roland and Sharon Roy, Kwok-Wai Ho, Ken Stevens, Nancy Williams, Marjorie Middel, Laura Zydbel, Robin Krause, Fayann Lippincott, Bob Holmes, Bob Wells, Albert Chov, Barbara Binns, and my mother and father-in-law Kenneth and Dorothy Jones.

I owe a debt of thanks for reaching this goal to my mother, father, and Aunt Doris, and even Sister Mary Collette, and Father Bob Dahlke S. J.

A most important person whom I have not forgotten is my new bride Susie Jones (Hickson). She has been understanding, patient, and instrumental in assisting me toward the completion of this dissertation.

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CHAPTER I

THE PROBLEM

Histochemical observations of mammalian skeletal muscle have demonstrated a heterogeneous distribution of metabolic characteristics. Attempts have been made to classify skeletal muscle fibers according to cellular characteristics (93, 77). These investigations have produced a taxonomy of skeletal muscle, predicated primarily on the identification of three fiber types. The definitions of the metabolic characteristics of red, white, or intermediate fibers, however, have not been entirely consistent with each other (9, 14, 15, 21, 26, 27, 28, 42, 74).

Physiological studies, based on contractile properties of muscle, coupled with histochemical and biochemical determinations have provided evidence for an innovative reclassification of fibers to fast-twitch red, fast-twitch white, and slow-twitch red (10). Additional biochemical research on skeletal muscle fiber types warranted a further modification in nomenclature to fast-twitch oxidative-glycolytic (FOG), fast-twitch glycolytic (FG), and slow-twitch oxidative (SO) respectively (82).

Fiber populations of five mammals were categorized histochemically according to their percentages of FOG, FG, and SO fibers (1).

Whole muscles showed little fiber-type homogeneity, although certain muscles were able to meet the criteria of being predominantly composed of one fiber population.

Some evidence has been presented to indicate a coordination of metabolism at the molecular level. Determinations of glycolytic enzyme activity in skeletal muscle have indicated that the five enzymes of the "phosphotriose glycerate phosphate group" are represented in constant proportions and in equimolar concentrations in all types of skeletal muscle (99, 83, 70). However, a recent paper by Dalrymple (19) has challenged this concept. Constant proportions of groups of mitochondrial enzymes also were established (84); but unlike the succession of glycolytic enzymes from triose phosphate isomerase to enolase, no sequential metabolic arrangement of constant-proportion mitochondrial enzymes was found.

A comparison of enzyme-activity ratios in various muscle types has exposed both a metabolic constancy and a metabolic differentiation (13, 85). Arduous endurance training can induce constant-proportion increases in the activity of the citric acid cycle, the electron transport system, and related enzymes and constituents in skeletal muscle (54, 56, 57, 25). The level of aerobic stress appears to be the primary stimulus in evoking appropriate metabolic responses. Most of the studies involving moderate aerobic exercise programs have not shown any resultant modifications of enzymatic activity.

Experimentation with anaerobic training programs is rare at this time. Staudte, Exner, and Pette (92) subjected rats to a high-intensity, short-duration running program and discovered that the soleus, a slow-twitch oxidative muscle, responded with a shift toward

glycolytic metabolism as judged by enzyme levels more than did the rectus femoris muscle which contains almost no slow-oxidative characteristics. It is worthwhile to postulate that the normal glycolytic capacity of the rectus femoris may be sufficient to cope with this degree of stress. If this is the case, adaptation of the rectus femoris might be observed only following more strenous workloads generating a high level of oxygen debt.

In light of these findings, this study was undertaken to determine the effects of specifically designed aerobic and anerobic endurance interval training programs on enzymatic activities which are representative of appropriate pathways of energy-supplying metabolism in skeletal muscle. Specific exercise regimens should represent a form of metabolic specialization. This raised the question of how the constant-proportion as well as the differentiated enzymatic activity ratios of skeletal muscle metabolism would respond to these different stresses. The responses of specific fiber types to the training programs also were investigated.

Statement of the Problem

The purpose of this study was to determine the effects of aerobic and anaerobic programs of endurance running on selected enzymatic activities in the left plantaris, the left soleus, and the "white" area of the left vastus lateralis muscles of the adult male albino rat. The training regimens were the Controlled Running Wheel programs previously reported from this laboratory (101). Various durations of the exercise programs were incorporated in the study. Biochemical determinations were made on the levels of activity of

phosphoglucomutase, an enzyme of glycogenolysis; phosphoglucoisomerase, an enzyme of glycolysis; lactate dehydrogenase, an enzyme concerned with anaerobic metabolism of glycolytically-formed pyruvate, and furmarase, a mitochondrial enzyme of the tricarboxylic acid cycle. Enzyme activity ratios also were investigated in each of the fiber types.

Rationale

The muscles designated for this study were selected to represent fast-twitch oxidative-glycolytic, fast-twitch glycolytic, and slow-twitch oxidative fiber types. Another criteria for selection was anatomical location. It was assumed that any resultant changes due to the exercise programs would be best reflected by muscles "actively" affected by the training regimens. Since "whole" muscle fiber homogeneity appears to be the exception, the muscles were chosen with emphasis toward a prevalent fiber-type population. In the rat, the plantaris was reported to contain 53% FOG, 41% FG, and 6% SO fibers; the soleus, 16% FOG, 0% FG, and 84% SO; and the vastus lateralis, 56% FOG, 42% FG, and 2% SO (1). Only the "white" area of the vastus lateralis which has predominantly FG fiber characteristics was used for this investigation.

It was hypothesized that the response to the increased energy requirements imposed by the training programs would be specific. Therefore, the enzymes were chosen to represent enzyme activity states in four separate biochemical pathways. Since the different exercise regimens could be expected to result in some degree of metabolic specialization, an adaptation of both constant and variable enzymatic

activity ratios was expected. A differential response by fiber types to the training regimens also was anticipated.

Exercise-induced biochemical alterations probably are dependent upon the duration of the training program. Thus, zero-week, eight-week, and sixteen-week periods of training were incorporated. Furthermore, the responses to training may be reflected differently immediately after an exercise bout and following a recovery period. To test this hypothesis, animals were sacrificed fifteen minutes and seventy-two hours after their last exercise bout within each duration group.

Significance of the Problem

Determinations of the biochemical effects of various training programs should contribute to an understanding of the functional nature of the aerobic-anaerobic continuum. The study of exercise-related alterations by muscle fiber types may provide further insight into the mechanisms of metabolic adaptation. A potential overall contribution of this study is that the information obtained may provide a basis for the appropriate prescription of exercise to improve human health and work performance.

Limitations of the Study

1. Although specific muscle fiber populations were employed, the results may reflect an integrated response of all fiber types.
2. The results of animal studies cannot be applied directly to humans.
3. In vitro enzymatic assays may not be representative of in vivo cellular activities.

4. The exercise programs were designed to represent specific aerobic and anaerobic interval training routines. However, at this time it is impossible to design and conduct a program of exercise for animals which is either purely aerobic or anaerobic in nature.
5. No control over the shock stimulus to run was included in the study.
6. The small sample size may have limited the power of the statistical analyses.
7. Enzymatic activities were expressed as Units/gm of muscle. It appears from the literature that biochemical differences in activity levels are not masked when expressed in this manner, but that may not be the case.

CHAPTER II

REVIEW OF RELATED LITERATURE

In an attempt to facilitate a discussion of the interrelationships between various biochemical parameters, especially enzymatic activities, and exercise in skeletal muscle, this chapter has been organized into three sub-divisions. First, an overview is presented of enzymatic studies that are uniquely connected with skeletal muscle fiber types. Second, there is a discussion of the effects of exercise on glycogenolytic, glycolytic, gluconeogenic and related constituents. Finally, the focus is on the influence of exercise on mitochondrial enzymes and related constituents.

Overview of Skeletal Muscle Enzyme Activities

An early investigation of skeletal muscle was performed on the African migratory locust (99). Three types of voluntary muscles were compared with respect to structure, function and enzymatic activity. A flight muscle used for enduring work performance exhibited high levels of aerobic metabolism, maximum mitochondria, and a moderate but specific endoplasmic reticulum. A hind leg muscle used for jumping had anaerobic metabolic patterns with minimum mitochondria, a maximum number of myofibrils, and a moderate endoplasmic reticulum. The

flexor tibiae exhibited contractile tonus, a specific arrangement of pairs of mitochondria in the vicinity of the Z discs and an extended endoplasmic reticulum. All three muscles had a constant relation between cytochrome c content and the volume fraction of mitochondria estimated by electron microscopic pictures. Lactate dehydrogenase activity was very low in the flight muscle and highest in the jumping muscle. Qualitative differences in locust muscles were previously reported by Delebruck, Zebe, and Bücher (22). The relationships between the five enzymes of the glycolytic pathway from triose-3-phosphate to phosphoenolpyruvate were relatively constant in the three types of muscle.

Subsequent examination of the same "phosphotriose-glyceratephosphate group" of enzymes again showed nearly constant ratios in various muscle types from phylogenetically different animals which suggested a coordination at the molecular level (83). It was suggested that since this quintet forms the only major unbranched segment of the glycolytic chain, the limited variability of the ratios of their activities might be explained by the close functional connection of these enzymes. Phosphoglucomutase and several other enzymes exhibited similar constant proportions with this quintet of enzymes. Supportive evidence for the constant-proportion group of the Embden-Meyerhof pathway was presented by Mier and Cotton (70). A significant finding which may have escaped notice by Vogell and coworkers (99) is that the actual pattern represents equimolar amounts of these enzymes. They concluded that the cell necessarily produces molecules of each of these five enzymes in precisely equal numbers and that synthesis is controlled by a single operator gene.

Moreover, one single molecule of mRNA is synthesized from the DNA strand per operon rather than a separate mRNA molecule for each structural gene.

The constant-proportion concept for the glycolytic enzymes was studied in developing red (trapezius) and white (longissimus) muscles of pigs from the fetal stage to 24 weeks (19). Adult levels of enzymes were evident two weeks postnatal. Enzyme activities were two- to three-fold greater in the longissimus than in the trapezius muscles. Enzyme activity ratios based on glyceraldehyde-3-phosphate dehydrogenase were not constant in fetal and day-one samples but were constant during later stages of growth. At 105 days gestation, glyceraldehyde-3-phosphate dehydrogenase and enolase activities were different from triose phosphate isomerase, phosphoglycerate kinase, and phosphoglycerate mutase enzyme patterns. From this evidence, the single operon hypothesis of Mier and Cotton was questioned.

A constancy of the respiratory chain molar ratio of cytochrome a to cytochrome c has been found in different types of muscle (84), and the DPN content in various types of mitochondria has been shown to be constant when expressed as a ratio of molar concentrations to cytochrome c (61). When expressed relative to cytochrome c turnover and not activity per gm tissue, malate dehydrogenase and glutamate-oxaloacetate transaminase were constant, succinic dehydrogenase and pyruvate oxidase varied slightly and TPN-specific isocitrate dehydrogenase, glutamate dehydrogenase, and glycerol-1-P oxidase varied in the range of two orders of magnitude in different mitochondria.

The constant-proportion enzymes in the mitochondria were not sequential as was observed for the five glycolytic enzymes. The

importance of auxiliary enzymes of the tricarboxylic acid cycle was emphasized in the constant ratio of glutamate-oxaloacetate transaminase to cytochrome c.

Hexokinase, an enzyme linked with glucose phosphorylation, was found to be highest in red muscle (semitendinosus) of the rabbit and to vary inversely with phosphorylase which is maximally active in white muscle (adductor magnus) (16). Hexokinase activities correlate approximately with citric acid cycle capacities as determined by succinate oxidase. Observations from the literature (8, 15) suggest that muscles with an active hexokinase may preferentially accumulate glycogen when excess glucose is present. It also was concluded that phosphorylase plays a minor role in muscles with predominantly oxidative metabolism and that there is a significant increase in phosphorylase activity levels with muscle dissuse.

Crabtree and Newsholme (18) measured the maximal activities of hexokinase, phosphorylase, and phosphofructokinase in extracts from a variety of muscles. In all vertebrate muscles examined, the activity of phosphofructokinase was very similar to that of phosphorylase which was highest in white muscle. These findings are consistent with the concept that glycogenolytic and glycoltyic control are mediated by phosphorylase and phosphofructokinase respectively (103). In addition, the work supported and extended the conclusion of Burleigh and Schimke (16) that utilization of glycogen is more important and utilization of glucose is less important in white than in red muscles. In vertebrates the activity of hexokinase is greater in red (rabbit semitendinosus and rat heart) than in white muscles. This observation suggests that glucose is a more important energy source for contraction

in red than in white muscle. However, phosphorylase and phosphofructokinase levels are higher than hexokinase levels in all red and most white mammalian muscles. An examination of human skeletal muscle disclosed lower levels of lactate dehydrogenase and total phosphorylase in the soleus than in either the gastrocnemius or vastus lateralis (41). No intermuscular differences were found to exist for levels of activity of hexokinase, succinate dehydrogenase and creatine phosphokinase. Biopsy samples from the soleus contained approximately 80% slow-twitch (ST) fibers. Fibers from the gastrocnemius and vastus lateralis averaged 57% ST.

In the guinea pig, the LDH activity level of a fast-twitch white muscle (white vastus lateralis) was found to be higher than in either fast-twitch red (red vastus lateralis) or slow-twitch red (soleus) muscles (81). The LDH activity in the red vastus lateralis was significantly higher than in the soleus. The LDH_5 (muscle-type) was shown to be predominant in both fast-twitch white and fast-twitch red fibers, whereas LDH_1 (heart-type) was predominant in the slow-twitch red fibers.

In a classical study Bass et al. (13) attempted to uncover the systems of energy-supplying metabolism which are subject to differentiation in muscle. This question was examined by comparing enzyme activity levels and ratios of these activities in various muscle types of higher animals. The ratios were intended to reflect quantitative relations of metabolic systems at the level of enzymatic organization. Nearly constant values were found for the following ratios:

Enzymes	Pathways Represented
Phosphorylase	Glycogenolysis
Triosephosphate Dehydrogenase	Glycolysis
Glycerophosphate Dehydrogenase	Mitochondrial Glycerophosphate Oxidation
Triosephosphate Dehydrogenase	Glycolysis
Triospehosphate Dehydrogenase	Glycolysis
Lactate Dehydrogenase	Lactate Fermentation
Hexokinase	Glucose Phosphorylation
Citrate Synthetase	Citric Acid Cycle
3-Hydroxyl-CoA Dehydrogenase	Fatty Acid Oxidation
Citrate Synthetase	Citric Acid Cycle

The lack of variability in these ratios was assumed to indicate constant organization of enzyme activity patterns. It was postulated, therefore, that the relationships between these metabolic systems are not subject to differentiation.

Metabolic variability was observed in the following ratios:

Enzymes	Pathways Represented
Triosephosphate Dehydrogenase	Glycolysis
3-Hydroxyl-CoA Dehydrogenase	Fatty Acid Oxidation
Triosephosphate Dehydrogenase	Glycolysis
Citrate Synthetase	Citric Acid Cycle
Lactate Dehydrogenase	Lactate Fermentation
Citrate Synthetase	Citric Acid Cycle
Phosphorylase	Glycogenolysis
Hexokinase	Glucose Phosphorylation
Hexosediphosphatase	Gluconeogenesis
Hexokinase	Glucose Phosphorylation

It was concluded that these variable enzymatic activity ratios are discriminative and can be used to classify distinct metabolic types of muscle.

Fast-twitch white muscles exhibit high levels of glycogenolytic, glycolytic, lactate fermentation, mitochondrial glycerophosphate oxidation, and gluconeogenic enzyme activities. Low activities of glucose phosphorylation, citric acid cycle, and fatty acid oxidation are found in white muscle. An opposite pattern of these enzyme activities is found in red (slow-twitch) muscle. Hexokinase activity parallels that of citrate synthetase in both muscle types. However, mitochondrial glycerophosphate dehydrogenase and hexosediphosphatase activities are high in white muscle indicating the importance of gluconeogenesis starting from glycerophosphate or triosephosphate in this type of fiber. Higher levels of fructose 1,6-diphosphatase have been previously reported in white muscle than in red muscle, and a plausible relationship between gluconeogenesis activity and glycerophosphate content is implied (62, 75).

Ratios of these enzyme activities might help to clarify the pattern of metabolic differentiation not only in red and white muscles but in mixed-fiber populations of heterogeneous muscles.

Pette (85) further illustrated metabolic differentiation in two muscles with similar functional characteristics. The energy expenditures of the flight muscles of the Apis Mellifera (honey bee) and the Bombyx Mori (silk moth) are both based on aerobic metabolism. However, the predominant substrates for catabolism are carbohydrate and fat respectively.

It was concluded that further clarification of the differences and similarities between the energy metabolism of red and white muscle is needed. The constancy of some ratios suggests a fixed pattern of the metabolic systems at the level of molecular organization. However,

specific metabolic specialization may alter the constant-proportion enzyme activity groups. These variations can be regarded as the expression of metabolic differentiation. Differentiation then could be interpreted as an optimal adaptation of the energy supply to the requirements of muscle function, and different metabolic patterns could be associated with different functional activities. The optimal way to investigate differences in metabolic type is at the level of enzymatic organization.

Staudte and Pette (91) studied fifty-one muscle specimens from a variety of animals of different species and phyla. Linear correlations of enzyme activities were observed between glycogen phosphorylase and triosephosphate dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase and citrate synthetase, and hexokinase and citrate synthetase. In muscles containing creatine kinase, a linear correlation existed between the activity of that enzyme and the activity of triosephosphate dehydrogenase. Although these results reinforce the general validity of the concept that constant proportions do exist between certain metabolic enzymes in muscle, deviations were found which indicate the possibility of independent regulation of the constant-proportion enzymes.

Administration of thyroid hormones has been shown to induce changes in energy metabolism in red (soleus), white (rectus femoris), and heart muscles of rats (63). Hexokinase activity increased in white muscle, while both hexokinase and glycerophosphate dehydrogenase activities increased in red muscle. In heart muscle, only glycerophosphate dehydrogenase activity increased. The ratio of hexokinase/citrate synthetase increased in white and red muscle only; whereas,

the ratio of glycerophosphate dehydrogenase/triosephosphate dehydrogenase increased only in red and heart muscle. These results indeed suggest that various metabolic factors can affect the constant-proportion enzymatic pattern.

Glycogenolytic, Glycolytic, Gluconeogenic Enzymes and Related Muscle Constituents

Introductory investigations using biopsy samples of the vastus lateralis muscle showed phosphorylase a activity to increase with prolonged training (96). The experimental subjects were healthy adult males who were trained to increase maximum VO_2 for sixteen weeks on a bicycle ergometer. After exercise to fatigue, both the trained subjects and a group of sedentary controls had decreased activities of phosphorylase a. Neither training nor exercise to fatigue affected phosphorylase b activity.

Endurance training on a treadmill for six months produced no changes in the activities of phosphorylase, lactate dehydrogenase, or mitochondrial α-glycerophosphate dehydrogenase in either the semimembranosus or vastus lateralis of the lesser bushbaby (Galago senegalensis) (29). Forced swimming in rats for 6 hours did not produce any changes in phosphorylase (43). Intermittent isometric activity has induced elevated phosphorylase activities in the rectus femoris muscles of female and male rats (33, 34).

Peter, Jeffress, and Lamb (80) demonstrated increases in activity levels of hexokinase in red and white quadriceps of adult guinea pigs who had been trained every other day for 21 days on a treadmill driven at 1.9 km/hr. The results were significant when expressed as Units/gm muscle and Units/mg protein. Lamb et al. (64)

also observed increases in levels of hexokinase activity in the same muscles of guinea pigs both immediately and 48 hours after exercise. The results of both studies revealed hexokinase activity to be higher in red than in white skeletal muscle.

A similar adaptation to endurance exercise in rats was reported by Holloszy (57). The levels of activity of hexokinase increased two-fold in exercised gastrocnemius muscle. In contrast, the levels of activity of phosphorylase, phosphofructokinase, pyruvate kinase, and lactate dehydrogenase were unaffected by the exercise program. Baldwin et al. (6) also has shown that rats who were endurance trained by treadmill running had increased levels of hexokinase: 170% in the red portion of quadriceps muscle, 50% in the soleus, and 30% in the white portion of the quadriceps as well as a 15% decrease of lactate dehydrogenase in this area. In the red muscle decreases of approximately 20% occurred in the activity levels of phosphorylase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, lactate dehydrogenase and cytoplasmic α-glycerophosphate dehydrogenase of the trained group. A 50% increase in cytoplasmic α-glycerophosphate dehydrogenase activity and 18-35% increases in the levels of phosphorylase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase and pryuvate kinase was evident in the soleus muscle of the exercised animals. The increases of hexokinase and phosphorylase activities in soleus muscle were inconsistent with previous results (18).

Gollnick et al. (40) subjected six healthy male subjects to a five-month program of endurance training on a bicycle ergometer. The level of activity of phosphofructokinase (PFK) increased 117% in the

biopsied vastus lateralis muscle. Histochemical analysis showed percentages of slow-twitch and fast-twitch fibers to remain unchanged after training when identified by myosin ATPase. Oxidative potential (DPNH-Diaphorase) and glycolytic capacity (α-glycerophosphate dehydrogenase) increased in both fiber types. Slow-twitch fibers were larger after training than before training. The relative area the slow-twitch fibers occupied in the muscle was higher after training. The soleus fiber type in the rat is similar to the slow-twitch human type. These findings suggest increased glycolytic capacity of slow-twitch fibers after training. Increased levels of activities of PFK (83%) also have been shown in boys 11-13 years old after an endurance training program (31).

Staudte, Exner, and Pette (92) imposed a high-intensity, short-duration (80 m/min, 30° incline, 45 sec) training program on female rats four times daily for three weeks. Increases in hexokinase and citrate synthetase activities occurred with training in the rectus femoris and soleus muscles. The adaptability of the soleus muscle was again more pronounced. It acquired a shorter contraction time and increases in creatine kinase and glycolytic triosephosphate dehydrogenase activities. The authors noted that physiological and biochemical changes in muscle may not correlate with higher performance. However, different responses of fast- and slow-twitch muscles can be representative of specific adaptations.

Aldolase activity in rat skeletal muscle has been shown to increase after prolonged treadmill running (49). However, in another study, training plus sustained running raised aldolase activity in red muscle only of rats on a low vitamin C diet (23). Hearn (51)

reported no differences in aldolase levels of the gastrocnemius muscle after a five-week swimming program and detraining.

Lactate dehydrogenase (LDH) activity was found to increase in the vastus lateralis muscles of four male subjects after prolonged severe exercise (59). These findings are similar to those of Dieter, Altland, and Highman (24) who showed increased activity levels of LDH in red and white muscles of cold-acclimated rats that were exercised for up to nine hours. LDH levels of unacclimated rats increased up to five hours and then dropped off. However, the levels of activity of LDH were decreased in the gastrocnemius muscles of rats trained by swimming (37). Acute exercise did not alter these levels. Molé et al. (73) observed no significant shift in LDH isozyme patterns in the gastrocnemius muscles of endurance-trained rats. Mitochondrial and cytoplasmic glutamate-pyruvate transaminase activities (GPT) increased in the same muscle with training. This result led to the conclusion that muscle which has adapted to endurance exercise has an increased capacity to generate alanine from pyruvate and citric acid cycle intermediates from glutamate.

Elevated glycogen synthetase activities in both the I (independent or active) and D (dependent or inactive) forms were elicited in human vastus lateralis muscle following a five-month aerobic training program (95). After exercise to fatigue, both I and D values were lower in all groups with the sedentary group demonstrating the greatest decline in synthetase I values. Synthetase I and D values were markedly elevated after exhaustive exercise of 3-5 min duration. Jeffress, Peter, and Lamb (58) also demonstrated increased I and I + D forms of glycogen synthetase in skeletal muscle of guinea pigs after

training. Total synthetase activity was higher in the red area than in the white area of the vastus lateralis muscle. Sustained swimming for six hours raised glycogen synthetase I and D activities in hindleg muscle of rats (17). This effect was still evident after six hours of rest. Glycogen content and glycogen synthetase I activity (% of total) has been shown to vary inversely in skeletal muscle (20). This concept was investigated by Roch-Norlund (89) who found that glycogen depletion by exercise increased I activity in normal subjects and in diabetics to a lesser extent.

Various intracellular ATPase's are responsible for forming ATP from appropriate precursors as immediate sources of energy. Study of these enzymes when related to exercise has yielded a diversity of results. Low-intensity endurance swim training produced an increase in myosin ATPase activity of the soleus muscle, but not the extensor digitorum longus muscle, in young rats (94). Histochemical analysis showed an 11.8% increase in the number of muscle fibers with high actomyosin ATPase activity in the soleus muscles of the trained animals. These effects were not apparent if the exercise was initiated in adult animals. Bagby, Sembrowich, and Gollnick (3) determined the effects of endurance training (28.4 m/min, 60 min/day, five days/week) and sprint training (80.4 m/min, 30-sec alternated exercise and rest periods, 18 bouts) for 11 weeks on myosin ATPase activity and fiber composition of rat gastrocnemius muscle. Neither exercise program altered the levels of myosin ATPase. It was calculated that the small percentage (8%) of slow-twitch fibers in the gastrocnemius could account for only minor changes in myosin ATPase activity were they all to change from slow-twitch to fast-twitch fibers. These results agree

with those of previous studies (87) in which swimming 30 min/day for six weeks produced no changes in calcium-activated myosin ATPase. Neither myosin ATPase nor actomysin ATPase in vastus lateralis and vastus intermedius muscles of the lesser bushbaby were affected by endurance training (29). Contrasting results of increased myosin ATPase activity were obtained by Marikova (68) and Wilkerson and Evonuk (102) in response to moderate and exhaustive swimming programs respectively.

The response to exercise of creatine phosphokinase (CPK), an enzyme involved in the release of stored ATP and an immediate source of energy, has not been consistent. Kendrick-Jones and Perry (60) demonstrated increased levels of CPK activity in rat hind-leg muscle as a result of prolonged treadmill exercise. Their activity values were related to the wet weight of the total protein-nitrogen content after removal of all enzyme from the tissue. Dieter (23) also found increased CPK activity per mg protein in homogenates of red (soleus) and white (anterior portion of biceps femoris) muscles of guinea pigs. The animals were maintained on low levels of vitamin C and were exercised for 10 hours following six weeks of treadmill training. Rawlinson and Gould (87) did not find any change in CPK activity in skeletal muscle of rats swim-trained 30 min/day for six 5-day weeks.

Oscai and Holloszy (76) studied the effects of endurance training in rats on enzymes involved in the regeneration of ATP. Both mitochondrial ATPase activity and cytochrome C content, which served as a marker for the respiratory chain, increased two-fold in the gastrocnemius muscles of the exercised animals. These effects were viewed as a coordinated, quantitatively related increase in the

components of mitochondrial cristae as an adaptation to exercise. The levels of activity of mitochondrial creatine phosphokinase and adenylate kinase did not increase with training, and were reduced when expressed per mg of mitochondrial protein. These results provided evidence for a change in mitochondrial composition. Cytoplasmic creatine phosphokinase and adenylate kinase were unchanged. These findings lend credence to the hypothesis that endurance exercise can increase the ability of the muscle to regenerate ATP aerobically while not altering the ability of muscle to form ATP anerobically.

Mitochondrial Enzymes and Related Muscle Constituents

Moderate exercise regimens do not produce changes in mitochondrial enzyme levels. Hearn and Waino (48) subjected rats to 30 minutes of daily swimming for five to eight weeks and found no increase in succinic dehydrogenase (SDH) activity in gastrocnemius muscle. Under similar training procedures, Gould and Rawlinson (43) and Hearn (51) found no differences in malic dehydrogenase and cytochrome oxidase activities respectively. Succinate cytochrome c reductase activity was elevated in the latter study. Neither cold acclimation nor treadmill running caused any change in SDH activity in the gastrocnemius muscles of white mice (46). These results were expressed as activity per mg of protein.

Strenuous aerobic endurance training has been shown to markedly influence mitochondrial capacity. Holloszy (54) subjected rats to treadmill running for 12 weeks with the workload gradually being increased to 120 minutes at 31 m/min on a 8° incline with twelve 30-sec. intervals at 42 m/min interspersed every 10 minutes. The

total protein content of the mitochondrial fraction of the gastrocnemius muscle increased approximately 60% and the capacity of the mitochondrial fraction to oxidize pyruvate expressed as µl O_2 hr/g, doubled in the trained animals. SDH, DPNH dehydrogenase, DPNH cytochrome c reductase, succinate oxidase, and cytochrome oxidase, when expressed per gm of muscle, increased approximately two-fold in hindlimb muscles in response to training. The change in DPNH dehydrogenase activity was not significant when expressed as activity per mg of mitochondrial protein. Cytochrome c content also doubled which suggested that the rise in respiratory enzyme activity was due to an increase in enzyme protein. The increase in electron transport capacity was associated with a concommitant rise in capacity to produce ATP. SDH activity values after mild exercise were not changed. This data reinforces the hypothesis that mild or moderate exercise is not sufficient to increase respiratory enzyme activity in rats. By comparison, it has been shown that SDH activity (Vo_2 ml/gm/min) in human vastus lateralis muscle is increased by six weeks of activity such as occasional football, bicycling 15-30 minutes daily, or long distance walks on Sunday (98). Bicycle ergometer training for five months (40) and six weeks (31) induced increased levels of SDH activity in the vastus lateralis muscles of adults by 95% and in boys 11-13 years old by 30% respectively.

The adaptive increase in respiratory enzymes due to strenuous exercise led Holloszy et al. (56) to investigate exercise-related effects on the citric acid cycle and related enzymes. Cytochrome content and SDH activity, which served as respiratory chain markers, increased two-fold in the gastrocnemius muscles of trained rats. The

activities of citrate synthetase and DPN-specific isocitrate dehydrogenase also doubled. These results suggested a quantitative coordination of the respiratory chain enzymes with some citric acid cycle enzymes. The levels of activity of α-ketoglutarate dehydrogenase and mitochondrial malate dehydrogenase increased approximately 50% while glutamate dehydrogenase increased only 35% providing further evidence of an exercise adaptation of muscle mitochondria. These findings showed that all citric acid cycle enzymes do not undergo parallel changes in conjunction with respiratory constituents in response to exercise.

Barnard and Peter (11) also have investigated the effects of exercise on cytochromes. Concentrations of cytochromes a and c were not increased until the sixth week of training in the gastrocnemius muscles of guinea pigs. The cytochrome c changes were not has high as those reported by Holloszy (54); however, the experimental animals were different. The coefficients of correlation were 0.70 between cytochrome c concentration and performance of isolated muscle (electrical stimulation), 0.37 between cytochrome c concentration and running time to exhaustion, and 0.26 between running time to exhaustion and performance of isolated muscle. Cytochrome c concentration increased more in young guinea pigs that were trained (87%) than in adults that were trained (68%). The results indicated that improvement in performance capacity of the whole animal, as measured by running time to exhaustion, is largely independent of cytochrome concentration or performance capacity of the isolated muscle. The importance of relating biochemical adaptations in skeletal muscle to the performance of the muscle itself and not to the whole animal was emphasized.

The oxidative capacity of six trained adult non-human primates (Galago senegalensis) was shown to increase after six months of endurance running (29). Cytochrome a and c in the semimembranosus and vastus lateralis muscles and SDH in the vastus lateralis muscle were elevated as a result of training. The tibialis anterior muscle showed a decrease in the number of fast-twitch glycolytic fibers which paralleled an increase in fast-twitch oxidative-glycolytic fibers. The training program did not elicit any changes in soleus and plantaris fiber-type populations, but fast-twitch oxidative-glycolytic fibers were significantly larger in the plantaris and soleus muscles of the trained Galagos than in a group of untrained control animals. These observations demonstrated that muscles vary in response to treadmill exercise depending on their anatomical location and action as well as fiber-type composition.

Dohm et al. (25) studied the effects of training, exercise, and diet on citric acid cycle activity in skeletal muscle of the rat. High carbohydrate and high fat diets did not produce any alteration of citric acid cycle enzyme activity. However, training did increase the activities of all citric acid cycle enzymes and cytochrome oxidase in gastrocnemius muscle. The increased activities of citrate synthetase, isocitrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase with training were parallel to those reported by Holloszy and coworkers (56). This was to be expected since the training programs were similar. The magnitude of increase of cytochrome oxidase in Dohm's study was not as marked as previously reported (54). Relative mitochondrial yield and succinate oxidation by isolated mitochondria also increase in quadriceps muscle with training. The enzyme activities, relative

mitochondrial yield and mitochondrial succinate oxidation were the same in exhausted untrained rats and rested untrained animals. Exhausted trained rats had significantly lower values for isocitrate dehydrogenase, succinate dehydrogenase, cytochrome oxidase, relative mitochondrial yield and succinate oxidation by mitochondria than did rested trained rats. The results were the same whether activity was expressed per gm of tissue or per milligram of protein.

Baldwin et al. (4) studied which specific fiber type or types contribute to the increase in oxidative capacity following a program of strenuous endurance running. The soleus muscle and the arbitrarily divided superfical white and deep-red portion of the quadriceps were selected as being representative of intermediate, white and red muscle of the rat. The activities of cytochrome oxidase and citrate synthetase and the concentration of cytochrome c increased approximately two-fold in all three muscle types. This data was not in agreement with that from histochemical studies which suggested that the exercise-induced increase in oxidative capacity of muscles is due to the conversion of white into red fibers (9, 26, 35). Since the respiratory capacity response to exercise was approximately two-fold in all three muscles, the proportionate contribution from each fiber population remained unchanged.

Holloszy and Oscai (55) studied the influence of endurance exercise on α-glycerophosphate dehydrogenase activity. Although cytochrome oxidase levels and cytochrome c concentration doubled in the gastrocnemius muscles of trained rats, neither mitochondrial nor cytoplasmic α-glycerophosphate dehydrogenase activity was affected by the training program. The mitochondrial protein fraction of the

gastrocnemius muscle increased 60% in the exercised group. Thus, expressing cytochrome oxidase activity per milligram of mitochondrial protein eliminated any differences due to exercise. In addition, mitochondrial α-glycerophosphate dehydrogenase activity, when expressed per milligram of mitochondrial protein, was significantly lower in the trained group than in the sedentary group. These findings support the position that the adaptive response of skeletal muscle to exercise involves a change in mitochondrial cristae rather than simply an increase in size or number of mitochondria. The fact that glycerophosphate shuttle activity was not increased and may even have been decreased, led to the hypothesis of lower glycolytic activity and/or a shift in carbon source for the citric acid cycle from carbohydrate to fat. Edgerton et al. (29) also reported no training differences in α-glycerophosphate dehydrogenase activity in vastus lateralis and semimembranosus muscles of the lesser bushbaby.

Molé, Oscai, and Holloszy (72) have provided enzymatic evidence of a shift toward fatty acid oxidation as an adaptive response of skeletal muscle to endurance exercise. The levels of activity of carnitine palmityltransferase, palmityl CoA dehydrogenase, and mitochondrial ATP-dependent palmityl CoA synthetase doubled in gastrocnemius and quadriceps muscles of trained rats. Mitochondrial protein increased 60%. In contrast, lipoprotein lipase activity was unaffected by endurance training in the quadriceps muscle groups of rats (2). In rat muscle stimulated to contract repetitively in situ, the level of activity of carnitine palmityltransferase was greater in the slow-twitch soleus than in the fast-twitch tibialis anterior (5). Both muscles had similar rates of decline of work during prolonged

stimulation, although the soleus maintained greater relative and absolute work capacities. It was suggested that the primary energy source for the sustained work was the capacity of the red and intermediate fibers in the muscles to oxidize fatty acids. Fatty acid oxidation has been shown to increase significantly after a program of endurance training (71, 72, 105). Triglyceride concentrations have been shown to decrease more in red muscle than in white or intermediate muscle after exhaustive exercise (88).

Mitochondrial protein, as reported by Barnard, Edgerton, and Peter (9), did not increase in guinea pig gastrocnemius and plantaris muscles after nine weeks of training. Training for eighteen weeks did result in a significant increase in mitochondrial yield. Electron microscopic studies have provided conflicting results. Gollnick et al. (38) observed that the fine structure of human vastus lateralis muscle after exhaustive exercise was not different from that of rested muscle, although the electron micrographs of fatigued muscles displayed an almost complete absence of glycogen particles. In a subsequent study, Gollnick and King (39) found greater numbers of mitochondria in the gastrocnemius muscles of trained rats. These mitochondria also appeared to be larger and to have more densely packed cristae than did those of the sedentary animals. In animals that were sacrificed immediately after exhaustive running, the mitochondria showed swelling, cristae degeneration and tissue edema. Exhaustive swimming did not produce any of these effects. Terjung et al. (97) also ran trained rats to exhaustion. No evidence was found of leakage of soluble enzymes from the mitochondria of gastrocnemius muscle. Electron

microscopic inspection revealed the mitochondria to be well preserved in the exhausted animals with no evidence of disruption or impairment of function.

CHAPTER III

METHODS AND MATERIALS

In classical exercise physiology, gross measurements of total-body oxygen debt and oxygen uptake have been used to reflect metabolic responses to physical activity. Exhaustive or nearly exhaustive training programs lead to greater tolerance of oxygen debt which presumably reflects a greater capacity for generation of metabolic energy via anaerobic (e.g., glycolytic) metabolism. Such programs are described as being of the "anaerobic endurance" type and are characterized by maximal workloads maintained for relatively short periods of time. In contrast, training of an "aerobic endurance" nature is thought to be dependent chiefly upon oxidative muscle metabolism and tends to increase total-body oxygen uptake capacity. Moderate or light workloads maintained for relatively long periods of time are typical of aerobic endurance exercise programs. The current study was designed to investigate cellular level alterations in selected working muscles following various durations of aerobic and anaerobic endurance training.

Sampling Procedures

Eighty-four normal, male, albino rats of the Sprague-Dawley strain were used for this study. The animals were received in two

shipments of 72 and 12 animals respectively. All of the animals were randomly assigned to treatment groups and were given a period of ten days to adjust to laboratory conditions before the treatments were begun. Shipment-one animals were trained for eight and sixteen weeks duration, while those in shipment-two served as zero-week controls. Initiation of treatments for all animals began at 84 days of age.

Research Design

The study was organized as a 3x3x2 factorial design. The first factor, Treatment, consisted of three groups: (Short) an exercise group which was subjected to an anaerobic endurance training program, (Long) an exercise group which was subjected to an aerobic endurance training program, and (Control) a sedentary-control group. The second factor, Duration, consisted of three groups: (0-wk) a group that was trained for zero weeks, (8-wk) a group that was trained for eight weeks, and (16-wk) a group that was trained for sixteen weeks. The third factor, Sacrifice Time After Exercise, consisted of two groups: (Acute) those animals sacrificed fifteen minutes after their last exercise bout, and (Chronic) those animals sacrificed seventy-two hours after their last exercise bout.

For the trained animals, percent of expected meters run (PEM) served as the major performance criterion for selection of the final sample (101). In order to achieve a cell size of n=4, animals with the highest training performance over each duration period were selected. A representation of the experimental design with final cell frequencies can be seen in Table 1.

Table 1.--Experimental design with final cell frequencies.

Treatment	0-weeks Sacrifice Time		8-weeks Sacrifice Time		16-weeks Sacrifice Time	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
Control	4	4	4	4	4	4
Short	4	4	4	4	4	4
Long	4	4	4	4	4	4

Treatment Groups

The exercise treatments were performed on a Controlled Running Wheel (CRW) developed at the Human Energy Research Laboratory, Michigan State University. The CRW apparatus can be described as, ". . . a unique animal-powered wheel which is capable of inducing small laboratory animals to participate in highly specific programs of controlled, reproducible exercise" (101). The animals learn to run by avoidance-response operant conditioning. A low-intensity controlled shock current provides motivation for the animals to run.

Following body weight recording at the start of each treatment period, each animal was placed in an individual running wheel. A light above the running wheel signaled the start of each work interval. If the animal responded to the light by running at or faster than a preset speed, the light was extinguished and shock was avoided. The initial time during which the light was on is termed the "acceleration period." If the animal was not running at the predetermined speed by the end of the acceleration period, the light was turned off and a current was applied to the grid which serves as the running surface.

If the animal attained the prescribed speed while being shocked, the shock was immediately discontinued. If, when running, the animal slowed down below the prescribed speed, the light and shock sequence was repeated. A typical running program consisted of alternate work and rest periods. During the work periods, the wheel was free to turn; whereas, during the rest periods, the wheel was braked automatically to prevent spontaneous activity. A specified number of alternated work and rest periods (repetitions) constituted one bout of exercise. A single training period would include several such bouts separated by a relatively long time between bouts.

The three training groups in the study were as follows:

Short (SHT)

This group was subjected to a high-intensity, short-duration endurance program which was progressive in nature. That is, the intensity of the program was gradually increased until on the thirty-seventh day of training, and thereafter, the animals were expected to complete eight bouts of exercise with 2.5 minutes of inactivity between bouts. Each bout consisted of six repetitions of 10 seconds of work alternated with 40 seconds of rest. During the work intervals, these animals were required to run at the relatively fast speed of 99 m/min. A description of the complete training program is given in Appendix A.

Long (LON)

This group was subjected to a low-intensity, long-duration endurance program which was also progressive in nature. By the thirty-seventh day of training, and thereafter, the animals were expected to complete four bouts of exercise with 2.5 minutes of inactivity between

bouts. Each bout consisted of one repetition of 12.5 minutes of continuous work. This group ran at a speed of 36 m/min during the work intervals. A description of the complete training program is given in Appendix A. Table 2 illustrates the controlled running wheel regimens for both exercise groups which was imposed on the thirty-seventh and all ensuing days of training.

Control (CON)

These animals did not receive any type of forced exercise and were maintained in individual, sedentary cages during the adjustment and treatment periods.

Duration Groups

Three durations of the treatments were studied. Eight animals from each treatment group were killed at zero, eight, and sixteen weeks. These groups are referred to as 0-wk, 8-wk, and 16-wk animals respectively. The animals were 84, 140, and 196 days of age at sacrifice.

Acute and Chronic Groups

Four animals from each duration group were sacrificed at fifteen minutes (Acute) and at seventy-two hours (Chronic) after their last exercise bout. Acute and chronic measures were incorporated into the experimental design to distinguish immediate and transitory responses to exercise from gradual metabolic adaptations to training. In the current investigation only the data from the chronically exercised animals are reported.

Table 2.--Controlled running wheel exercise programs for SHT and LON groups at the thirty-seventh day of training.

Program	Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (m/min)	Total Time of Prog. (min: sec)	Total Exp. Meters TEM	Total Work Time (sec) TWT
SHT	2.0	00:10	40	6	8	2.5	1.0	99	52:10	792	480
LON	1.0	12:30	0	1	4	2.5	1.0	36	57:30	1800	3000

Experimental Procedures

All exercise treatments were conducted five days per week between 12:00 noon and 9:00 p.m. Body weights of the exercised animals were recorded before and after each exercise period. The intensity of the exercise was gradually increased up to eight weeks and then held constant through sixteen weeks.

The performance data for each trained animal were recorded daily. Total meters run (TMR) and total expected meters (TEM) were used to calculate percent to expected meters (PEM): PEM = 100 (TMR/TEM). PEM values were used to evaluate performance and to select animals for inclusion in the final sample. Total work time (TWT) and cumulative duration of shock (CDS) were used to calculate percent shock-free time (PSF): PSF = 100 - 100 (CDS/TWT). PSF values served as an additional criterion to evaluate performance in some cases.

Animal Care

All of the animals were housed in standard, individual, sedentary cages (24 cm x 18 cm x 18 cm) throughout the entire investigation. Since rats normally are more active at night than during daylight hours, the light sequence in the animal quarters was automatically set to reverse the rat's active period by having the lights off between 1:00 p.m. and 1:00 a.m.

A relatively constant environment was maintained for the animals by daily handling as well as by temperature, barometric pressure and humidity control. The cages were cleaned regularly. All animals had access to food (Wayne Laboratory Blox) and water *ad libitum*.

Sacrifice Procedures

Four sacrifices of twelve animals each were conducted either fifteen minutes or seventy-two hours after eight and sixteen weeks of treatments. An additional sacrifice of twelve 0-wk animals was conducted following the 10-day adjustment period. Originally, each 0-wk animal was assigned randomly to one of the three training groups as an Acute animal (see table 1). Since no training had occurred at this time, it was decided that these same 12 animals could be used as 0-wk Chronic subjects. Therefore, to provide a balanced statistical design, each 0-wk animal also was assigned randomly to a second but different training group as a Chronic animal.

On the sacrifice day, final body weights were recorded and each animal was anesthetized by an intraperitoneal injection (4 mg/100g body weight) of a 6.48% Halatal solution (sodium pentobarbital). After several tissues had been removed by other investigators, the left hindlimb was skinned and the superior posterior crural muscles were exposed by reflecting the overlying tissues. The left plantaris and soleus muscles were removed separately. The quadriceps group then was exposed and a portion from the white area of the vastus lateralis muscle was removed. Each muscle was weighed, held in forceps and quick frozen in 2-methylbutane (isopentane). The isopentane had been previously cooled to a viscous fluid (-140 to -185°C) by liquid nitrogen. The frozen muscles were placed in aluminum 35-mm film containers and stored at -20°C until the biochemical analyses could be run.

Biochemical Procedures

The glycogenolytic enzymes, phosphorylase and phosphoglucomutase, are responsible for the conversion of glycogen to glucose-6-phosphate. Furthermore, the activity of phosphoglucomutase has been shown to be proportional to the activities of the "phosphotriose glycerate phosphate group" of glycolytic enzymes in various types of muscles (83). Phosphoglucomutase was selected to represent glycogenolytic activity in this investigation.

Glucose-6-phosphate is the common intermediate produced by glycogenolysis and phosphorylation of blood glucose and may be metabolized further via either the glycolytic or the hexose monophosphate shunt (HMS) pathways. However, metabolism via the HMS is low in skeletal muscle (47). Thus, the relative levels of phosphoglucoisomerase, presumed to reflect glycolytic capacity, and phosphoglucomutase were used to estimate the relative contributions of blood glucose and glycogen as sources of glucose-6-phosphate for glycolytic metabolism.

It generally is accepted that mammalian lactate dehydrogenase exists as five tetrameric isozymes. One isozyme, LDH_5, is predominate in muscle and catalyzes the anaerobic conversion of pyruvate to lactate. It also appears that this enzyme is regulatory in function (36). In this study, total lactate dehydrogenase was chosen to represent anaerobic glycolysis.

Fumarase and citrate synthetase, enzymes of the tricarboxylic acid cycle, can serve as markers for the mitochondrial matrix (67). Wilson (104) has shown that fumarase and citrate synthetase exist in an approximately constant ratio in all types of rat brain mitochondria.

Unpublished observations by Wilson have disclosed rather invariant cytochrome/fumarase ratios in mitochondria from a variety of mammalian tissues. Furthermore, in pilot work for this investigation, relatively constant cytochrome oxidase/fumarase ratios were found in different types of rat skeletal muscle. Fumarase was used in this study to represent tricarboxylic acid cycle activity.

All biochemicals required for the assays were obtained from the Sigma Chemical Co. except for glucose 1,6-diphosphate which was obtained from the Boehringer-Mannheim Corp.

Preparation of Homogenates

Muscle homogenates were prepared in 10-15 volumes of 0.1 M potassium phosphate (pH 7.0). The muscles were chopped into a fine mince with scissors and homogenized in a Servall Omnimixer at maximum power for 15 seconds. This procedure was followed by 15 passes in a glass-Teflon homogenizer. The samples were maintained in an ice bath during the preparation and kept at -20°C until assayed for enzyme activity.

Enzyme Assays

Phosphoglucomutase, phosphoglucoisomerase, and lactate dehydrogenase assays were performed in a Turner Spectrophotometer. Fumarase activity was determined in a Coleman-Hitachi 124 Spectrophotometer. The sample compartment was thermostated at 25°C for all assays. Enzyme activities were determined by analyzing the muscles of each animal at the same time as those of the other animals in its duration group. All enzyme activities were recorded on a Sargent Recorder and have been expressed as Units/g fresh wt. of muscle. An

enzyme unit is the amount of enzyme catalyzing the formation of one μ mole of product per minute.

Phosphoglucomutase (PGM) catalyzes the following reaction of glycogenolysis:

Glucose 1-phosphate ⇄ Glucose 6-phosphate

Phosphoglucomutase (α-D-glucose-1,6 bisphosphate:α-D-glucose-1-phosphate phosphotransferase EC 2.7.5.1) was assayed by the method of Shonk and Boxer (90). The enzyme activity was determined by measuring the reduction of $NADP^+$ at 340 nm. The assay medium contained: 55 mM HEPES (pH 7.6), 5.55 mM EDTA, 15.55 mM $MgCl_2$, 0.34 mM $NADP^+$ 1.0 U glucose-6-phosphate dehydrogenase, 3.2 mM glucose-1-phosphate, and 5.0 μM glucose-1,6-diphosphate. Samples of homogenate (10-25 μl) plus water were added to the reaction medium for a total volume of 1 ml.

Phosphoglucoisomerase (PGI) catalyzes the following reaction of glycolysis:

α-D-Glucose 6-phosphate ⇄ α-D-fructose 6-phosphate

Phosphoglucoisomerase (D-glucose-6-phosphate ketolisomerase EC 5.3.1.9) was assayed by the method of Shonk and Boxer (90). The enzyme activity was determined by measuring the reduction of $NADP^+$ at 340 nm. The assay medium contained: 50 mM HEPES (pH 7.6), 5 mM EDTA, 10 mM $MgCl_2$, 0.34 mM $NADP^+$, 1.0 U glucose-6-phosphate dehydrogenase, and 3.0 mM fructose-6-phosphate. Samples of homogenate (1-5 μl) plus water were added to the reaction medium for a total volume of 1 ml.

Lactate dehydrogenase (LDH) catalyzes the following anaerobic reaction of glycolytically formed pyruvate:

$$\text{Pyruvate} + \text{NADH+H}^+ \rightleftarrows \text{Lactate} + \text{NAD}^+$$

Lactate dehydrogenase (L-Lactate:NAD^+oxidoreductase EC 1.1.1.27) was assayed by a method adapted from Shonk and Boxer (90). The enzyme activity was determined by measuring the oxidation of NADH at 340 nm. The assay medium contained: 0.03 M potassium phosphate (pH 7.4), 1.0 mM sodium pyruvate (pH 7), and 0.17 mM NADH. Samples of homogenate (1-5 µl) plus water were added to the reaction medium for a total volume of 1 ml.

Fumarase (FUM) catalyzes the following reaction of the tricarboxylic acid cycle:

$$\text{Fumarate} + H_2O \rightleftarrows \text{L-malate}$$

Fumarase (L-Malate hydro-lyase EC 4.2.1.2) was assayed by the method of Racker (86). The enzyme activity was determined by measuring the formation of fumarate from malate at 240 nm. The assay medium contained: 56 mM potassium phosphate (pH 7.4) and 56 mM L-malate. Samples of homogenate (10-50 µl) plus water were added to the reaction medium for a total volume of 1 ml. No further increase in fumarase activity was observed after treatment of the homogenates with 0.5% (v/v) Triton x-100. Thus, complete disruption of the mitochondria apparently had occurred during homogenization.

Enzyme Activity Ratios

The following three enzyme activity ratios, reflecting constant metabolic organization, were calculated for each of the three voluntary muscles:

Phosphoglucomutase / Phosphoglucoisomerase	representing	Glycogenolysis / Glycolysis
Phosphoglucoisomerase / Lactate Dehydrogenase	representing	Glycolysis / Lactate Fermentation
Phosphoglucomutase / Lactate Dehydrogenase	representing	Glycogenolysis / Lactate Fermentation

Three enzyme activity ratios, reflecting variable metabolic organization, also were calculated for each of the three voluntary muscles:

Lactate Dehydrogenase / Fumarase	representing	Lactate Fermentation / Tricarboxylic Acid Cycle
Phosphoglucoisomerase / Fumarase	representing	Glycolysis / Tricarboxylic Acid Cycle
Phosphoglucomutase / Fumarase	representing	Glycogenolysis / Tricarboxylic Acid Cycle

Statistical Procedures

The experimental design satisfied the assumptions for multivariate analysis of variance (MANOVA) procedures. The data were analyzed using the Finn's Multivarance routine as modified and adapted for the Control Data Computer 6500 by V. M. Scheifley and W. H. Schmidt. Treatment and duration of treatment constituted the category variables.

Separate overall MANOVA analyses were performed for the following seven sets of dependent variables: plantaris and soleus muscle weights, enzyme activities for each of the three muscles, and enzyme activity ratios for each of the three muscles. Significant interaction effects were followed by appropriate one-way multivariate analyses of variance across all treatments within each duration and across all durations within each treatment. Significant main-effect

contrasts, with a concomitant nonsignificant interaction effect, also justified use of the one-way fixed-effects MANOVA across levels of that independent variable within each level of the other category variable.

An F-ratio for the multivariate test of the equality of mean vectors was used to determine a significant multivariate effect. Univariate F's were used to obtain an estimate of the relative contribution of each variable involved in the analysis. Step-down F's will not be reported since no a priori knowledge of the importance of the dependent variables was available and thus no logical ordering the variables could be accomplished prior to the analyses. An experiment-wide alpha level of .05 was used for all multivariate procedures. Significant contrasts were reported at the .10 level in order to avoid overlooking potentially meaningful differences which may have been hidden by the sample size.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter is divided into five main sections. The training results from the controlled running wheel programs are given first. Data on muscle weights, enzyme activities, and enzyme activity ratios follow in that order. An overall discussion of the more important findings is presented last.

Training Results

For both training groups, a criterion of 75 was set for the percent of expected meters (PEM). This value represents the minimum acceptable performance level of the animals on the CRW programs. The training results for the SHT group are shown in Fig. 1. The data indicate that these animals maintained a PEM of approximately 80 during the most vigorous stages of training when running velocities of 90 and 99 m/min were required. A search of the literature has revealed no other programs for the training of small animals which have incorporated such high running velocities. Percent shock-free time (PSF) values were approximately 85 to 90 during the more strenuous portions of the training program. These results show that the animals generally responded to the conditioned light stimuli rather than to the

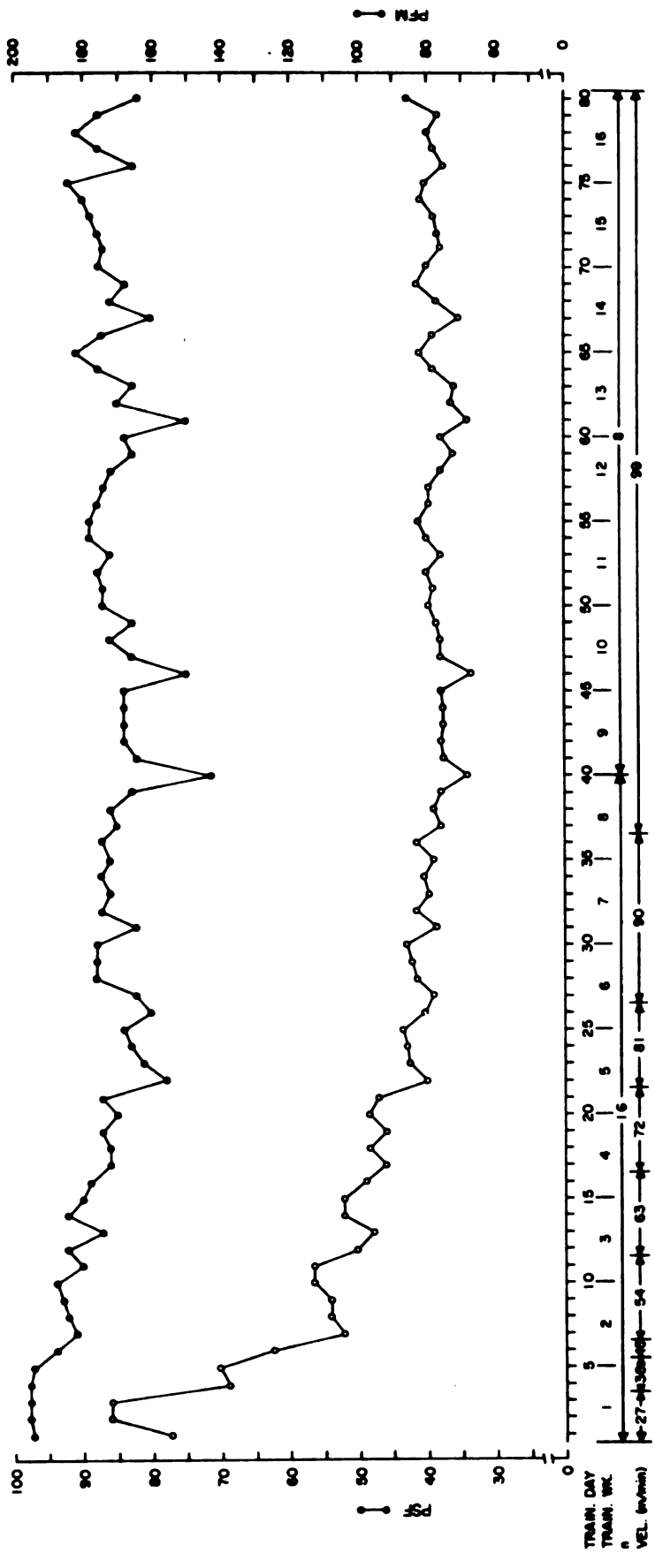

Fig. 1 Mean Daily Per Cent Shock Free Time (PSF) and Per Cent Expected Meters (PEM) for CRW SHORT

unconditioned shock stimuli. It is evident that the SHT running program is a satisfactory interval training regimen of high intensity endurance exercise.

Training data for the LON program are presented in Fig. 2. PEM values were approximately 85 for the last fourteen weeks of training. A running velocity of 36 m/min was required throughout this period. The PSF values were in the range of 85 to 95 during the last eleven weeks of training. These results again provide evidence that the animals responded well to the training regimen. The running speed used in the LON program is similar to that of 31 m/min employed by Holloszy (54). However, Holloszy ran animals continuously for periods of up to two hours. By contrast, the LON program was designed to simulate long-duration low-intensity interval training and is characterized by a maximum work time of fifty minutes split up into a small number of bouts of running with a recovery interval between bouts. The physiological changes induced by these two programs need not coincide.

Muscle Weight Results

Plantaris and soleus muscle weights are shown across all treatments and durations of treatments in Fig. 3. The vertical bars represent standard errors of the means. Overall MANOVA results for treatment and duration of treatment are presented in Table 3. Neither treatment nor the treatment x duration interaction produced a significant multivariate effect. An overall significant effect was found for duration of treatment. Subsequent one-way MANOVA results for duration within each of the treatment groups are shown in Table 4. A summary of these effects is is included in Table 5.

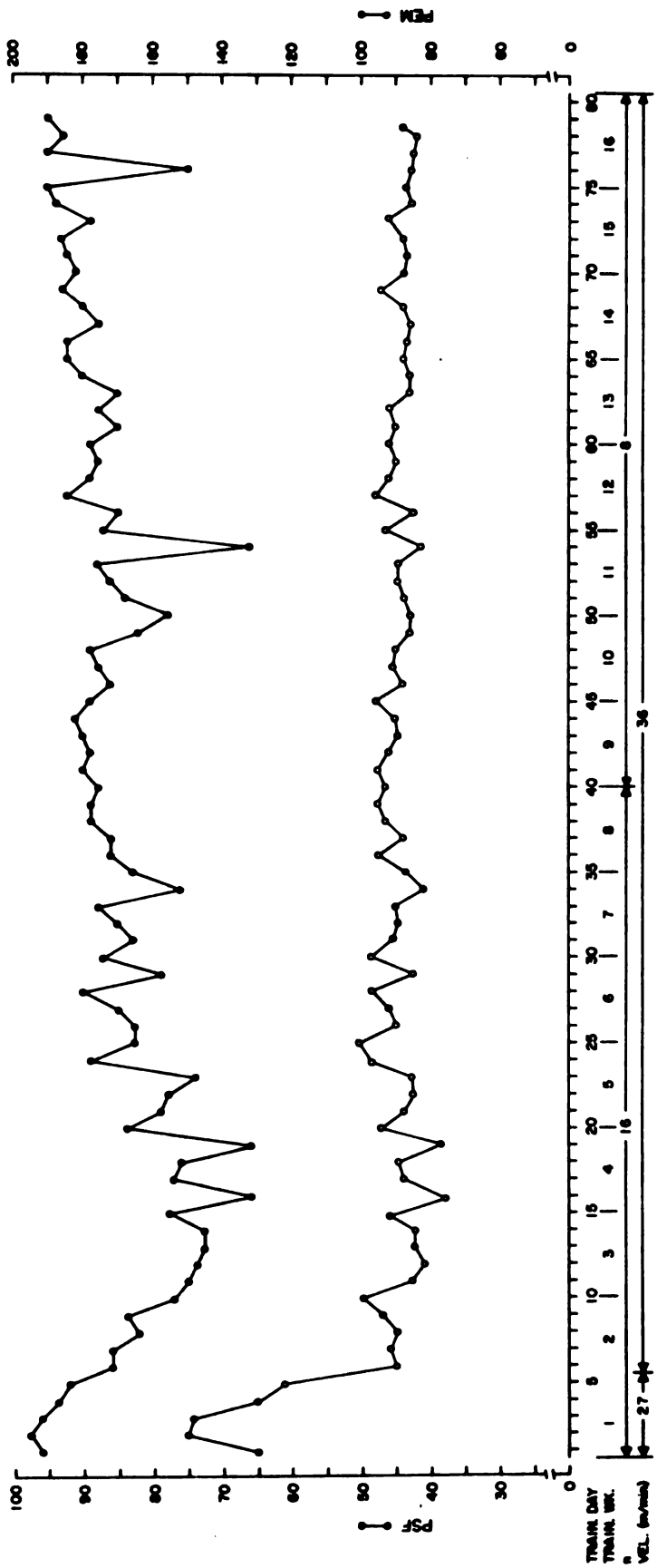

Fig. 2 Mean Daily Per Cent Shock Free Time (PSF) and Per Cent Expected Meters (PEM) for CRW LONG

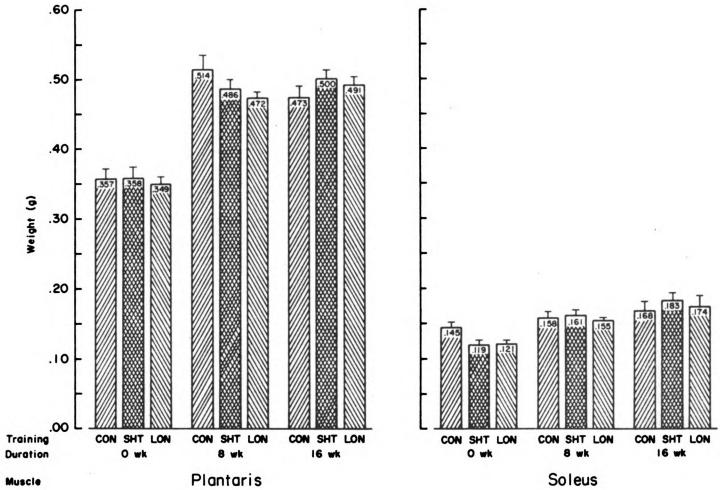

Fig. 3 Plantaris and soleus muscle weights across all treatments and durations of treatments.

Table 3.--Two-way multivariate analysis of variance for plantaris and soleus muscle weights demonstrating the effects of treatment and duration of treatment.

Effect	Helmert Contrast	Multivariate	
		F-Ratio	P Less Than
Treatment	TRAIN[a]-CON	.35	.710
	SHT-LON	.52	.602
Duration	0 vs 8,16	92.91	.001**
	8 vs 16	2.33	.117
Interaction	. .	1.27	.279

[a]TRAIN group contains pooled muscle weights of SHT and LON groups.

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 4.--One-way multivariate analysis of variance for muscle weights, demonstrating the effects of treatment duration.

Contrast (wks)	Treatment Group	Multivariate Results		Muscle	Univariate Results	
		F-Ratio	P Less Than		F	P Less Than
0 vs 8	CON	10.38	.006**	Plantaris	21.83	.001**
				Soleus	.02	.894
	SHT	4.66	.046**	Plantaris	10.32	.011**
				Soleus	.94	.357
	LON	10.83	.005**	Plantaris	16.72	.003**
				Soleus	.47	.509
0 vs 16	CON	13.20	.003**	Plantaris	22.33	.001**
				Soleus	2.45	.152
	SHT	28.13	.001**	Plantaris	47.80	.001**
				Soleus	25.78	.001**
	LON	45.25	.001**	Plantaris	91.67	.001**
				Soleus	16.47	.003**
8 vs 16	CON	1.30	.325	Plantaris	2.84	.127
				Soleus	.44	.523
	SHT	1.35	.313	Plantaris	.46	.517
				Soleus	2.88	.124
	LON	.98	.417	Plantaris	1.55	.244
				Soleus	2.05	.186

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 5.--Summary of duration effects for muscle weights with significant mean differences.

Duration Contrast (wks)	Treatment Groups	Multivariate Results		Mean Differences[a]	
		F-Ratio	P Less Than	Plantaris	Soleus
0 vs 8	CON	10.38	.006**	*+45*	
	SHT	4.66	.046**	*+35*	
	LON	10.83	.005**	*+35*	
0 vs 16	CON	13.20	.003**	*+30*	
	SHT	28.13	.001**	*+40*	*+55*
	LON	42.25	.001**	*+40*	*+45*
8 vs 16	CON	1.30	.325		
	SHT	1.35	.313		
	LON	.98	.417		

[a]Significant mean differences for muscle weights are tabulated as percent increase over time. Percentages are rounded to nearest multiple of five. Effects which are attributed to training appear in boldface. Maturation-related effects are shown in italics. Effects which may be due to unexplained or measurement artifacts are given in small type.

*Significant at $p < .10$.

**Significant at $p < .05$.

Significant multivariate effects between 0 wks and 8 wks were evident in all treatment groups. Both plantaris and soleus weights were greater after 8 wks. However, examination of the univariate F statistics revealed that the plantaris weight was the source of the multivariate effect in all three groups.

Statistically significant multivariate effects also were observed in all training groups between 0 wks and 16 wks. Greater weights were found in both muscles after 16 wks. The plantaris weight again was the source of the significance in the CON group; however, in the SHT and LON groups both the plantaris and soleus weights were responsible for the "significant effect." These results might be interpreted as evidence that the training programs produced increases in soleus weight between 0 and 16 wks beyond those normally expected to occur with maturation. However, the large random variability between groups at 0 wks (see Fig. 3) makes this conclusion unwarranted.

No multivariate effects were observed in the muscle weights between 8 wks and 16 wks.

Enzyme Activity Results

Mean enzyme activity responses in the three voluntary muscle fiber types, across all treatments and duration of treatments, appear graphically in Fig. 4. The vertical bars represent standard errors of the means. Overall MANOVA results for treatment and duration of treatment are shown in Table 6. The multivariate test for the treatment x duration interaction was significant in all muscle types. Separate treatment and duration effects also were significant for each

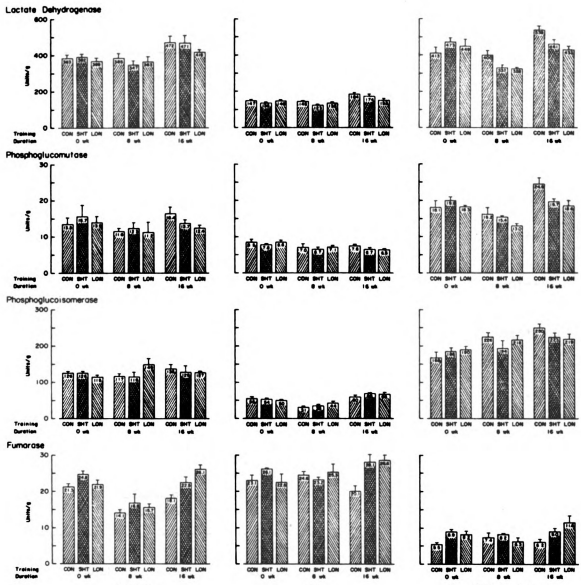

Fig. 4 Enzyme activities in the plantaris, soleus, and "white" vastus lateralis muscles across all treatments and durations of treatments.

Table 6.--Two-way multivariate analysis of variance for enzyme activities in three voluntary muscle types demonstrating the effects of treatment and duration of treatment.

Muscle	Effect	Helmert Contrast	Multivariate Results	
			F-Ratio	P Less Than
Plantaris	Treatment	TRAIN[a]-CON	5.84	.003**
		SHT-LON	1.13	.365
	Duration	0 vs 8,16	7.00	.004**
		8 vs 16	19.95	.001**
	Treatment x Duration	. .	2.11	.017**
Soleus	Treatment	TRAIN[a]-CON	7.22	.001**
		SHT-LON	.22	.922
	Duration	0 vs 8,16	6.24	.001**
		8 vs 16	27.37	.001**
	Treatment x Duration	. .	2.14	.015**
"White" Vastus Lateralis	Treatment	TRAIN[a]-CON	10.17	.001**
		SHT-LON	1.38	.269
	Duration	0 vs 8,16	7.34	.001**
		8 vs 16	14.42	.001**
	Treatment x Duration	. .	2.37	.007**

[a]TRAIN represents pooled enzyme activities of SHT and LON group.

*Significant at $p < .10$.

**Significant at $p < .05$.

muscle; however, these main effects are statistically dependent upon the treatment x duration interactions.

Treatment Effects

One-way MANOVA results for enzyme activities, comparing treatment effects at 8 wks and 16 wks, are presented in Table 7. A summary of all treatment contrasts, including identification of enzymes which account for the significant effects, is shown in Table 8.

SHT-CON

There were no significant SHT training effects, when the enzyme levels were tested simultaneously, other than marginal significance in the soleus muscle at 8 wks. Decreased lactate dehydrogenase activity in the SHT group was the source of that effect. Nonsignificant but specific metabolic activity patterns were apparent. The SHT group had levels of lactate dehydrogenase activity which were slightly lower than those of the CON group in all fiber types at 8 and 16 wks with the exception of the plantaris at 16 wks. The high-intensity trained animals, as compared to the controls, displayed decreased activity levels of phosphoglucomutase in the plantaris and "white" vastus lateralis muscles at 16 wks. Glycolytic activity, as measured by phosphoglucoisomerase levels, tended to be greater in the soleus of the SHT group but lower in the plantaris and vastus muscles of that group at 8 and 16 wks. Fumarase activities of the SHT group generally were higher than those of the CON group in all muscles for both durations of training.

Table 7.--One-way multivariate analysis of variance for enzyme activities in three voluntary muscle fiber types demonstrating the treatment effects.

Muscle	Contrast	Duration (wks)	Multivariate Results		Enzyme	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
Plantaris	SHT-CON	8	.88	.530	FUM	.92	.362
					LDH	.57	.470
					PGI	1.01	.341
					PGM	.29	.605
		16	.75	.595	FUM	.09	.767
					LDH	.43	.531
					PGI	.11	.753
					PGM	.18	.686
	LON-CON	8	1.42	.334	FUM	.48	.505
					LDH	.30	.597
					PGI	2.95	.120
					PGM	.03	.862
		16	9.20	.010**	FUM	22.93	.001**
					LDH	1.43	.262
					PGI	.45	.521
					PGM	5.02	.052*
	LON-SHT	8	1.40	.338	FUM	.23	.640
					LDH	.14	.713
					PGI	2.99	.118
					PGM	.31	.593
		16	2.05	.207	FUM	4.54	.062
					LDH	1.35	.274
					PGI	.00	.959
					PGM	.58	.468
	TRAIN[a]-CON	8	.89	.522	FUM	1.17	.307
					LDH	.73	.417
					PGI	.97	.351
					PGM	.01	.913
		16	7.89	.014**	FUM	18.49	.002**
					LDH	.51	.495
					PGI	.55	.477
					PGM	4.62	.060*

[a]TRAIN represents pooled enzyme activities of SHT and LON groups.

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 7.--Continued.

Muscle	Contrast	Duration (wks)	Multivariate Results		Enzyme	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
Soleus	SHT-CON	8	3.57	.081*	FUM	.68	.432
					LDH	9.16	.014**
					PGI	.22	.652
					PGM	.14	.715
		16	2.13	.195	FUM	3.21	.107
					LDH	.17	.690
					PGI	1.01	.342
					PGM	1.09	.323
	LON-CON	8	3.13	.103	FUM	.08	.789
					LDH	2.13	.178
					PGI	6.36	.033
					PGM	.01	.925
		16	13.92	.004**	FUM	12.01	.007**
					LDH	4.35	.066*
					PGI	2.07	.184
					PGM	7.47	.023**
	LON-SHT	8	3.22	.098*	FUM	.72	.417
					LDH	3.57	.091*
					PGI	2.77	.130
					PGM	.14	.717
		16	.68	.629	FUM	.03	.859
					LDH	1.96	.195
					PGI	.02	.884
					PGM	.21	.655
	TRAIN[a]-CON	8	3.49	.084*	FUM	.03	.867
					LDH	7.72	.022**
					PGI	3.80	.083*
					PGM	.01	.919
		16	15.36	.003**	FUM	15.18	.004**
					LDH	2.56	.144
					PGI	3.06	.115
					PGM	8.35	.018**

[a]TRAIN represents pooled enzyme activities of SHT and LON groups.

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 7.--Continued.

Muscle	Contrast	Duration (wks)	Multivariate Results		Enzyme	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
"White" Vastus Lateralis	SHT-CON	8	1.22	.392	FUM	1.28	.287
					LDH	2.16	.176
					PGI	2.06	.185
					PGM	.33	.578
		16	.72	.607	FUM	.01	.941
					LDH	.81	.392
					PGI	.56	.474
					PGM	1.33	.278
	LON-CON	8	1.54	.302	FUM	.73	.416
					LDH	8.35	.018
					PGI	.14	.721
					PGM	4.12	.073
		16	17.24	.002**	FUM	9.48	.013**
					LDH	10.81	.010**
					PGI	3.11	.112
					PGM	9.91	.012**
	LON-SHT	8	.95	.498	FUM	1.98	.193
					LDH	.03	.867
					PGI	1.12	.318
					PGM	2.30	.164
		16	1.92	.227	FUM	2.17	.175
					LDH	.75	.410
					PGI	.06	.820
					PGM	.33	.580
	TRAIN[a]-CON	8	1.82	.244	FUM	.03	.867
					LDH	10.48	.010
					PGI	1.07	.327
					PGM	2.16	.176
		16	16.05	.002**	FUM	7.31	.024**
					LDH	10.87	.009**
					PGI	3.61	.090*
					PGM	10.91	.009**

[a]TRAIN represents pooled enzyme activities of SHT and LON groups.

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 8.--Summary of treatment effects for enzyme activities with significant mean differences.

Treatment Contrast	Muscle	Duration (wks)	Multivariate Results		Mean Differences[a]			
			F-Ratio	P Less Than	Fumarase	Lactate Dehydrogenase	Phosphogluco-isomerase	Phosphogluco-mutase
SHT-CON	Plantaris	8	.88	.530				
		16	.75	.595				
	Soleus	8	3.57	.081*		-15		
		16	2.13	.195				
	"White" Vastus Lateralis	8	1.22	.392				
		16	.72	.607				
LON-CON	Plantaris	8	1.42	.334				
		16	9.20	.010**	45			-25
	Soleus	8	3.13	.103				
		16	13.92	.004**	40	-15		-15
	"White" Vastus Lateralis	8	1.54	.302				
		16	17.24	.002**	85	-20		-25
LON-SHT	Plantaris	8	1.40	.338				
		16	2.05	.207				
	Soleus	8	3.22	.098*		+10		
		16	.68	.629				
	"White" Vastus Lateralis	8	.95	.498				
		16	1.92	.227				
TRAIN[b]-CON	Plantaris	8	.89	.522				
		16	7.89	.014**	35			-20
	Soleus	8	3.49	.084*		-10	30	
		16	15.36	.003**	40			-15
	"White" Vastus Lateralis	8	1.82	.244				
		16	16.05	.002**	65	-15	-10	-20

[a]Significant mean differences in enzyme activity are tabulated as percent increase or decrease for the contrast shown. Percentages are rounded to nearest multiple of five. Effects which are attributed to training appear in boldface. Maturation-related effects are shown in italics. Effects which may be due to unexplained or measurement artifacts are given in small type.

[b]TRAIN represents pooled enzyme activities of SHT and LON groups.

*Significant at $p < .10$.
**Significant at $p < .05$.

LON-CON

Examination of the generalized multivariate F-ratios indicated significant LON training effects at 16 wks in all three muscles.

Plantaris.--The enzyme activities of fumarase and phosphoglucomutase contributed most to the significant multivariate effect in the plantaris. The activity level of fumarase was increased and that of phosphoglucomutase was decreased after 16 wks of LON training. These results must be viewed with some reservation. The moderate random variabilities between treatment groups at 0 wks would support the existence of real fumarase and phosphoglucomutase differences at 16 wks (see Fig. 4). On the other hand, the relatively large variabilities that were observed between durations within the CON group must be considered. The question is whether the patterns of the CON-group fumarase and phosphoglucomutase means across time were due to systematic or random factors.

Data on the levels of these two enzymes in similar animals sacrificed at the same time for a companion study suggest that small but systematic measurement errors depressed all of the plantaris fumarase and phosphoglucomutase values obtained in this investigation at 8 wks. If that were the case, it would appear that maturation tended to decrease fumarase and increase phosphoglucomutase activities over time. Under this hypothesis, there is no reason to question the existence of significant LON training effects in those two enzymes at 16 wks.

There is no evidence to support the alternative hypothesis that the variabilities in the CON group fumarase and phosphoglucomutase

means across time are reflective of purely random factors, but that possibility cannot be ignored. If only random factors were operating, the assumption of meaningful LON training effects on fumarase and phosphoglucomutase at 16 wks is not justified.

Soleus.--Fumarase, lactate dehydrogenase and phosphoglucomutase enzyme activities were responsible for the LON training effect at 16 wks in the soleus. Fumarase activity increased while the activities of lactate dehydrogenase and phosphoglucomutase decreased as a result of long-endurance running.

"White" Vastus Lateralis.--The LON training effect in the "white" vastus lateralis was produced by alterations in fumarase, lactate dehydrogenase, and phosphoglucomutase at 16 wks. Fumarase activity almost doubled in the LON group; however, the magnitude of this increase should be viewed in light of the rather large random variability observed between groups at 0 wks. The activities of lactate dehydrogenase and phosphoglucomutase were lower in this group than in the CON group.

LON-SHT

The only significant difference between enzyme levels in LON groups was observed in the soleus at 8 wks. The activity of lactate dehydrogenase was slightly lower in the SHT group than in the LON group at that time. The nonsignificant but consistent results across muscles in each of the four enzymes might suggest a metabolic divergence between the training groups at 16 wks.

TRAIN-CON

This contrast was established to determine if an overall interval training response, obtained by combining enzyme activities for the SHT and LON groups, would be reflected by some general metabolic adaptations. It would appear that this analysis was justified. That is, although the results of the LON-SHT contrast suggest differential training effects, the final enzyme levels in both training groups were consistently either above or below those of the CON group in all muscles.

Several important overall training effects were identified. Significant multivariate F-values were obtained for the soleus at 8 wks and for all three skeletal muscles at 16 wks.

Plantaris.--Fumarase and phosphoglucomutase activities were identified as reflecting a general training effect in the plantaris at 16 wks. The direction and magnitude of the enzyme changes were similar to those found in the LON-CON contrast, and the interpretation of these results is subject to the limitation mentioned in the discussion of that contrast.

Soleus.--At 8 wks, there was a significant overall training effect of decreased lactate dehydrogenase activity in the soleus. The apparent general increase in soleus phosphoglucoisomerase activity at that time must be regarded cautiously due to the large variability found in the CON group means across durations.

The activities of fumarase and phosphoglucomutase accounted for the multivariate overall training effect in the soleus at 16 wks.

Changes in these enzymes were similar to those observed in the LON-CON contrast.

"White" Vastus Lateralis.--The significant overall training effect in the "white" vastus muscle was mediated by alterations in all of the enzymes. Fumarase, lactate dehydrogenase, and phosphoglucomutase activities responded in the same direction as they did in the LON-CON contrast. In addition, the activity of phosphoglucoisomerase decreased with training.

Duration Effects

One-way multivariate analysis of variance results for enzyme activities, comparing duration effects within each of the treatment groups, are presented in Table 9. A summary of all duration contrasts, including identification of enzymes which account for significant effects, is shown in Table 10.

0 vs 8 wks

The one-way multivariate F-ratios indicate the existence of significant 0 vs 8-wk effects for all treatment groups in the plantaris, for the CON and SHT groups in the soleus, and for all groups in the "white" vastus lateralis.

Plantaris.--The activity of fumarase in the plantaris was significantly affected in all treatment groups with lower levels being observed at 8 wks than at 0 wks. As discussed earlier, these changes probably were due to a systematic maturation effect which operated across all groups. The activity of phosphoglucomutase was lower in the CON group after 8 wks. This observation also appears to be

Table 9.--One-way multivariate analysis of variance for enzyme activities in three voluntary muscle fiber types demonstrating the effects of treatment duration.

Muscle	Contrast (wks)	Treatment Group	Multivariate Results		Enzyme	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
Plantaris	0 vs 8	CON	5.89	.028	FUM	30.96	.001**
					LDH	1.63	.234
					PGI	2.69	.135
					PGM	3.41	.098*
		SHT	8.36	.013**	FUM	10.65	.010**
					LDH	5.27	.047**
					PGI	.42	.532
					PGM	.86	.378
		LON	11.72	.005**	FUM	36.64	.001**
					LDH	1.14	.314
					PGI	4.36	.066*
					PGM	1.05	.333
	0 vs 16	CON	2.84	.123	FUM	7.70	.022
					LDH	5.34	.046
					PGI	1.33	.279
					PGM	1.77	.216
		SHT	2.02	.211	FUM	.86	.377
					LDH	3.52	.094
					PGI	.01	.915
					PGM	.423	.532
		LON	2.09	.201	FUM	7.11	.026
					LDH	2.13	.178
					PGI	.56	.472
					PGM	.48	.508
	8 vs 16	CON	3.15	.102	FUM	11.78	.008
					LDH	5.10	.050
					PGI	3.99	.077
					PGM	5.13	.050
		SHT	8.44	.012**	FUM	5.58	.043**
					LDH	8.56	.017**
					PGI	.38	.552
					PGM	.23	.644
		LON	12.55	.005**	FUM	43.24	.001**
					LDH	2.73	.133
					PGI	2.05	.186
					PGM	.29	.602

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 9.--Continued.

Muscle	Contrast (wks)	Treatment Group	Multivariate Results		Enzyme	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
Soleus	0 vs 8	CON	7.60	.016**	FUM	2.51	.147
					LDH	4.23	.070*
					PGI	23.61	.001**
					PGM	1.36	.273
		SHT	18.35	.022**	FUM	6.13	.035**
					LDH	10.43	.010**
					PGI	71.71	.001**
					PGM	1.87	.205
		LON	3.10	.105	FUM	.01	.912
					LDH	2.47	.151
					PGI	12.85	.006
					PGM	.76	.405
	0 vs 16	CON	12.25	.005**	FUM	1.79	.214
					LDH	10.46	.010**
					PGI	.61	.453
					PGM	.75	.410
		SHT	10.82	.007**	FUM	1.26	.291
					LDH	10.80	.010**
					PGI	16.09	.003**
					PGM	7.51	.023**
		LON	5.24	.037**	FUM	4.46	.064*
					LDH	.47	.511
					PGI	13.50	.005**
					PGM	14.31	.004**
	8 vs 16	CON	8.55	.012**	FUM	4.17	.072*
					LDH	11.54	.008**
					PGI	21.16	.001**
					PGM	.34	.577
		SHT	27.16	.001**	FUM	7.32	.024**
					LDH	19.71	.002**
					PGI	87.22	.001**
					PGM	.03	.857
		LON	4.89	.043**	FUM	1.33	.278
					LDH	2.90	.123
					PGI	24.42	.001**
					PGM	1.29	.286

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 9.--Continued.

Muscle	Contrast (wks)	Treatment Group	Multivariate Results		Enzyme	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
"White" Vastus Lateralis	0 vs 8	CON	3.34	.091*	FUM	2.07	.184
					LDH	5.34	.046**
					PGI	1.29	.286
					PGM	5.77	.040**
		SHT	30.09	.001**	FUM	.79	.396
					LDH	28.45	.001**
					PGI	.28	.609
					PDM	21.32	.001**
		LON	8.37	.013**	FUM	4.94	.053*
					LDH	13.01	.006**
					PGI	.85	.380
					PGM	21.52	.001**
	0 vs 16	CON	16.59	.002**	FUM	.34	.575
					LDH	10.82	.009**
					PGI	23.84	.001**
					PGM	6.94	.027**
		SHT	1.44	.329	FUM	.01	.930
					LDH	.18	.679
					PGI	2.95	.120
					PGM	.04	.856
		LON	2.22	.183	FUM	3.07	.114
					LDH	.20	.665
					PGI	3.10	.112
					PGM	.04	.839
	8 vs 16	CON	6.68	.021**	FUM	.91	.365
					LDH	13.28	.005**
					PGI	1.98	.193
					PGM	11.53	.008**
		SHT	18.79	.002**	FUM	.67	.435
					LDH	19.41	.002**
					PGI	1.74	.220
					PGM	15.25	.004**
		LON	4.44	.052*	FUM	7.85	.021**
					LDH	8.41	.018**
					PGI	.01	.938
					PGM	16.99	.003**

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 10.--Summary of duration effects for enzyme activities with significant mean differences.

Duration Contrast (wks)	Muscle	Treatment Group	Multivariate Results		Mean Differences[a]			
			F-Ratio	P Less Than	Fumarase	Lactate Dehydrogenase	Phosphogluco-isomerase	Phosphogluco-mutase
0 vs 8	Plantaris	CON	5.89	.028**	-35			-15
		SHT	8.36	.013**	-30	-10		
		LON	11.72	.005**	-30		30	
	Soleus	CON	7.60	.016**			-45	
		SHT	18.35	.002**	-10	-10	-35	
		LON	3.10	.105				
	"White" Vastus Lateralis	CON	3.34	.091*		-5		-10
		SHT	30.09	.001**		-30		-25
		LON	8.37	.013**	-25	-25		-30
0 vs 16	Plantaris	CON	2.84	.123				
		SHT	2.02	.210				
		LON	2.09	.201				
	Soleus	CON	12.25	.005**		25		
		SHT	10.82	.007**		30	30	-15
		LON	5.24	.037**	25		35	-25
	"White" Vastus Lateralis	CON	16.59	.002**		30	50	35
		SHT	1.44	.329				
		LON	2.22	.183				
8 vs 16	Plantaris	CON	3.15	.102				
		SHT	8.44	.012**	35	35		
		LON	12.55	.005**	65			
	Soleus	CON	8.55	.012**	-20	25	95	
		SHT	27.16	.001**	20	40	90	
		LON	4.89	.043**			55	
	"White" Vastus Lateralis	CON	6.68	.021**		35		55
		SHT	18.79	.002**		40		30
		LON	4.44	.052*	85	30		45

[a]Significant mean differences for enzymes activities are tabulated as percent increase or decrease over time. Percentages are rounded to the nearest multiple of five. Effects which are attributed to training appear in boldface. Maturation-related effects are shown in italics. Effects which may be due to unexplained or measurement artifacts are given in small type.

*Significant effect $p < .10$.

**Significant effect $p < .05$.

maturation related. The level of activity of lactate dehydrogenase was lower in the SHT group, but phosphoglucoisomerase activity was somewhat higher in the LON group after 8 wks. These changes most likely are indicative of real training effects.

Soleus.--A lower level of phosphoglucoisomerase at 8 wks was primarily responsible for the significant 0 vs 8-wk duration effect in the soleus muscle of the CON group. The significant multivariate effect in the SHT group was the result of decreased activities of fumarase, lactate dehydrogenase, and phosphoglucoisomerase at 8 wks. The phosphoglucoisomerase results found in the CON and SHT groups are suspect. A maturation effect does not seem likely since the 0 to 8-wk trend seen in the CON group was reversed by 16 wks. No claim can be made for a training effect in the SHT group since the decrease in the SHT group was less than that in the CON group. At present, the depressed phosphoglucoisomerase values obtained at 8 wks should be considered to be unexplained artifacts. The fumarase level decrease in the SHT group also should be discounted due to the large random variability observed between groups at 0 wks.

"White" Vastus Lateralis.--The multivariate 0 vs 8-wks duration effects in the three treatment groups were primarily due to lower lactate dehydrogenase and phosphoglucomutase activities in the "white" vastus muscle after 8 wks. In addition, the fumarase level of the LON group was decreased at this time. The lactate dehydrogenase decreases in the two training groups were of considerably greater magnitude than that in the CON group and probably represent training effects. The change in phosphoglucomutase level in the LON group also

seems to be exercise related, but no claim can be made for that in the SHT group. The fumarase results should be ignored because of the large random variability observed between groups at 0 wks.

0 vs 16 wks

Significant 0 vs 16 wks duration effects were evident for all treatment groups in the soleus muscle and for the control group in the "white" vastus muscle.

Soleus.--Lactate dehydrogenase activity in the soleus was increased between 0 and 16 wks in the CON group. The overall SHT-group effect was attributable to increased lactate dehydrogenase and phosphoglucoisomerase levels and to a decreased phosphoglucomutase level after 16 wks. The enzymes alterations observed in the LON group after 16 wks were increased fumarase and phosphoglucoisomerase activities and decreased phosphoglucomutase activity. All of these alterations in lactate dehydrogenase and phosphoglucoisomerase activities probably were maturation dependent, whereas those seen in fumarase and phosphoglucomutase levels appear to have been produced by the training regimens.

"White" Vastus Lateralis.--The significant response in the "white" vastus lateralis of the CON group at 16 wks can be attributed to maturative increases in levels of activity of lactate dehydrogenase, phosphoglucoisomerase, and phosphoglucomutase. It should be noted that the two training programs apparently delayed these age-related changes.

8 vs 16 wks

Significant multivariate contrasts between 8 and 16 wks were observed for the SHT and LON groups in the plantaris and for all three treatment groups in the other two muscles.

Plantaris.--The univariate F results indicate that fumarase activity in the plantaris was higher in both the SHT and LON groups after 16 wks than after 8 wks. However, the apparent change in the SHT group could have been an artifact resulting from systematic factors operating at 8 wks. The increase in the LON group may have been inflated by these same factors but probably reflects a true training effect. The SHT group had a training-related increase in lactate dehydrogenase activity during this period of time.

Soleus.--The enzyme activities that were altered in the soleus muscles of the CON and SHT groups between 8 and 16 wks of training were those of fumarase, lactate dehydrogenase, and phosphoglucoisomerase. Activity levels of lactate dehydrogenase increased greatly and levels of phosphoglucoisomerase almost doubled in both groups between 8 and 16 wks. In contrast, fumarase activity decreased in the CON group and increased in the SHT group. For the LON group, an increase in phosphoglucoisomerase activity was the only source of the multivariate effect. Of all of these changes, the only one which definitely can be attributed to training is the increased fumarase activity in the SHT group.

"White" Vastus Lateralis.--Increases in lactate dehydrogenase and phosphoglucomutase activities were accountable for the multivariate

effect in the "white" vastus lateralis muscles of all three treatment groups between 8 and 16 wks. All of these changes appear to be due to maturation. Fumarase levels were nearly doubled as a result of the LON program during the second half of the training period.

Enzyme Activity Ratio Results

The term "variable metabolic organization," when used to describe an enzyme activity ratio, refers to a ratio that has essentially different ranges of values in various types of skeletal muscles. "Constant metabolic organization" indicates an enzyme activity ratio that has overlapping ranges of values that do not differentiate skeletal-muscle types.

Mean enzyme activity ratios representing variable metabolic organization and constant metabolic organization in the three voluntary muscle-fiber types, across all treatments and durations of treatments, are found in Figs. 5 and 6. The vertical bars represent standard errors of the means. Overall MANOVA results for treatment and duration of treatment appear in Table 11. The multivariate test for the treatment x duration interaction was significant in the soleus muscle with borderline significance in the plantaris muscle. Separate treatment and duration effects also were significant for each muscle.

Treatment Effects

One-way MANOVA results for enzyme activity ratios, comparing treatment effects at 8 and 16 wks, are presented in Table 12. A summary of all treatment contrasts, including identification of enzyme ratios which account for the significant effects, is shown in Table 13.

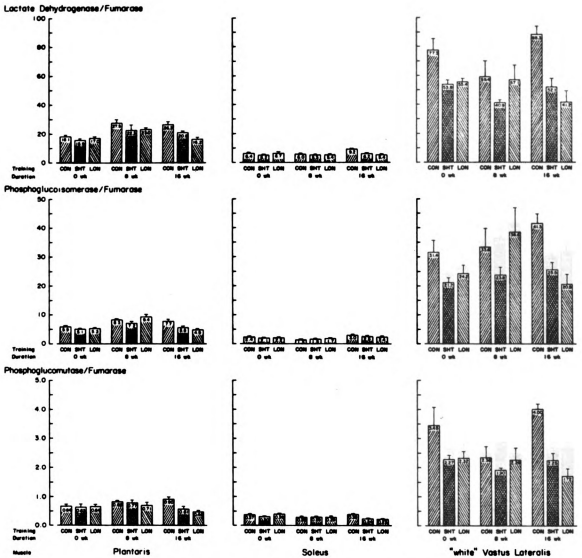

Fig. 5 Enzyme activity ratios representing variable metabolic organization in the plantaris, soleus, and "white" vastus lateralis muscles across all treatments and durations of treatments.

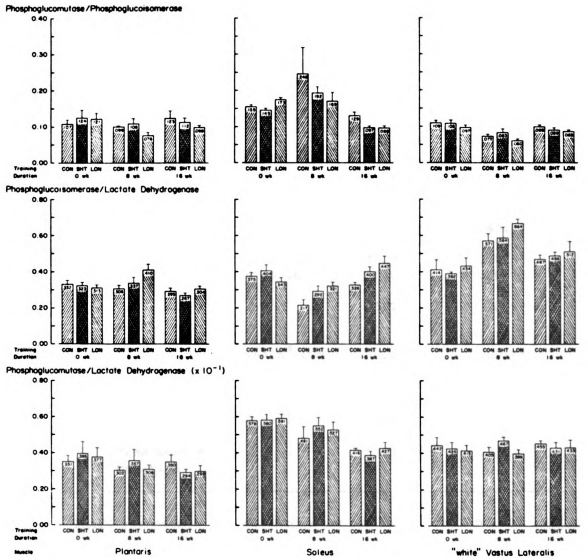

Fig. 6 Enzyme activity ratios representating constant metabolic organization in the plantaris, soleus, and "white" vastus lateralis muscles across all treatments and durations of treatments.

Table 11.--Two-way multivariate analysis of variance for enzyme activity ratios in three voluntary muscle types demonstrating the effects of treatment and duration of treatment.

Muscle	Effect	Helmert Contrast	Multivariate Results	
			F-Ratio	P Less Than
Plantaris	Treatment	TRAIN[a]-CON	2.71	.040**
		SHT-LON	1.12	.381
	Duration	0 vs 8,16	4.26	.005**
		8 vs 16	5.72	.001**
	Treatment x Duration	. .	1.50	.095*
Soleus	Treatment	TRAIN[a]-CON	5.97	.001**
		SHT-LON	1.03	.431
	Duration	0 vs 8,16	10.39	.001**
		8 vs 16	16.08	.001**
	Treatment x Duration	. .	3.29	.001**
"White" Vastus Lateralis	Treatment	TRAIN[a]-CON	7.23	.001**
		SHT-LON	1.06	.417
	Duration	0 vs 8,16	4.65	.004**
		8 vs 16	6.40	.001**
	Treatment x Duration	. .	1.17	.299

[a]TRAIN represents pooled enzyme activities of SHT and LON group.

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 12.--One-way multivariate analysis of variance for enzyme activity ratios in three voluntary muscle fiber types demonstrating the treatment effects.

Muscle	Contrast	Duration (wks)	Multivariate Results		Enzyme Ratios	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
Plantaris	SHT-CON	8	1.14	.469	LDH/FUM	.79	.397
					PGI/FUM	4.69	.059
					PGM/FUM	.00	.982
					PGI/LDH	.40	.544
					PGM/LDH	2.06	.185
					PGM/PGI	2.94	.120
		16	.78	.626	LDH/FUM	.05	.834
					PGI/FUM	.96	.352
					PGM/FUM	1.42	.263
					PGI/LDH	2.66	.137
					PGM/LDH	.69	.426
					PGM/PGI	.01	.929
	LON-CON	8	.72	.660	LDH/FUM	1.37	.271
					PGI/FUM	1.52	.249
					PGM/FUM	1.02	.339
					PGI/LDH	7.25	.025
					PGM/LDH	.01	.934
					PGM/PGI	2.69	.136
		16	5.51	.060*	LDH/FUM	21.50	.001**
					PGI/FUM	11.22	.009**
					PGM/FUM	32.98	.001**
					PGI/LDH	.24	.634
					PGM/LDH	1.47	.256
					PGM/PGI	1.58	.240
	LON-SHT	8	1.02	.518	LDH/FUM	.03	.859
					PGI/FUM	6.21	.034
					PGM/FUM	.28	.612
					PGI/LDH	3.58	.091
					PGM/LDH	1.44	.261
					PGM/PGI	5.32	.047
		16	.92	.562	LDH/FUM	4.54	.062
					PGI/FUM	.68	.431
					PGM/FUM	3.38	.099
					PGI/LDH	2.76	.131
					PGM/LDH	.01	.911
					PGM/PGI	.50	.497
	TRAIN[a]-CON	8	.84	.594	LDH/FUM	2.13	.179
					PGI/FUM	.00	.988
					PGM/FUM	.75	.410
					PGI/LDH	4.06	.075
					PGM/LDH	.62	.450
					PGM/PGI	.32	.588
		16	5.38	.063*	LDH/FUM	17.00	.003**
					PGI/FUM	11.51	.008**
					PGM/FUM	31.03	.001**
					PGI/LDH	.15	.707
					PGM/LDH	2.15	.176
					PGM/PGI	1.09	.324

[a]TRAIN represents pooled enzyme activity ratios of SHT and LON group.

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 12.--Continued.

Muscle	Contrast	Duration (wks)	Multivariate Results		Enzyme Ratios	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
Soleus	SHT-CON	8	.64	.705	LDH/FUM	1.10	.321
					PGI/FUM	.00	.971
					PGM/FUM	.02	.890
					PGI/LDH	.54	.480
					PGM/LDH	.58	.466
					PGM/PGI	.08	.787
		16	2.91	.160	LDH/FUM	5.92	.098
					PGI/FUM	.82	.388
					PGM/FUM	10.69	.010
					PGI/LDH	.15	.708
					PGM/LDH	1.19	.304
					PGM/PGI	3.21	.107
	LON-CON	8	5.30	.064*	LDH/FUM	1.73	.221
					PGI/FUM	4.69	.059*
					PGM/FUM	.02	.897
					PGI/LDH	8.45	.017**
					PGM/LDH	.41	.538
					PGM/PGI	1.48	.255
		16	10.85	.019**	LDH/FUM	49.74	.001**
					PGI/FUM	4.89	.054*
					PGM/FUM	44.64	.001**
					PGI/LDH	9.38	.014**
					PGM/LDH	.09	.774
					PGM/PGI	11.00	.009**
	LON-SHT	8	1.97	.267	LDH/FUM	.06	.807
					PGI/FUM	1.10	.321
					PGM/FUM	.00	.956
					PGI/LDH	.67	.436
					PGM/LDH	.12	.742
					PGM/PGI	.14	.722
		16	.54	.760	LDH/FUM	2.01	.190
					PGI/FUM	.10	.756
					PGM/FUM	.26	.623
					PGI/LDH	1.43	.262
					PGM/LDH	1.19	.303
					PGM/PGI	.01	.918
	TRAIN[a]-CON	8	3.97	.102	LDH/FUM	2.77	.131
					PGI/FUM	3.59	.091
					PGM/FUM	.03	.856
					PGI/LDH	8.33	.018
					PGM/LDH	.87	.374
					PGM/PGI	1.42	.263
		16	13.22	.013**	LDH/FUM	53.64	.001**
					PGI/FUM	5.61	.042**
					PGM/FUM	55.07	.001**
					PGI/LDH	8.10	.019**
					PGM/LDH	.08	.779
					PGM/PGI	14.21	.005**

[a]TRAIN represents pooled enzyme activity ratios of SHT and LON group.

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 12.--Continued.

Muscle	Contrast	Duration (wks)	Multivariate Results		Enzyme Ratios	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
"White" Vastus Lateralis	SHT-CON	8	.62	.717	LDH/FUM	2.69	.136
					PGI/FUM	2.67	.137
					PGM/FUM	1.04	.335
					PGI/LDH	.38	.551
					PGM/LDH	4.79	.057
					PGM/PGI	3.59	.091
		16	.57	.742	LDH/FUM	2.90	.123
					PGI/FUM	1.88	.204
					PGM/FUM	4.74	.058
					PGI/LDH	.01	.941
					PGM/LDH	.12	.737
					PGM/PGI	.13	.727
	LON-CON	8	1.75	.307	LDH/FUM	.03	.857
					PGI/FUM	.33	.580
					PGM/FUM	.04	.840
					PGI/LDH	2.26	.167
					PGM/LDH	.05	.829
					PGM/PGI	1.22	.299
		16	8.91	.027**	LDH/FUM	29.86	.001**
					PGI/FUM	20.76	.001**
					PGM/FUM	49.28	.001**
					PGI/LDH	.81	.393
					PGM/LDH	.26	.622
					PGM/PGI	3.09	.113
	LON-SHT	8	.90	.570	LDH/FUM	1.76	.217
					PGI/FUM	2.89	.123
					PGM/FUM	.61	.456
					PGI/LDH	1.66	.230
					PGM/LDH	4.02	.076
					PGM/PGI	4.80	.056
		16	1.32	.410	LDH/FUM	1.58	.240
					PGI/FUM	1.19	.304
					PGM/FUM	2.64	.139
					PGI/LDH	.27	.619
					PGM/LDH	.00	.966
					PGM/PGI	.32	.584
	TRAIN[a]-CON	8	1.47	.371	LDH/FUM	.96	.353
					PGI/FUM	.10	.757
					PGM/FUM	.48	.507
					PGI/LDH	.98	.348
					PGM/LDH	.81	.391
					PGM/PGI	.00	.994
		16	8.15	.031**	LDH/FUM	31.17	.001**
					PGI/FUM	21.45	.001**
					PGM/FUM	51.38	.001**
					PGI/LDH	.55	.478
					PGM/LDH	.38	.553
					PGM/PGI	2.90	.123

[a]TRAIN represents pooled enzyme activity ratios of SHT and LON group.

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 13.--Summary of treatment effects for enzyme activity ratios with significant mean differences.

Treatment Contrast	Muscle	Duration (wks)	Multivariate Results		Mean Differences[a]					
			F-Ratio	P Less Than	LDH/FUM	PGI/FUM	PGM/FUM	PGI/LDH	PGM/LDH	PGM/PGI
SHT-CON	Plantaris	8	1.14	.469						
		16	.78	.626						
	Soleus	8	.64	.705						
		16	2.91	.160						
	"White" Vastus	8	.62	.717						
	Lateralis	16	.57	.742						
LON-CON	Plantaris	8	.72	.660						
		16	5.51	.060*	-40	-35	-45			
	Soleus	8	5.30	.064*		*30*		*50*		
		16	10.85	.019**	-40	-20	-40	35		-25
	"White" Vastus	8	1.75	.307						
	Lateralis	16	8.91	.027**	-55	-50	-55			
LON-SHT	Plantaris	8	1.02	.518						
		16	.92	.562						
	Soleus	8	1.97	.267						
		16	.54	.760						
	"White" Vastus	8	.90	.570						
	Lateralis	16	1.32	.410						
TRAIN[b]-CON	Plantaris	8	.84	.594						
		16	5.38	.063*	-30	-35	-40			
	Soleus	8	3.97	.102						
		16	13.22	.013**	-35	-20	-40	30		-25
	"White" Vastus	8	1.47	.371						
		16	8.15	.031**	-45	-45	-50			

[a]Significant mean differences in enzyme activity ratios are tabulated as percent increase or decrease for the contrast shown. Percentages are rounded to nearest multiple of five. Effects which are attributed to training appear in boldface. Maturation-related effects are shown in italics. Effects which may be due to unexplained or measurement artifacts are given in small type.

[b]TRAIN represents pooled enzyme activity ratios of SHT and LON groups.

*Significant at $p < .10$.

**Significant at $p < .05$.

SHT-CON

No statistically significant SHT training effects were observed when all of the enzyme ratios were tested simultaneously. However, subjectively a distinct metabolic pattern appeared to exist in the three variable activity ratios. These ratios generally were lower in the SHT group than in the CON group, at both 8 wks and 16 wks in all three fiber types. No identifable pattern was found for the ratios of constant metabolic organization.

LON-CON

Inspection of the one-way multivariate F-ratios revealed significant multivariate training effects in the soleus and "white" vastus lateralis muscles at 16 wks. Marginal effects were observed in the plantaris at 16 wks and the soleus at 8 wks.

Plantaris.--In the plantaris muscle, enzyme activity ratios of variable metabolic organization were uniquely affected by the LON program. These ratios were approximately one-third to one-half lower in the LON group than in the CON group at 16 wks.

Soleus.--The LON program induced pronounced changes in the phosphoglucoisomerase/fumarase and phosphoglucoisomerase/lactate dehydrogenase ratios in the soleus after 8 wks of training. Both ratios appeared to be elevated in the LON group; however, these results should be overlooked due to the pattern of variability found in the CON group means across durations.

At 16 wks, the multivariate effect was at least partially attributable to training-related decreases in all three variable-ratio

enzymes. Activity ratios of constant metabolic organization behaved differently. Phosphoglucoisomerase/lactate dehydrogenase was increased by the LON program. Phosphoglucomutase/phosphoglucoisomerase was lower in the LON group than in the CON group, but this finding is suspect due to the variability observed in the 0-wk data as well as in the CON group values across durations.

"White" Vastus Lateralis.--The significant multivariate LON training effect at 16 wks in the "white" vastus lateralis was caused by decreases of approximately one-half in the variable enzyme ratios. These changes must be viewed with caution due to the random variability seen at 0 wks. However, that variability is the direct result of the single relatively low fumarase value obtained in the CON group at 0 wks (see Fig. 4). No such anomalities were observable in the 16-wk enzyme data, and thus it appears that these decreases in the variable enzyme ratios at 16 wks probably represent actual training effects.

LON-SHT

Neither 8 wks nor 16 wks of training produced any significant differences in enzyme activity ratios between the LON and SHT groups. Certain ratios appeared subjectively to be specifically altered by each training regimen in all three muscles. The phosphoglucomutase/phosphoglucoisomerase ratios were higher in the SHT group than in the LON group at both 8 and 16 wks. In contrast, the phosphoglucoisomerase/lactate dehydrogenase ratios were higher in the LON group at both of these durations.

TRAIN-CON

An overall interval training response was significant in the soleus and "white" vastus lateralis muscles at 16 wks. Marginal significance was observed in the plantaris at this duration.

Plantaris.--The overall training effect at 16 wks in the plantaris was modulated entirely by changes in enzyme activity ratios which are representative of variable metabolic organization. The adaptation was similar to that found in the LON-CON contrast for the plantaris.

Soleus.--Significant training changes at 16 wks in the soleus appear to be regulated by both activity ratios of variable and constant metabolic organization. The pattern of response was similar to that observed in the LON-CON contrast for this muscle, and the interpretation of these results is subject to the limitation mentioned in the discussion of that contrast.

"White" Vastus Lateralis.--The multivariate overall training effect at 16 wks in the "white" vastus lateralis was predominantly related to changes in the variable enzyme ratios. As in the plantaris and soleus, the shifts in these ratios were similar to those produced by the LON program alone.

Duration Effects

One-way MANOVA results for enzyme activity ratios, illustrating duration effects within each of the treatment groups, are presented in Table 14. A summary of all duration contrasts, including

Table 14.--One-way multivariate analysis of variance for enzyme activity ratios in three voluntary muscle fiber types demonstrating the effects of treatment duration.

Muscle	Contrast (wks)	Treatment Group	Multivariate Results		Enzyme Ratios	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
Plantaris	0 vs 8	CON	1.27	.426	LDH/FUM	5.20	.049
					PGI/FUM	4.81	.056
					PGM/FUM	.29	.602
					PGI/LDH	.08	.785
					PGM/LDH	1.62	.236
					PGM/PGI	.88	.375
		SHT	2.74	.175	LDH/FUM	1.72	.222
					PGI/FUM	10.02	.012
					PGM/FUM	1.63	.233
					PGI/LDH	2.30	.164
					PGM/LDH	..04	.838
					PGM/PGI	.22	.651
		LON	7.57	.035**	LDH/FUM	22.48	.001**
					PGI/FUM	48.20	.001**
					PGM/FUM	3.22	.106
					PGI/LDH	14.51	.004**
					PGM/LDH	.51	.494
					PGM/PGI	6.21	.034**
	0 vs 16	CON	2.43	.205	LDH/FUM	9.27	.014
					PGI/FUM	5.22	.048
					PGM/FUM	6.51	.031
					PGI/LDH	2.02	.189
					PGM/LDH	.00	.993
					PGM/PGI	.62	.452
		SHT	.57	.741	LDH/FUM	2.05	.186
					PGI/FUM	.62	.453
					PGM/FUM	.01	.913
					PGI/LDH	3.00	.117
					PGM/LDH	2.43	.154
					PGM/PGI	.26	.624
		LON	1.33	.410	LDH/FUM	.27	.616
					PGI/FUM	.26	.620
					PGM/FUM	2.75	.132
					PGI/LDH	.07	.792
					PGM/LDH	2.26	.167
					PGM/PGI	2.33	.161
	8 vs 16	CON	.26	.930	LDH/FUM	.20	.662
					PGI/FUM	.57	.469
					PGM/FUM	.65	.441
					PGI/LDH	.22	.651
					PGM/LDH	1.20	.302
					PGM/PGI	1.44	.260
		SHT	2.57	.190	LDH/FUM	.18	.684
					PGI/FUM	5.52	.043
					PGM/FUM	1.35	.275
					PGI/LDH	4.75	.057
					PGM/LDH	.92	.362
					PGM/PGI	.02	.882
		LON	5.09	.069*	LDH/FUM	19.06	.002**
					PGI/FUM	39.30	.001**
					PGM/FUM	5.68	.041**
					PGI/LDH	11.80	.008**
					PGM/LDH	.02	.896
					PGM/PGI	1.95	.197

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 14.--Continued.

Muscle	Contrast (wks)	Treatment Group	Multivariate Results		Enzyme Ratios	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
Soleus	0 vs 8	CON	6.85	.042**	LDH/FUM	19.51	.002**
					PGI/FUM	36.18	.001**
					PGM/FUM	8.64	.017**
					PGI/LDH	26.57	.001**
					PGM/LDH	.12	.741
					PGM/PGI	4.10	.074*
		SHT	5.27	.065*	LDH/FUM	1.75	.219
					PGI/FUM	17.86	.002**
					PGM/FUM	1.39	.269
					PGI/LDH	8.85	.016**
					PGM/LDH	2.45	.152
					PGM/PGI	30.27	.001**
		LON	1.12	.479	LDH/FUM	.77	.403
					PGI/FUM	9.51	.013
					PGM/FUM	.29	.606
					PGI/LDH	4.66	.059
					PGM/LDH	.18	.684
					PGM/PGI	3.43	.097
	0 vs 16	CON	10.11	0.21**	LDH/FUM	34.84	.001**
					PGI/FUM	5.13	.050**
					PGM/FUM	.12	.739
					PGI/LDH	2.61	.141
					PGM/LDH	8.47	.017**
					PGM/PGI	.19	.675
		SHT	4.31	.090*	LDH/FUM	8.15	.019**
					PGI/FUM	3.68	.087*
					PGM/FUM	8.57	.017**
					PGI/LDH	.01	.918
					PGM/LDH	14.69	.004**
					PGM/PGI	10.16	.011**
		LON	6.66	.044**	LDH/FUM	2.22	.171
					PGI/FUM	.44	.526
					PGM/FUM	14.46	.004**
					PGI/LDH	6.75	.029**
					PGM/LDH	10.47	.010**
					PGM/PGI	13.19	.006**
	8 vs 16	CON	7.75	.034**	LDH/FUM	45.92	.001**
					PGI/FUM	40.22	.001**
					PGM/FUM	7.39	.024**
					PGI/LDH	13.37	.005**
					PGM/LDH	1.35	.276
					PGM/PGI	3.89	.080*
		SHT	6.30	0.48**	LDH/FUM	6.62	.030**
					PGI/FUM	21.34	.001**
					PGM/FUM	6.17	.035**
					PGI/LDH	6.37	.033**
					PGM/LDH	10.71	.010**
					PGM/PGI	40.43	.001**
		LON	3.05	.150	LDH/FUM	.00	.988
					PGI/FUM	9.00	.015
					PGM/FUM	2.07	.184
					PGI/LDH	10.05	.011
					PGM/LDH	3.93	.079
					PGM/PGI	11.69	.008

*Significant at $p < .10$.

**Significant at $p < .05$.

Table 14.--Continued.

Muscle	Contrast (wks)	Treatment Group	Multivariate Results		Enzyme Ratios	Univariate Results	
			F-Ratio	P Less Than		F	P Less Than
"White" Vastus Lateralis	0 vs 8	CON	2.23	.228	LDH/FUM	5.11	.050
					PGI/FUM	.22	.654
					PGM/FUM	6.49	.031
					PGI/LDH	7.12	.026
					PGM/LDH	1.20	.302
					PGM/PGI	16.28	.003
		SHT	3.63	.092*	LDH/FUM	10.95	.009**
					PGI/FUM	.03	.877
					PGM/FUM	2.84	.127
					PGI/LDH	10.19	.011**
					PGM/LDH	.00	1.000
					PGM/PGI	2.08	.183
		LON	3.97	.102	LDH/FUM	.85	.381
					PGI/FUM	5.95	.037
					PGM/FUM	.37	.557
					PGI/LDH	14.35	.004
					PGM/LDH	.48	.508
					PGM/PGI	28.17	.001
	0 vs 16	CON	1.42	.384	LDH/FUM	.86	.378
					PGI/FUM	2.08	.183
					PGM/FUM	.77	.402
					PGI/LDH	.89	.370
					PGM/LDH	.08	.788
					PGM/PGI	1.37	.272
		SHT	3.01	.126	LDH/FUM	.17	.691
					PGI/FUM	1.73	.221
					PGM/FUM	.00	.948
					PGI/LDH	3.19	.108
					PGM/LDH	.00	1.000
					PGM/PGI	2.28	.165
		LON	1.29	.421	LDH/FUM	1.65	.231
					PGI/FUM	.22	.650
					PGM/FUM	1.68	.227
					PGI/LDH	1.81	.211
					PGM/LDH	.16	.701
					PGM/PGI	3.29	.103
	8 vs 16	CON	2.71	.177	LDH/FUM	5.86	.039
					PGI/FUM	1.26	.290
					PGM/FUM	7.00	.027
					PGI/LDH	3.38	.099
					PGM/LDH	1.18	.305
					PGM/PGI	8.46	.017
		SHT	1.77	.273	LDH/FUM	7.08	.026
					PGI/FUM	.27	.615
					PGM/FUM	2.03	.188
					PGI/LDH	3.50	.094
					PGM/LDH	.00	1.000
					PGM/PGI	.24	.633
		LON	2.91	.160	LDH/FUM	2.08	.183
					PGI/FUM	5.51	.044
					PGM/FUM	1.38	.270
					PGI/LDH	6.80	.028
					PGM/LDH	.63	.446
					PGM/PGI	13.61	.005

*Significant at $p < .10$.

**Significant at $p < .05$.

identification of enzyme ratios which account for the significant effects, is shown in Table 15.

0 vs 8 wks

A check of the multivariate F-ratios for 0 vs 8 wks revealed significant multivariate effects for the LON group in the plantaris and for the CON group in the soleus. Marginal significance was found for the SHT group in both the soleus and "white" vastus lateralis muscles.

Plantaris.--Increases in the enzyme activity ratios of lactate dehydrogenase/fumarase, phosphoglucoisomerase/fumarase, and phosphoglucoisomerase/lactate dehydrogenase as well as a decrease in phosphoglucomutase/phosphoglucoisomerase all contributed to the overall significant effect of the LON training regimen at 8 wks. Only the phosphoglucoisomerase/lactate dehydrogenase increase appears to be definitely attributable to training.

Soleus.--All of the variable enzyme ratios were decreased in the soleus muscles of the CON group at 8 wks. In the constant-ratio organizational scheme, phosphoglucoisomerase/lactate dehydrogenase was decreased and phosphoglucomutase/phosphoglucoisomerase was increased in the soleus muscles of the CON group at 8 wks. The changes in the soleus associated with the SHT group at 8 wks were decreased phosphoglucoisomerase/fumarase, decreased phosphoglucoisomerase/lactate dehydrogenase, and increased phosphoglucomutase/phosphoglucoisomerase. No importance can be attached to these alterations in the soleus. The lactate dehydrogenase/fumarase and phosphoglucomutase/ fumarase results must be discounted due to the large random variabilities observed

Table 15.--Summary of duration effects for enzyme activity ratios with significant mean differences.

Duration Contrast (wks)	Muscle	Treatment Group	Multivariate Results		Mean Differences[a]					
			F-Ratio	P Less Than	LDH/FUM	PGI/FUM	PGM/FUM	PGI/LDH	PGM/LDH	PGM/PGI
0 vs 8	Plantaris	CON	1.27	.426						
		SHT	2.74	.175						
		LON	7.57	.035**	35	75		30		-35
	Soleus	CON	6.85	.042**	-5	-45	-25	-40		60
		SHT	5.27	.065*		-30		-30		40
		LON	1.12	.479						
	"White"	CON	2.23	.228						
	Vastus	SHT	3.63	.092*	-25			50		
	Lateralis	LON	3.97	.102						
0 vs 16	Plantaris	CON	2.43	.205						
		SHT	.57	.741						
		LON	1.33	.410						
	Soleus	CON	10.11	.021**	35	25			-30	
		SHT	4.31	.090*	20	20	-20		-35	-35
		LON	6.66	.044**			-45	30	-30	-45
	"White"	CON	1.42	.384						
	Vastus	SHT	3.01	.126						
	Lateralis	LON	1.29	.421						
8 vs 16	Plantaris	CON	.26	.930						
		SHT	2.57	.190						
		LON	5.09	.069*	-30	-50	-35	-25		
	Soleus	CON	7.75	.034**	55	130	35	50		-50
		SHT	6.30	.048**	15	65	-20	35	-30	-50
		LON	3.05	.150						
	"White"	CON	2.71	.177						
	Vastus	SHT	1.77	.273						
	Lateralis	LON	2.91	.160						

[a]Significant mean differences for enzyme activity ratios are tabulated as percent increase or decrease over time. Percentages are rounded to nearest multiple of five. Effects which are attributed to training appear in boldface. Maturation-related effects are shown in italics. Effects which may be due to unexplained or measurement artifacts are given in small type.

*Significant at $p < .10$.
**Significant at $p < .05$.

between groups at 0 wks. Possible measurement artifacts in phosphoglucoisomerase at 8 wks cast doubt on the changes in the other three ratios.

"White" Vastus Lateralis.--At 8 wks, the SHT group had a decreased lactate dehydrogenase/fumarase ratio and an increased phosphoglucoisomerase/lactate dehydrogenase ratio. Only the latter effect can be ascribed to training. It should be noted that both this increase in the SHT group and the parallel nonsignificant increase in the LON group were caused by significant training-related decreases in lactate dehydrogenase levels between 0 and 8 wks. The seemingly similar change in the CON group, on the other hand, was the result of a maturative increase in phosphoglucoisomerase activity.

0 vs 16 wks

Significant multivariate F-values for 0 vs 16 wks were obtained only in the soleus muscle. All three of the treatment groups exhibited overall duration effects.

The variable enzyme ratios of lactate dehydrogenase/fumarase and phosphoglucoisomerase/fumarase were increased in the soleus muscles of the CON group at 16 wks. However, the constant enzyme ratios of phosphoglucomutase/lactate dehydrogenase was decreased at this time. These changes appear to be related to age.

All of the variable enzyme ratios in the soleus were altered significantly in the SHT group between 0 and 16 wks. The lactate dehydrogenase/fumarase and phosphoglucoisomerase/fumarase ratios were increased while the phosphoglucomutase/fumarase ratio was decreased. The constant proportion ratios of phosphoglucomutase/lactate dehydrogenase and phosphoglucomutase/phosphoglucoisomerase both were decreased

approximately one-third at the termination of the study. Only the phosphoglucomutase/phosphoglucoisomerase decrease appears to be a training effect.

The LON group had a decreased phosphoglucomutase/fumarase ratio in the soleus at 16 wks. The three constant-organization enzyme ratios also were affected in the LON group. The phosphoglucoisomerase/lactate dehydrogenase ratio was increased, whereas the changes of the other two ratios were similar to those found in the SHT group. Again, the only change which can be attributed to training is that which occurred in the phosphoglucomutase/phosphoglucoisomerase ratio.

8 vs 16 wks

The multivariate F-values for 8 vs 16 wks confirmed the enzyme-ratio changes in the plantaris muscles of the LON group to be marginally significant. The CON and SHT groups experienced significant enzyme-ratio changes in the soleus muscle during the latter half of the treatment period.

Plantaris.--The three enzyme ratios of variable metabolic organization and the phosphoglucoisomerase/lactate dehydrogenase ratio all decreased between 8 and 16 wks as a result of the LON training program.

Soleus.--Between 8 and 16 wks, all three of the enzyme ratios of variable metabolic organization significantly increased in the soleus muscles of both the CON and SHT groups except for the phosphoglucomutase/fumarase ratio in the SHT group which decreased. Alterations in enzyme ratios representing constant metabolic

organization varied considerably. The CON and SHT groups each displayed an increase in the phosphoglucoisomerase/lactate dehydrogenase ratio and a decrease in the phosphoglucomutase/phosphoglucoisomerase ratio between 8 and 16 wks of training. In addition, the phosphoglucomutase/lactate dehydrogenase ratio decreased in the SHT group. None of these 8 to 16-wk ratio changes can be ascribed definitely to training.

Discussion

The results of this investigation suggest that over time the LON and SHT training programs tend to produce similar metabolic adaptations in skeletal muscle. This observation is supported by the large number of significant overall training (TRAIN) effects found at 16 wks and by the fact that there were no significant LON vs SHT contrasts at that time (Tables 8 and 13). It also can be seen that at 16 wks the LON and SHT groups exhibited parallel responses across all three muscles in all four enzymes (Fig. 4). The patterns of change found in the enzyme ratios are nearly as conclusive (Figs. 5 and 6). The mean value of the CON group separated those of the LON and SHT groups at 16 wks in only two instances: phosphoglucoisomerase/lactate dehydrogenase in the plantaris and phosphoglucomutase/lactate dehydrogenase in the soleus (Fig. 6).

Unpublished data from a companion study tend to both confirm and refute the theory that there may be no lasting differential effects of the LON and SHT training regimens. The mean numbers of split fibers in the soleus muscle, although quite different after 4 wks of training, were similar in LON and SHT groups of animals at 8 and 12 wks. The functional significance of fiber splitting as an adaptation to

training has yet to be clarified, but there is little doubt that at least transient metabolic adjustments must take place during the splitting phenomenon. In the same study, phosphorylase activity was demonstrated histochemically in ten preselected areas of the plantar flexor muscles. After 4 wks of training, histochemical photometry revealed differences between the LON and SHT groups in two areas of the gastrocnemius and in one area each of the plantaris and soleus muscles. By 8 wks, the number of muscle areas in which there were LON vs SHT differences had decreased to two. A difference in phosphorylase activity was discernible in only one mixed-fiber area of the gastrocnemius at 12 wks. It is clear that these histochemical phosphorylase observations are in general agreement with the biochemical phosphoglucomutase results obtained in the present investigation. In contrast to the evidence which suggests that no differences or only temporary differences exist between the effects of the LON and SHT programs, other histochemical data from the companion study support the concept of at least some continuing differential training responses. For example, Sudan Black B was used to demonstrate total lipid content. Higher values were obtained for the LON group than for the SHT group in all ten muscle areas after 8 wks of training. Several of these differences in lipid content were magnified at 12 wks. No 16-wk histochemical results are available as yet.

Regardless of whether or not there are permanent differences in the effects of the LON and SHT programs, the parameters selected for study in this investigation did not reflect a differentiation of metabolic activities between the two groups. Therefore, this

discussion will be based chiefly upon the results obtained in the CON and combined TRAIN groups.

Since no training had occurred at 0 wks, the means of the three treatment groups all reflect control values, and the patterns of the group means at that time are representative of random group variability. Furthermore, for those parameters in which a smooth maturation effect is not apparent in the CON group across time (Figs. 4, 5, and 6), alterations in the CON group means at 8 and 16 wks may be suspected of being due to either random factors or measurement artifacts. These multiple control values have been used subjectively to discriminate between those statistically significant effects that may be due to maturation or artifacts and those that actually appear to be caused by training.

The pattern of responses of the TRAIN group in all muscles at 16 wks suggests a decrease in glycogenolytic capacity as measured by phosphoglucomutase levels. In addition, increases in fumarase levels and decreases in enzyme ratios of variable metabolic organization in all muscles, as well as a decrease in the lactate dehydrogenase level of the "white" vastus lateralis muscle, imply increased dependence upon oxidative metabolism by the TRAIN group.

Burleigh and Schimke (16) have observed an inverse relationship between phosphorylase and hexokinase activities in mammalian skeletal muscles. A similar relationship has been found between phosphoglucomutase and mitochondrial hexokinase levels in rat liver, diaphragm, kidney, heart, brain, testis, spleen, lung, small intestine, and pancreas (Wilson, unpublished report). Thus, in this investigation the decreased phosphoglucomutase levels in the TRAIN group might reflect

an increased dependence upon blood glucose as a source of carbon for glycolysis. This hypothesis is plausible since the decreases in phosphoglucomutase levels in the plantaris and soleus muscles of the TRAIN group were not accompanied by decreases in the activity of phosphoglucoisomerase. However, it should be noted that the decreases in the variable enzyme ratios, particularly those in phosphoglucoisomerase/fumarase, indicate that other sources of fuel besides carbohydrates may have been at least partially responsible for the increases in tricarboxylic acid cycle activity as measured by fumarase. There is strong evidence from other studies that endurance training can elicit a shift toward increased fatty acid oxidation with subsequent participation of triglycerides as well as carbohydrates for energy supply (71, 72, 105, 88). Unfortunately, the current data do not provide a means of detecting alterations in beta oxidation.

Various investigators have concluded that glucose is more important than glycogen as an energy source for contraction in red muscle and, conversely, that glycogen is more important in white muscle (16, 18). The decreased phosphoglucomutase results that occurred with endurance training seem to indicate that the relative importance of glucose utilization can be increased in all muscle fiber types. It should be noted that the percentage decrease in phosphoglucomutase activity was approximately the same in the three muscles of the TRAIN group.

The decreased dependence on glycogenolysis in all muscle types which was found in the present study is in partial disagreement with the results of Baldwin et al. (6) who reported that endurance exercise did not induce phosphorylase changes in white quadriceps, whole

quadriceps, and soleus muscles of the rat. Only the red quadriceps, a portion of muscle with a fiber population similar to that of the plantaris, displayed decreased levels of phosphorylase in the exercised group. Baldwin and his coworkers also reported that decreased lactate dehydrogenase levels occurred in red, white, and whole quadriceps muscles as a result of training. In the current study, lower lactate dehydrogenase activity was found only in the "white" vastus lateralis of the TRAIN group at 16 wks.

There have been several other studies of the effects of exercise on glycogenolytic capacity. All of these have yielded results which conflict with the data obtained in this investigation. No changes in phosphorylase levels were found when the exercise regimen used was of a endurance nature (29, 43, 57). Isometric strength exercise even produced increases in phosphorylase activity (33, 34).

Holloszy et al. (56) observed a 50 percent increase in mitochondrial malate dehydrogenase activity in rat gastrocnemius muscle after twelve weeks of long-duration endurance running. As described by Ariano, Armstrong, and Edgerton (1), the medial and lateral aspects of the gastrocnemius muscle have fiber-type characteristics that resemble those of the plantaris. The reaction which fumarase catalyzes in the tricarboxylic acid cycle is just prior to that which is catalyzed by malate dehydrogenase. Thus, the 45 percent increase in fumarase activity that occurred in the plantaris muscle of the LON group at 16 wks is almost an identical manifestation of training as that previously reported.

Endurance exercise has been shown to cause a two-fold increase in the activity of citrate synthetase, an enzyme of the tricarboxylic

acid cycle, and similar increases in cytochrome oxidase activity and cytochrome c content in three types of rat muscle (4). These results were interpreted as being incompatible with the histochemically based hypothesis that an exercise-related increase in the oxidative capacity of muscle may be due to a mutation of white to red fibers (9, 26, 35). In this investigation, increases in fumarase levels of different magnitudes were observed in the three muscles of the TRAIN group at 16 wks. Furthermore, the fact that statistically significant decreases in lactate dehydrogenase activity were found only in the soleus at 8 wks and in the "white" vastus lateralis at 16 wks suggests that unequal alterations in anaerobic metabolism occurred in the three muscles. It would appear from these results that the possibility of fiber-type conversions in skeletal muscle cannot be discounted as yet.

For a given carbohydrate molecule, anaerobic metabolism releases much less chemical energy than does aerobic metabolism. The observed increases at 16 wks in fumarase activity appear to indicate that the efficiency of energy production was increased in all three muscles of the TRAIN group. The increased availability of energy acquired through the tricarboxylic acid cycle might help to explain the results of other studies in which there were no exercise-related changes in creatine phosphokinase, adenylate kinase, and ATPase--enzymes involved in the regeneration of ATP (50, 76, 87).

Staudte and Pette (91) suggest that deviations in the constant proportion enzymes are possible. Kubišta, Kubištová, and Pette (63) administered thyroid hormone to male rats and found markedly altered constant enzyme ratios in the soleus muscle but not in the rectus femoris muscle. The results of the present study reinforce the

general concept of constant proportion enzyme groups (Fig. 6). However, some of the constant enzyme ratios do appear to be altered either by maturation or training (Tables 13 and 15).

The 0 vs 16-wks contrast for enzyme activities in the "white" vastus lateralis muscle suggests that there were significant maturational increases in lactate dehydrogenase, phosphoglucoisomerase, and phosphoglucomutase levels (Table 10). From inspection of the 16-wk TRAIN vs CON contrast for this muscle, it seems that the overall training effect was to delay the age-related increases in these enzymes.

Studies by Bass et al. (13) and Pette (85) have shown that the variable enzyme activity ratios can be used to classify distinct types of muscle. Examination of these ratios in Fig. 5 illustrates their usefulness. The highest ratios are seen in the "white" vastus lateralis, a fast-twitch glycolytic muscle; and the lowest ratios are seen in the soleus, a slow-twitch oxidative muscle. Intermediate values are seen in the plantaris, a fast-twitch oxidative-glycolytic muscle. The variable enzyme ratio data support the validity of the histochemical fiber typing methods (1, 82).

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this study was to determine the effects of aerobic and anaerobic programs of endurance running on selected enzymatic activities in the left plantaris, the left soleus, and the "white" area of the left vastus lateralis muscles of the adult male albino rat. The training regimens were the Controlled Running Wheel programs previously reported from this laboratory (101). Biochemical determinations of the muscle homogenates were made on the levels of activity of phosphoglucomutase, an enzyme of glycogenolysis; phosphoglucoisomerase, an enzyme of glycolysis; lactate dehydrogenase, an enzyme concerned with anaerobic metabolism of glycolytically-formed pyruvate; and fumarase, a mitochondrial enzyme of the tricarboxylic acid cycle. Enzyme activity ratios also were investigated in each of the fiber types.

Animals were randomly assigned to CON, SHT, and LON treatment groups. Initiation of treatments for all animals began at 84 days of age. Performance criteria were used as the basis of animal selection for subsequent investigation. Animals were sacrificed 72 hours after their last training period. Biochemical analyses were performed

before the initiation of treatments and after eight and sixteen weeks of exercise. The final sample size consisted of 36 animals with four animals in each treatment-duration subgroup.

The results suggested that, over time, the two training programs tend to produce similar metabolic adaptations in skeletal muscle. This observation is supported by the large number of significant overall training (TRAIN) effects found at 16 wks and by the fact that there were no significant LON vs SHT contrasts at that time.

An inverse relationship of decreased glycogenolytic capacity, as measured by phosphoglucomutase, and increased tricarboxylic acid cycle activity, as measured by fumarase, was evident in all muscles of the TRAIN group at 16 wks. In addition, decreases in enzyme ratios of variable metabolic organization in all muscles, along with a decrease in the lactate dehydrogenase activity of the white "vastus" lateralis muscle, imply an increased dependence upon oxidative metabolism by the TRAIN group.

Some of the constant proportion enzyme ratios were altered either by maturation or training. The variable enzyme ratio data support the validity of histochemical fiber typing methods.

Conclusions

The results of this study have led to the following conclusions:

1. The parameters selected for study did not reflect a differentiation of metabolic activities between the SHT and LON groups.
2. The pattern of responses of the TRAIN group in all muscles at 16 wks suggests a decrease in glycogenolytic capacity as

measured by phosphoglucomutase levels. In addition, increases in fumarase levels and decreases in enzyme ratios of variable metabolic organization in all muscles, as well as a decrease in the lactate dehydrogenase level of the "white" vastus lateralis muscle, imply increased dependence upon oxidative metabolism by the TRAIN group.

3. The decreased phosphoglucomutase results that occurred with endurance training seem to indicate that the relative importance of glucose utilization can be increased in all muscle fiber types.
4. The results of the present study reinforce the general concept of constant proportion enzyme groups. However, some of the constant enzyme ratios do appear to be altered either by maturation or training.
5. The variable enzyme ratio data support the validity of histochemical fiber typing methods.

Recommendations

1. Studies of the biochemical responses to specific exercise regimens of acetylocholinesterase and related enzymes at the motor end plate are needed.
2. Biochemical, histochemical, and contractile investigations of the effects of exercise on the different types of motor units are needed.
3. Studies of the hormonal influences on exercise performance and metabolism are necessary.

4. High-intensity exercise regimens for animals should be developed to facilitate study of the adaptations to anaerobic training.
5. Power-type events such as high-jumping and weight-lifting are needed for animals so that resultant adaptations can be compared with those resulting from activities across the endurance continuum.
6. Both biochemical and histochemical analyses are needed for complete muscle evaluations.
7. The effects of specific exercise regimens should be studied through muscle biopsys in humans. These studies should include various age groups as well as both sexes.
8. In any follow-up of this investigation, mitochondrial hexokinase activity should be included as a dependent variable. The incorporation of some parameter of fatty acid oxidation also would be beneficial.

REFERENCES

REFERENCES

1. Ariano, M. A., R. B. Armstrong, and V. R. Edgerton. Hindlimb muscle fiber populations of five mammals. *J. Histochem. Cytochem.* 21:51-55, 1973.

2. Askew, E. W., G. L. Dohm, R. L. Huston, T. W. Sneed, and R. P. Dowdy. Response of rat tissue lipases to physical training and exercise. *Proc. Soc. Exp. Biol. Med.* 141:123-129, 1972.

3. Bagby, G. J., W. L. Sembrowich, and P. D. Gollnick. Myosin ATPase and fiber composition from trained and untrained rat skeletal muscle. *Am. J. Physiol.* 223:1415-1417, 1972.

4. Baldwin, K. M., G. H. Klinkerfuss, R. L. Terjung, P. A. Molé, and J. O. Holloszy. Respiratory capacity of white, red, and intermediate muscle: adaptive response to exercise. *Am. J. Physiol.* 222:373-378, 1972.

5. Baldwin, K. M., and C. M. Tipton. Work and metabolic patterns of fast and slow twitch skeletal muscle contracting in situ. *Pflügers Arch.* 334:345-356, 1972.

6. Baldwin, K. M., W. W. Winder, R. L. Terjung, and J. O. Holloszy. Glycolytic enzymes in different types of skeletal muscle: adaptation to exercise. *Am. J. Physiol.* 225:962-966, 1973.

7. Bancroft, T. A. Analysis and inference for incompletely specific models involving the use of preliminary test(s) of significance. *Biometrics.* 20:427-442, 1964.

8. Bär, U., and M. C. Blanchaer. Glycogen and CO_2 production from glucose and lactate by red and white skeletal muscle. *Am. J. Physiol.* 209:905-909, 1965.

9. Barnard, R. J., V. R. Edgerton, and J. B. Peter. Effect of exercise on skeletal muscle. I. Biochemical and histochemical properties. *J. Appl. Physiol.* 28:762-766, 1970.

10. Barnard, R. J., V. R. Edgerton, and J. B. Peter. Effect of exercise on skeletal muscle. II. Contractile properties. *J. Appl. Physiol.* 28:767-770, 1970.

11. Barnard, R. J., and J. B. Peter. Effect of exercise on skeletal muscle. III. Cytochrome changes. *J. Appl. Physiol.* 31:904-908, 1971.

12. Barnard, R. J., V. R. Edgerton, T. Furukawa, and J. B. Peter. Histochemical, biochemical and contractile properties of red, white and intermediate fibers. *Am. J. Physiol.* 220:410-414, 1971.

13. Bass, A., D. Brdiczka, P. Eyer, S. Hofer, and D. Pette. Metabolic differentiation of distinct muscle types at the level of enzymatic organization. *Eur. J. Biochem.* 10:198-206, 1969.

14. Beatty, C. H., R. D. Peterson, and R. M. Bocek. Metabolism of red and white muscle fiber groups. *Am. J. Physiol.* 204: 939-942, 1963.

15. Bocek, R. M., R. D. Peterson, and C. H. Beatty. Glycogen metabolism in red and white muscle. *Am. J. Physiol.* 210: 1101-1107, 1966.

16. Burleigh, I. G., and R. T. Schimke. The activities of some enzymes concerned with energy metabolism in mammalian muscles of differing pigmentation. *Biochem. J.* 113:157-166, 1969.

17. Canal, N., and L. Frattola. Effect of exercise and denervation on glycogen synthetase of skeletal muscle. *Med. Exp.* 18: 313-320, 1968.

18. Crabtree, B., and E. A. Newsholme. The activities of phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase and the glycerol 3-phosphate dehydrogenases in muscles from vertebrates and invertebrates. *Biochem. J.* 126:49-58, 1972.

19. Dalrymple, R. H., R. G. Cassens, and L. L. Kastenschmidt. Glycolytic enzyme activity in developing red and white muscle. *J. Cell. Physiol.* 83:251-258, 1973.

20. Danforth, W. H. Glycogen synthetase activity in skeletal muscle. *J. Biol. Chem.* 240:588-593, 1965.

21. Dawson, D. M., and F. C. A. Romanul. Enzymes in muscle. II. Histochemical and quantitative studies. *Arch. Neurol.* 2:369-378, 1964.

22. Delebrück, A., E. Zebe, and T. Bücher. Uber Verteilungsmuster von Enzymen des Energie liefernden Stoffwechsels im Flugmuskel, Sprungmuskel und Fettkörper von Locusta migratoria und ihre cytologische Zuordnung. *Biochem. Z.* 331:273-296, 1959.

23. Dieter, M. P. Enzyme changes in red and white muscle, liver, and plasma of exercised guinea pigs maintained on high or low levels of vitamin C. *Life Sci.* 9:301-311, 1970.

24. Dieter, M. P., P. D. Highland, and B. Highman. Tolerance of unacclimated and cold-acclimated rats to exercise in the cold: serum, red and white muscle enzymes, and histological changes. *Can. J. Physiol. Pharmacol.* 48:723-731, 1970.

25. Dohm, G. L., R. L. Huston, E. W. Askew, and H. L. Fleshwood. Effects of exercise, training, and diet on muscle citric acid cycle enzyme activity. *Can. J. Biochem.* 51:849-854, 1973.

26. Edgerton, V. R., L. Gerchman, and R. Carrow. Histochemical changes in rat skeletal muscle after exercise. *Exptl. Neurol.* 24:110-123, 1969.

27. Edgerton, V. R., and D. R. Simpson. The intermediate muscle fiber of rats and guinea pigs. *J. Histochem. Cytochem.* 17:828-838, 1969.

28. Edgerton, V. R., D. R. Simpson, R. J. Barnard, and J. B. Peter. Phosphorylase activity in acutely exercised muscle. *Nature.* 225:866-867, 1970.

29. Edgerton, V. R., R. J. Barnard, J. B. Peter, C. A. Gillespie, and D. R. Simpson. Overloaded skeletal muscles of a nonhuman primate (Galago senegalensis). *Exptl. Neurol.* 37:322-339, 1972.

30. Edström, J. E. Effects of increased motor activity on the dimensions and the staining properties of the neuron soma. *J. Comp. Neurol.* 1107:295-304, 1957.

31. Eriksson, B. O., P. D. Gollnick, and B. Saltin. Muscle metabolism and enzyme activities after training in boys 11-13 years old. *Acta. Physiol. Scand.* 87:485-497, 1973.

32. Eversole, L. R., and S. M. Standish. Histochemical demonstration of muscle fiber types. *J. Histochem. Cytochem.* 18:590-593, 1970.

33. Exner, G. U., H. W. Staudte, and D. Pette. Isometric training of rats--effects upon fast and slow muscle and modification by an anabolic hormone (Nandrolone Decanoate). I. Female rats. *Pflügers Arch.* 345:1-14, 1973.

34. Exner, G. U., H. W. Staudte, and D. Pette. Isometric training of rats--effects upon fast and slow muscle and modification by and anabolic hormone (Nandrolone Decanoate). II. Male rats. *Pflügers Arch.* 345:15-22, 1973.

35. Faulkner, J. A., D. C. Maxwell, D. A. Brook, and D. A. Lieberman. Adaptation of guinea pig plantaris muscle fibers to training. *Am. J. Physiol.* 221:291-297, 1971.

36. Fritz, P. J. Rabbit muscle lactate dehydrogenase 5. A regulatory enzyme. *Science.* 150:364-366, 1965.

37. Gollnick, P. D., P. J. Struck, and T. P. Bogyo. Lactic dehydrogenase activities of rat heart and skeletal muscle after exercise and training. *J. Appl. Physiol.* 22:623-627, 1967.

38. Gollnick, P. D., C. D. Ianuzzo, C. Williams, and T. R. Hill. Effect of prolonged, severe exercise on the ultrastructure of human skeletal muscle. *Int. Z. angew. Physiol.* 27: 257-265, 1969.

39. Gollnick, P. D., and D. W. King. Effect of exercise and training on mitochondria of rat skeletal muscle. *Am. J. Physiol.* 216:1502-1509, 1969.

40. Gollnick, P. D., R. B. Armstrong, B. Saltin, C. W. Saubert IV, W. L. Sembrowich, and R. E. Shepherd. Effect of training on enzyme activity and fiber composition of human skeletal muscle. *J. Appl. Physiol.* 34:107-111, 1973.

41. Gollnick, P. D., B. Sjödin, J. Karlsson, E. Jansson, and B. Saltin. Human soleus muscle: a comparison of fiber composition and enzyme activities with other leg muscles. *Pflügers Arch.* 348:247-255, 1974.

42. Gillespie, C. A., D. R. Simpson, and V. R. Edgerton. High glycogen content of red as apposed to white skeletal muscle fibers of guinea pigs. *J. Histochem. Cytochem.* 18:552-558, 1970.

43. Gould, M. K., and W. A. Rawlinson. Biochemical adaptation as a response to exercise. I. Effect of swimming on the levels of lactic dehydrogenase, malic dehydrogenase, and phosphorylase in muscles of 8-, 11-, and 15-week old rats. *Biochem. J.* 73:41-44, 1959.

44. Guth, L., and F. J. Samaha. Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Exptl. Neurol.* 25:138-152, 1969.

45. Guyton, A. C. *Textbook of Medical Physiology.* Philadelphia: W. B. Saunders Co., 1971, pp. 821-824.

46. Hamilton, M. J., and J. H. Ferguson. Effects of exercise and cold acclimation of the ventricular and skeletal muscles of white mice (Mus Musculus). I. Succinic dehydrogenase activity. *Comp. Biochem. Physiol.* 43:815-824, 1972.

47. Harper, H. A. *Review of Physiological Chemistry.* Los Altos, Calif.: Lange Medical Publications, 1973, p. 247.

48. Hearn, G. R., and W. W. Wainio. Succinic dehydrogenase activity of the heart and skeletal muscle of exercised rats. *Am. J. Physiol.* 185:348-350, 1956.

49. Hearn, G. R., and W. W. Wainio. Aldolase activity in the heart and skeletal muscle of exercised rats. *Am. J. Physiol.* 190:206-208, 1957.

50. Hearn, G. R., and P. D. Gollnick. Effects of exercise on the adenosinetriphosphatase activity in skeletal and heart muscle of rats. *Int. Z. angew. Physiol.* 19:23-26, 1961.

51. Hearn, G. R. The effects of terminating and detraining on enzyme activities of heart and skeletal muscle of trained rats. *Int. Z. angew. Physiol.* 21:190-194, 1965.

52. Henneman, E., G. Somjen, and D. O. Carpenter. Functional significance of cell size in spinal motoneurons. *J. Neurophysiol.* 28:560-580, 1965.

53. Henneman, E., and C. B. Olson. Relations between structure and function in the design of skeletal muscle. *J. Neurophysiol.* 28:581-598, 1965.

54. Holloszy, J. O. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J. Biol. Chem.* 242: 2278-2282, 1967.

55. Holloszy, J. O., and L. B. Oscai. Effect of exercise on α-glycerophosphate dehydrogenase activity in skeletal muscle. *Arch. Biochem. Biophys.* 130:653-656, 1969.

56. Holloszy, J. O., L. B. Oscai, I. J. Don, and P. A. Molé. Mitochondrial citric acid cycle and related enzymes: Adaptive response to exercise. *Biochem. Biophys. Res. Comm.* 40:1368-1373, 1970.

57. Holloszy, J. O., L. B. Oscai, P. A. Molé, and I. J. Don. Biochemical adaptations to endurance exercise in skeletal muscle. In: *Muscle Metabolism During Exercise.* Edited by B. Pernow and B. Saltin. New York: Plenum, 1971, pp. 51-61.

58. Jeffress, R. N., J. B. Peter, and D. R. Lamb. Effects of exercise on glycogen synthetase in red and white skeletal muscle. *Life Sci.* 7:957-960, 1968.

59. Karlsson, J., B. Diamant, and B. Saltin. Lactate dehydrogenase activity in muscle after prolonged severe exercise in man. *J. Appl. Physiol.* 25:88-91, 1968.

60. Kendrick-Jones, J., and S. V. Perry. Enzymatic adaptation to contractile activity in skeletal muscle. *Nature.* 208: 1068-1070, 1965.

61. Klingenberg, M., and D. Pette. Proportions of mitochondrial enzymes and pyridine nucleotides. *Biochem. Biophys. Res. Comm.* 7:430-432, 1962.

62. Krebs, H. A., and M. Woodford. Fructose 1,6-diphosphatase in striated muscle. *Biochem. J.* 94:436-445, 1965.

63. Kubišta, V., J. Kubištová, and D. Pette. Thyroid hormone induced changes in the enzyme activity pattern of energy-supplying metabolism of fast (white), slow (red), and heart muscle of the rat. *Eur. J. Biochem.* 18:553-560, 1971.

64. Lamb, D. L., J. B. Peter, R. N. Jeffress, and H. A. Wallace. Glycogen, hexokinase, and glycogen synthetase adaptations to exercise. *Am. J. Physiol.* 217:1628-1632, 1969.

65. Lawrie, R. A. Biochemical differences between red and white muscles. *Nature.* 170:122-123, 1952.

66. Lehninger, A. L. *Biochemistry.* New York: Worth Publishing Co., 1970, pp. 337-387.

67. Mahler, H. R., and E. H. Cordes. *Biological Chemistry.* New York: Harper and Row Publishers, 1971, p. 453.

68. Marikova, A. F. Effects of various types of muscular activity on the ATPase activity of myosin. *Ukr. Biokhim. Zh.* 30: 230-239, 1958.

69. McPhedran, A. M., R. B. Wuerker, and E. Henneman. Properties of motor units in a homogeneous red muscle (soleus) of the cat. *J. Neurophysiol.* 28:71-84, 1965.

70. Mier, P. D., and D. W. K. Cotton. Operon hypothesis. New evidence from the "constant proportion" group of the Embden-Meyerhof pathway. *Nature.* 209:1022-1023, 1966.

71. Molé, P. A., and J. O. Holloszy. Exercise-induced increase in the capacity of skeletal muscle to oxidize palmitate. *Proc. Soc. Exptl. Biol. Med.* 134:789-792, 1970.

72. Molé, P. A., L. B. Oscai, and J. O. Holloszy. Adaptation of muscle to exercise. Increase in levels of palmityl CoA synthetase, carnitine palymityltransferase, and palmityl CoA dehydrogenase, and in the capacity to oxidize fatty acids. *J. Clin. Invest.* 50:2323-2330, 1971.

73. Molé, P. A., K. M. Baldwin, R. L. Terjung, and J. O. Holloszy. Enzymatic pathways of pyravate metabolism in skeletal muscle: Adaptations to exercise. *Am. J. Physiol.* 224:50-54, 1973.

74. Moody, W. G., and R. G. Cassens. Histochemical differentiation of red and white muscle fibers. *J. Anim. Sci.* 27:961-967, 1968.

75. Opie, L. H., and E. A. Newsholme. The activities of fructose 1,6-diphosphatase, phosphofructokinase, and phosphoenolpyruvate carboxykinase in white muscle and red muscle. *Biochem. J.* 103:391-399, 1967.

76. Oscai, L. B., and J. O. Holloszy. Biochemical adaptations in muscle. II. Response of mitochondrial adenosine triphosphatase, creatine phosphokinase, and adenylate kinase activities in skeletal muscle to exercise. *J. Biol. Chem.* 246: 6968-6972, 1971.

77. Padykula, H. A., and G. F. Gauthier. Morphological and cytochemical characteristics of fiber types in normal mammalian skeletal muscle. In: *Exploratory Concepts in Muscular Dystrophy.* Edited by A. T. Milhorat. New York: Exerpta Medica Foundation, 1967.

78. Paull, A. E. On a preliminary test for pooling mean squares in the analysis of variance. *Annals. Math. Stat.* 21:539-556, 1950.

79. Pesce, A., T. P. Fondy, F. Stolzenbach, F. Castillo, and N. O. Kaplan. The comparative enzymology of lactic dehydrogenases. III. Properties of the H_4 and M4 enzymes from a number of vertebrates. *J. Biol. Chem.* 242:2151-2167, 1967.

80. Peter, J. B., R. N. Jeffress, and D. R. Lamb. Exercise: Effects on hexokinase activity in red and white skeletal muscle. *Science.* 160:200-201, 1968.

81. Peter, J. B., S. Sawaki, R. J. Barnard, V. R. Edgerton, and C. A. Gillespie. Lactate dehydrogenase isoenzymes: Distribution in fast-twitch red, fast-twitch white, and slow-twitch intermediate fibers of guinea pig skeletal muscle. *Arch. Biochem. Biophys.* 144:304-307, 1971.

82. Peter, J. B., R. J. Barnard, V. R. Edgerton, C. A. Gillespie, and K. E. Stempel. Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry.* 11:2627-2633, 1972.

83. Pette, D., N. Luh, and T. Bücher. A constant proportion group in the enzyme activity pattern of the Embden-Meyerhof Chain. *Biochem. Biophys. Res. Comm.* 7:419-424, 1962.

84. Pette, D., M. Klingenberg, and T. Bücher. Comparable and specific proportions in the mitochondrial enzyme activity pattern. *Biochem. Biophys. Res. Comm.* 7:425-429, 1962.

85. Pette, D. Metabolic differentiation of distinct muscle types at the level of enzymatic organization. In: *Muscle Metabolism During Exercise.* Edited by B. Pernow and B. Saltin. New York: Plenum, 1971, pp. 33-49.

86. Racker, E. Spectrophotometric measurements of the enzymatic formation of fumaric and cis-asconitic acids. *Biochim. Biophys. Acta.* 4:211-214, 1950.

87. Rawlinson, W. A., and M. K. Gould. Biochemical adaptation as a response to exercise. 2. Adenosine triphosphatase and creatine phosphokinase activity in muscles of exercised rats. *Biochem. J.* 73:44-48, 1959.

88. Reitman, J., K. M. Baldwin, and J. O. Holloszy. Intramuscular triglyceride utilization by red, white, and intermediate skeletal muscle and heart during exhausting exercise. *Proc. Soc. Exp. Biol. Med.* 142:628-631, 1973.

89. Roch-Norlund, A. E. Muscle glycogen synthetase in patients with diabetes mellitus. Basal values, effect of glycogen depletion by exercise, and effect of treatment. *Scand. J. Clin. Lab. Invest.* 29:237-242, 1972.

90. Shonk, C. E., and G. E. Boxer. Enzyme patterns in human tissues. I. Methods for the determination of glycolytic enzymes. *Cancer Res.* 24:709-721, 1964.

91. Staudte, H. W., and D. Pette. Correlations between enzymes of energy-supplying metabolism as a basic pattern of organization in muscle. *Comp. Biochem. Physiol.* 41:533-540, 1972.

92. Staudte, H. W., G. U. Exner, and D. Pette. Effects of short-term, high intensity (sprint) training on some contractile and metabolic characteristics of fast and slow muscle of the rat. *Pflügers Arch.* 344:159-168, 1973.

93. Stein, J. M., and H. A. Padykula. Histochemical classification of individual skeletal muscle fibers in the rat. *Am. J. Anat.* 110:103-123, 1962.

94. Syrový, I., E. Gutmann, and J. Melichna. Effect of exercise on skeletal muscle myosin ATP-ase activity. *Physiol. Bohemoslov.* 21:633-638, 1972.

95. Taylor, A. W., R. Thayer, and S. Rao. Human skeletal muscle glycogen synthetase activities with exercise and training. *Can. J. Physiol. Pharmacol.* 50:411-415, 1972.

96. Taylor, A. W., M. A. Booth, and S. Rao. Human skeletal muscle phosphorylase activities with exercise and training. *Can. J. Physiol. Pharmacol.* 50:1038-1042, 1972.

97. Terjung, R. L., K. M. Baldwin, P. A. Molé, G. H. Klinkerfuss, and J. O. Holloszy. Effect of Running to exhaustion on skeletal muscle mitochondria: A biochemical study. *Am. J. Physiol.* 223:549-554, 1972.

98. Varnauskas, E., P. Björntorp, M. Fahlén, I. Přerovský, and J. Stenberg. Effects of physical training on exercise blood flow and enzymatic activity in skeletal muscle. *Cardiovas. Res.* 4:418-422, 1970.

99. Vogell, W., F. R. Bishai, T. Bücher, M. Klingenberg, D. Pette, and E. Zebe. Strukturelle und enzymatisch Muster in Muskeln von Locusta migratoria. *Biochem. Z.* 332:81-117, 1959.

100. Vrbová, G. The effect of motorneuron activity on the speed of contraction of striated muscle. *J. Physiol.* 169:513-526, 1963.

101. Wells, R. L., and W. W. Heusner. A controlled-running wheel for small animals. *Lab. Anim. Sci.* 21:904-910, 1971.

102. Wilkerson, J. E., and E. Evonuk. Changes in cardiac and skeletal muscle myosin ATPase activities after exercise. *J. Appl. Physiol.* 30:328-330, 1971.

103. Wilson, J. E., B. Sacktor, and C. G. Tiekert. In situ regulation of glycolysis in tetanized cat skeletal muscle. *Arch. Biochem. Biophys.* 120:542:546, 1967.

104. Wilson, J. E. The localization of latent brain hexokinase on synaptosomal mitochondria. *Arch. Biochem. Biophys.* 150: 96-104, 1972.

105. Winder, W. W., K. M. Baldwin, and J. O. Holloszy. Exercise-induced adaptative increase in rate of oxidation of β-hydroxybutyrate by skeletal muscle. *Proc. Soc. Exp. Biol. Med.* 143:753-755, 1973.

106. Wuerker, R. B., A. M. McPhedran, and E. Henneman. Properties of motor units in a heterogeneous pale muscle (m. gastrocnemius) of the cat. *J. Neurophysiol.* 28:85-99, 1965.

107. Yellin, H., and L. Guth. The histochemical classification of muscle fibers. *Exptl. Neurol.* 26:424-432, 1970.

APPENDIX A

TRAINING PROGRAMS

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TRAINING PROGRAMS

Standard Eight-Week, Short-Duration, High-Intensity Endurance Training Program for Postpubertal and Adult Male Rats in Controlled-Running Wheels

Wk.	Day of Wk.	Day of Tr.	Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (m/min)	Total Time of Prog. (min: sec)	Total Exp. Meters TEM	Total Work Time (sec) TWT
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	27	49:30	540	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	27	49:30	540	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	27	49:30	540	1200
	4=T	4	2.5	00:10	10	40	3	5.0	1.2	36	49:30	720	1200
	5=F	5	2.0	00:10	10	40	3	5.0	1.2	36	49:30	720	1200
2	1=M	6	1.5	00:10	10	28	4	5.0	1.2	45	51:40	840	1120
	2=T	7	1.5	00:10	15	27	4	5.0	1.2	54	59:00	972	1080
	3=W	8	1.5	00:10	15	27	4	5.0	1.2	54	59:00	972	1080
	4=T	9	1.5	00:10	15	27	4	5.0	1.2	54	59:00	972	1080
	5=F	10	1.5	00:10	15	27	4	5.0	1.2	54	59:00	972	1080
3	1=M	11	1.5	00:10	15	27	4	5.0	1.2	54	59:00	972	1080
	2=T	12	1.5	00:10	20	23	4	5.0	1.2	63	59:40	966	920
	3=W	13	1.5	00:10	20	23	4	5.0	1.2	63	59:40	966	920
	4=T	14	1.5	00:10	20	23	4	5.0	1.2	63	59:40	966	920
	5=F	15	1.5	00:10	20	23	4	5.0	1.2	63	59:40	966	920
4	1=M	16	1.5	00:10	20	23	4	5.0	1.2	63	59:40	966	920
	2=T	17	1.5	00:10	25	20	4	5.0	1.0	72	60:00	960	800
	3=W	18	1.5	00:10	25	20	4	5.0	1.0	72	60:00	960	800
	4=T	19	1.5	00:10	25	20	4	5.0	1.0	72	60:00	960	800
	5=F	20	1.5	00:10	25	20	4	5.0	1.0	72	60:00	960	800
5	1=M	21	1.5	00:10	25	20	4	5.0	1.0	72	60:00	960	800
	2=T	22	1.5	00:10	30	16	4	5.0	1.0	81	55:40	864	640
	3=W	23	1.5	00:10	30	16	4	5.0	1.0	81	55:40	864	640
	4=T	24	1.5	00:10	30	16	4	5.0	1.0	81	55:40	864	640
	5=F	25	1.5	00:10	30	16	4	5.0	1.0	81	55:40	864	640
6	1=M	26	1.5	00:10	30	16	4	5.0	1.0	81	55:40	864	640
	2=T	27	2.0	00:10	35	10	5	5.0	1.0	90	54:35	750	500
	3=W	28	2.0	00:10	35	10	5	5.0	1.0	90	54:35	750	500
	4=T	29	2.0	00:10	35	10	5	5.0	1.0	90	54:35	750	500
	5=F	30	2.0	00:10	35	10	5	5.0	1.0	90	54:35	750	500
7	1=M	31	2.0	00:10	35	10	5	5.0	1.0	90	54:35	750	500
	2=T	32	2.0	00:10	35	7	8	2.5	1.0	90	54:50	840	560
	3=W	33	2.0	00:10	35	7	8	2.5	1.0	90	54:50	840	560
	4=T	34	2.0	00:10	35	7	8	2.5	1.0	90	54:50	840	560
	5=F	35	2.0	00:10	35	7	8	2.5	1.0	90	54:50	840	560
8	1=M	36	2.0	00:10	35	7	8	2.5	1.0	90	54:50	840	560
	2=T	37	2.0	00:10	40	6	8	2.5	1.0	99	52.10	792	480
	3=W	38	2.0	00:10	40	6	8	2.5	1.0	99	52:10	792	480
	4=T	39	2.0	00:10	40	6	8	2.5	1.0	99	52:10	792	480
	5=F	40	2.0	00:10	40	6	8	2.5	1.0	99	52:10	792	480

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSF* and *PER* scores of 75 or higher during the final two weeks. Alterations in the work time, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training programs.

Standard Eight-Week, Long-Duration, Low-Intensity Endurance Training Program for Postpubertal and Adult Male Rats in Controlled-Running Wheels

Wk.	Day of Wk.	Day of Tr.	Acc-eler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repeti-tions per Bout	No. of Bouts	Time Bet-ween Bouts (min)	Shock (ma)	Run Speed (m/min)	Total Time of Prog. (min: sec)	Total Exp. Meters TEM	Total Work Time (sec) TWT
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	27	49:30	540	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	27	49:30	540	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	27	49:30	540	1200
	4=T	4	2.5	00:20	10	30	2	5.0	1.2	27	34:40	540	1200
	5=F	5	2.5	00:30	15	20	2	5.0	1.2	27	34:30	540	1200
2	1=M	6	2.0	00:40	20	15	2	5.0	1.2	36	34:20	720	1200
	2=T	7	2.0	00:50	25	12	2	5.0	1.2	36	34:10	720	1200
	3=W	8	1.5	01:00	30	10	2	5.0	1.2	36	34:00	720	1200
	4=T	9	1.5	02:30	60	4	2	5.0	1.2	36	31:00	720	1200
	5=F	10	1.0	02:30	60	4	2	5.0	1.2	36	31:00	720	1200
3	1=M	11	1.0	02:30	60	4	2	5.0	1.2	36	31:00	720	1200
	2=T	12	1.0	05:00	0	1	5	2.5	1.2	36	35:00	900	1500
	3=W	13	1.0	05:00	0	1	5	2.5	1.2	36	35:00	900	1500
	4=T	14	1.0	05:00	0	1	5	2.5	1.2	36	35:00	900	1500
	5=F	15	1.0	05:00	0	1	5	2.5	1.2	36	35:00	900	1500
4	1=M	16	1.0	05:00	0	1	5	2.5	1.2	36	35:00	900	1500
	2=T	17	1.0	07:30	0	1	4	2.5	1.0	36	37:30	1080	1800
	3=W	18	1.0	07:30	0	1	4	2.5	1.0	36	37:30	1080	1800
	4=T	19	1.0	07:30	0	1	4	2.5	1.0	36	37:30	1080	1800
	5=F	20	1.0	07:30	0	1	4	2.5	1.0	36	37:30	1080	1800
5	1=M	21	1.0	07:30	0	1	4	2.5	1.0	36	37:30	1080	1800
	2=T	22	1.0	07:30	0	1	5	2.5	1.0	36	47:30	1350	2250
	3=W	23	1.0	07:30	0	1	5	2.5	1.0	36	47:30	1350	2250
	4=T	24	1.0	07:30	0	1	5	2.5	1.0	36	47:30	1350	2250
	5=F	25	1.0	07:30	0	1	5	2.5	1.0	36	47:30	1350	2250
6	1=M	26	1.0	07:30	0	1	5	2.5	1.0	36	47:30	1350	2250
	2=T	27	1.0	10:00	0	1	4	2.5	1.0	36	47:30	1440	2400
	3=W	28	1.0	10:00	0	1	4	2.5	1.0	36	47:30	1440	2400
	4=T	29	1.0	10:00	0	1	4	2.5	1.0	36	47:30	1440	2400
	5=F	30	1.0	10:00	0	1	4	2.5	1.0	36	47:30	1440	2400
7	1=M	31	1.0	10:00	0	1	4	2.5	1.0	36	47:30	1440	2400
	2=T	32	1.0	10:00	0	1	5	2.5	1.0	36	60:00	1800	3000
	3=W	33	1.0	10:00	0	1	5	2.5	1.0	36	60:00	1800	3000
	4=T	34	1.0	10:00	0	1	5	2.5	1.0	36	60:00	1800	3000
	5=F	35	1.0	10:00	0	1	5	2.5	1.0	36	60:00	1800	3000
8	1=M	36	1.0	10:00	0	1	5	2.5	1.0	36	60:00	1800	3000
	2=T	37	1.0	12:30	0	1	4	2.5	1.0	36	57:30	1800	3000
	3=W	38	1.0	12:30	0	1	4	2.5	1.0	36	57:30	1800	3000
	4=T	39	1.0	12:30	0	1	4	2.5	1.0	36	57:30	1800	3000
	5=F	40	1.0	12:30	0	1	4	2.5	1.0	36	57:30	1800	3000

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSF* and *PER* scores of 75 or higher during the final two weeks. Alterations in the rest time, repetitions per bout, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.