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DEVELOPMENTAL AND SPRINT TRAINING EFFECTS ON FIBER COMPOSITION, PROTEIN, AND NUCLEIC ACIDS IN SUBCELLULAR FRACTIONS OF RAT GASTROCNEMIUS MUSCLE

by

JOHN G. WILKINSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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DEPARTMENT OF PHYSICAL EDUCATION

EDMONTON, ALBERTA

FALL, 1977

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Developmental and Sprint Training Effects on Fiber Composition, Protein, and Nucleic Acids in Subcellular Fractions of Rat Gastrocnemius Muscle", submitted by John G. Wilkinson in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physical Education.

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Date September 6, 1977

DEDICATION

To my mother and father who instilled in me
a need and a love for learning.

ABSTRACT

The effects of development and sprint training on protein and nucleic acid content of homogenates and subcellular fractions of rat gastrocnemius has been investigated. Fiber composition and mean fiber areas were also studied. Two groups of ten 5 week old animals were subjected to 10 and 20 weeks of interval running respectively, consisting of 10 sec sprints followed by 20 sec rest periods. At the end of the training program the rats were running up to 20 sprints per day at 90 m/min with a 30% grade. Four groups of animals served as 5, 15 and 25 week old sedentary controls. Significant developmental changes were observed for each variable measured in the control animals at different ages. Body weights, muscle weights, homogenate DNA, subcellular fractions of DNA and estimated numbers of nuclei were all unchanged after sprint training when compared with age-matched controls. Homogenate protein and RNA content of sprint trained muscle showed no net changes, however time-dependent training adaptations were found in selected subcellular fractions. Ten weeks of training significantly increased myofibrillar-nuclear protein and RNA concentrations in the gastrocnemius. Microsomal protein increased and soluble protein decreased after 20 weeks of sprint training, while a shift in RNA distribution from the myofibrillar-nuclear to the sarcoplasmic fractions (soluble, mitochondrial and microsomal) was found in 25 week old sprint trained animals. The percentage of fast-glycolytic (FG) fibers was 36% after 10 weeks of sprint training which was significantly different from the 21% found in age-matched controls. A concomitant decrease in fast-oxidative-glycolytic (FOG) fibers was evident in the sprint runners and these fiber distribution

patterns were maintained in the animals trained to 25 weeks of age. The slow-oxidative (SO) fiber percentages were not affected by training. Significant enlargement in the mean fiber areas of SO and FG fibers were found after sprint training. These findings suggested that high intensity intermittent work overloads the gastrocnemius muscle, which adapts to training by maintenance of FG fiber percentages and hypertrophy. These adaptations, however, are not revealed in whole gastrocnemius protein and nucleic acid contents, although selective training alterations are evident in the subcellular fractions.

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INTRODUCTION

The physiological and metabolic processes of higher animals, including man, respond to genetic control as well as environmental changes such as exercise. Exercise physiologists have been investigating human physiological responses to physical work and exercise for the last hundred years. In recent years, with the use of animal models and human biopsy techniques, a number of metabolic, histochemical, biochemical and ultrastructural adaptions have been demonstrated in mammalian skeletal muscle as a result of various types of exercise (reviewed by Close, 1972; Keul, 1972; Gollnick, 1973c; Holloszy, 1975a). A knowledge of these changes in skeletal muscle at the cellular level seems necessary for a complete understanding of man's functional adaptability to exercise.

Mammalian skeletal muscle from various species consists of three muscle fiber types (Barnard et al., 1970; Peter et al., 1972; Ariano et al., 1973; Burke et al., 1973; Burke and Edgerton, 1975; Edgerton et al., 1975). The fiber typing system used by Peter et al. (1972) classifies muscle fibers according to their contractile speed, which is dependent on myosin ATPase enzyme activity, and the predominant metabolic pathways used for energy production as characterised by oxidative or glycolytic enzymes. These inherent properties of the individual fibers allow selected fibers to be recruited at different intensities and durations of exercise (Baldwin et al., 1972, 1973, 1975; Costil et al., 1973; Gollnick et al., 1973a, 1973b, 1974a, 1974b; Staudte et al., 1973). Recruitment of different muscle fibers in turn regulates the speed at which the whole muscle contracts (Barnard et al., 1970; Close, 1972), the utilization of substrates (Pernow et al., 1971; Baldwin et al., 1973) and the enzymatic

pathways which are employed during energy production (Molé et al., 1971; Edington et al., 1973; Holloszy et al., 1973, 1975; Benzi et al., 1975).

Physical work capacity is ultimately limited by the amount of contractile protein in skeletal muscle and/or the ability of that muscle to produce the energy needed for contraction. During exercise the rate of energy production must equal the energy demands placed on skeletal muscle if work is to be continued at the same intensity (Simonson, 1971; Wenger and Reed, 1976). Skeletal muscle therefore adapts to exercise overloads by increasing the quantity of myofibrillar protein available for muscular contraction (Gordon et al., 1967b; Jaweed et al., 1974) or by increasing its capacity to supply energy to the contractile machinery (Wenger and Reed, 1976).

Fundamental to the metabolic alterations observed in muscle fibers are the changes in the concentrations as well as the activities of enzymes involved in intermediary metabolism (Bass et al., 1969; Staudte and Pette, 1972). Increases in oxidative enzyme capabilities with endurance exercise and training have been demonstrated by a number of researchers (Barnard et al., 1970, 1971; Edington et al., 1970, 1973; Gollnick et al., 1972; Peter et al., 1972; Terjung et al., 1972; Holloszy et al., 1973, 1975a, 1975b; Fitts et al., 1974). Isometric and sprint training seems to alter glycolytic as well as oxidative enzymes (Bagby et al., 1972; Exner et al., 1973a, 1973b; Saubert et al., 1973; Staudte et al., 1973; Thorstensson et al., 1975). These enzyme adaptations to different exercise overloads result in enhanced metabolic capability for energy production (Saltin, 1973; Holloszy, 1975a). Hence, the overall effect of these adaptions is an increased functional work capacity.

Harper (1975) has suggested that through the regulation of enzyme

activity, overall metabolic regulation is possible. Clearly, enzyme alterations are a regulating mechanism in skeletal muscle metabolism (previous paragraph). This implies the presence of suitable sensory devices, trigger mechanisms, and chemical messengers which ultimately affect enzyme activity (Harper, 1975). Protein synthesis and degradation must also play a role in the regulation of muscle metabolism because enzymes, contractile elements, membranes and other cell constituents are made up of proteins. Muscle hypertrophy and increased metabolic function is accompanied by a net gain in protein synthesis (Young, 1970, 1974). In this context the complexity of skeletal muscle adaptations to exercise becomes more evident. The metabolic and ultrastructural changes that have been observed in trained skeletal muscle are dependent on protein turnover which is in turn regulated by a number of complex factors (Young, 1970, 1974). An insight into net protein synthesis and its regulation in various subcellular fractions of muscle may give ergophysiologists a better understanding of the mechanisms governing adaptations in working muscle.

Basic protein metabolism in skeletal muscle has been reviewed by a number of authors (Goldberg, 1967, 1969; Pain and Manchester, 1970; Young, 1970; Millward et al., 1973; Thompson and Heywood, 1974). The effect of normal muscle growth and development on protein synthesis has also been a topic of research interest (Devi et al., 1963; Winnick and Noble, 1965; Srivastava and Chaudhary, 1969; Howarth and Baldwin, 1971, 1972; Edgerton, 1973; Young, 1974; Burleigh, 1974; Giovannetti and Stothers, 1975; Millward et al., 1975). Recently, work physiologists have become interested in the effects of exercise on protein metabolism. Inc eases in whole muscle weights, deoxyribonucleic acid (DNA), ribonu-

cleic acid (RNA), RNA polymerase activity, total muscle protein, low molecular weight proteins, and protein content of subcellular muscle fractions have been reported as a result of endurance exercise and training (Yakovlev et al., 1963; Gordon et al., 1967a, 1967b; Bailey et al., 1973; Bostrom et al., 1974; Hubbard et al., 1974; Jaweed et al., 1974; Goldberg et al., 1975; Poortmans, 1975; Rogozkin, 1976).

Two groups of researchers have monitored the effects of both growth and endurance training on the protein and DNA content of rat skeletal muscle although their results were not always in agreement (Bailey et al., 1973; Hubbard et al., 1974). Both groups reported an increase in muscle weights and the ratio of protein to DNA in control and trained animals. Bailey and co-workers (1973) reported a significant decrease in the DNA content (μg/mg wet wt) of gastrocnemius muscle in control animals and those trained between 3 and 12 weeks of age. However, no change was found in the whole muscle DNA content of these animals which is in accordance with the work of Gordon et al. (1967a, 1967b). Hubbard (1974) reported an increase in the protein composition of the sarcoplasmic and myofibrillar fractions of growing rat anterior tibialis muscle between 6 and 17 weeks of age while endurance training did not alter this developmental pattern. These results are contrary to the findings of Gordon et al. (1967a, 1967b) and Jaweed et al. (1974) who have observed large increases in the sarcoplasmic and myofibrillar protein content of adult rat skeletal muscle differentially trained with endurance and weight lifting exercises respectively.

There are still many unanswered questions concerning the effects of exercise on growth and protein metabolism. Selective hypertrophy of skeletal muscle fiber types and conversion of their metabolic and contractile

properties with growth and exercise is not completely understood (Goldberg et al., 1975; Wilkinson et al., 1976). The influence of high intensity sprint training on protein synthesis in developing muscle has not been determined. Furthermore, there are only a few studies which relate protein synthetic adaptations to the various subcellular compartments or fractions of skeletal muscle, and controversy still exists concerning exercise specific protein alterations in these different fractions (Hubbard et al., 1974; Jaweed et al., 1974).

With some of the above questions in mind, the purposes of this study were threefold:

To determine the effect of sprint training on fiber type composition of developing rat gastrocnemius muscle.

To determine the effect of sprint training on the whole muscle weights and muscle volumes as indicated by mean fiber areas of developing rat gastrocnemius.

To determine the effect of sprint training on protein and nucleic acid concentrations in four subcellular fractions of developing rat skeletal muscle.

TERMINOLOGY

Effects of Time - In this study references to the effects of time are related to development or growth of skeletal muscle between 5, 15 and 25 weeks of age.

Hypertrophy - An increase in the size of a muscle or muscle fiber caused by enlargement of its cellular components.

Hyperplasia - An increase in the number of cells composing a skeletal muscle caused by muscle fiber splitting.

Subcellular Fractions - Components or parts of a muscle cell

which, in this study, were separated by centrifugation at different gravitational forces.

Myofibrillar-Nuclear Fraction - That part of a muscle cell which contains myofibrillar protein, nuclei, and cell debris resulting from homogenation.

Mitochondrial Fraction - That part of a muscle cell which predominantly contains mitochondria. Other cytoplasmic particles such as lysosomes are found in this fraction when they are of similar size, shape and density as mitochondria.

Microsomal Fraction - That part of a muscle cell which predominantly contains vesicles and membrane fragments, of both the smooth and rough variety, derived from the endoplasmic reticulum.

Soluble Fraction - That part of a muscle cell which contains cytoplasmic proteins and particles which do not sediment at gravitational forces up to 105,000 x g.

METHODOLOGY

ANIMAL SELECTION AND CARE

Fifty male Wistar rats ([WOF (WI)], specific pathogen free) were obtained at approximately four weeks of age (85-100 g) through the Director of the Health Sciences Animal Center at the University of Alberta. The animals were housed in individual self-cleaning cages in an air-conditioned room maintained at 22°C, and the day/night cycle was reversed (eg. light from 6 PM to 6 AM) for the remainder of the experiment. The rats were fed ad libitum on a normal protein diet (23% crude protein) of Purina rat chow and water. Each morning all animals were handled, the cages were rotated on the cage rack, soiled papers were changed and food and water was replenished. The cages were washed and sterilized weekly and the rats were weighed every second week. After six days of orientation to the laboratory the animals were randomly assigned to one of six groups as indicated in Table I. The final number of animals in this investigation was reduced to forty-seven because three of the sprint training animals were injured and had to be removed from the study.

SPRINT TRAINING PROTOCOL

The animals were exercised on a motor driven treadmill (Quinton rodent treadmill) which was divided into ten 9.5 cm by 48 cm compartments each o[illegible] hich had a shock grid at the back of the compartment. Training was done [illegible] ys per week (eg. Mon., Tues., Thurs., and Fri.) at approximately [illegible]00 [illegible] 500 hours. Sprint trained rats were given a treadmill orientation p[illegible] for the first week which consisted of two 5 minute runs each day [illegible] 2[illegible] m/min and 8% grade. After this preliminary expo-

TABLE I GROUPS OF ANIMALS, TRAINING PERIOD AND AGE AT TIME OF SACRIFICE

ANIMAL GROUP	NUMBER OF ANIMALS (N)	AGE AT TIME OF SACRIFICE (N)
C5 - Control Group No Exercise	5	5
ST5[1] - Sprint Training Control Group	5	5
C15 - Control Group No Exercise	10	15
ST15 - Sprint Training from 5-15 weeks of age	8	15
C25 - Control Group No Exercise	10	25
ST25 - Sprint Training from 5-25 weeks of age	9	25

[1] Although the group called ST5 did not train they were designated as such to balance the design (see Experimental Design).

sure they then began the interval program of 10 bouts of 15 sec running, 20 sec rest at 30 m/min with a 30% grade. The treadmill speed was then progressively increased by 5 m/min increments every second day until all animals were running 10 bouts at 80 m/min, 30% grade twice daily (Reardon, 1975; Wilkinson et al., 1976). This progressive training increase took approximately four weeks after which both groups of runners continued at this intensity up until 15 weeks of age. The running speed was then further increased for the ST25 group up to 85 m/min and again to 90 m/min at 20 weeks of age, which was maintained until the time of sacrifice. Pre-

vious studies from our laboratory have shown that this interval running program results in significant anaerobic training effects (Reardon, 1975; Wilkinson et al., 1976; Jobin, 1977).

EXPERIMENTAL DESIGN

Six groups of experimental animals were used in this study (Table 1). Two groups received exercise treatment (ST15 and ST25) while the others remained as sedentary controls. C5 and ST5 groups served as initial control animals and the ST5 group was designated as such to balance the statistical design. Although they did not train it was necessary to establish this group so that the data could be efficiently handled with a two way analysis of variance. The other two groups, C15 and C25, served as age-matched controls for the training groups. Sedentary control groups provided data on normal skeletal muscle developmental patterns between 5, 15 and 25 weeks of age, while the two training groups elucidated the effects of sprint training on these developmental patterns. Selected parameters of growth, muscle fiber typing, and net protein synthesis were investigated as indicated by the dependent variables listed below.

1) Body Weight (grams) (g)
2) Muscle Weight (g)
3) Slow-Oxidative (SO) Fiber Types (% distribution)
4) Fast-Oxidative-Glycolytic (FOG) Fiber Types (%)
5) Fast-Glycolytic (FG) Fiber Types (%)
6) SO Fiber Areas (square micrometers) (μ^2)
7) FOG Fiber Areas (μ^2)
8) FG Fiber Areas (μ^2)
9) Total Homogenate Protein (milligrams) (mg)
10) Total Homogenate RNA (micrograms) (μg)
11) Total Homogenate DNA (μg)
12) Homogenate Protein Concentration (Conc.) (mg/g wet weight)

13) Homogenate RNA Conc. (μg/g wet wt)
14) Homogenate DNA Conc. (μg/g wet wt)
15) Total Myofibrillar-Nuclear Protein (mg)
16) Total Myofibrillar-Nuclear RNA (μg)
17) Total Myofibrillar-Nuclear DNA (μg)
18) Myofibrillar-Nuclear Protein Conc. (mg/g wet wt)
19) Myofibrillar-Nuclear RNA Conc. (μg/g wet wt)
20) Myofibrillar-Nuclear DNA Conc. (μg/g wet wt)
21) Total Mitochondrial Protein (mg)
22) Total Mitochondrial RNA (μg)
23) Total Mitochondrial DNA (μg)
24) Mitochondrial Protein Conc. (mg/g wet wt)
25) Mitochondrial RNA Conc. (μg/g wet wt)
26) Mitochondrial DNA Conc. (μg/g wet wt)
27) Total Microsomal Protein (mg)
28) Total Microsomal RNA (μg)
29) Total Microsomal DNA (μg)
30) Microsomal Protein Conc. (mg/g wet wt)
31) Microsomal RNA Conc. (μg/g wet wt)
32) Microsomal DNA Conc. (μg/g wet wt)
33) Total Soluble Protein (mg)
34) Total Soluble RNA (μg)
35) Total Soluble DNA (μg)
36) Soluble Protein Conc. (mg/g wet wt)
37) Soluble RNA Conc. (μg/g wet wt)
38) Soluble DNA Conc. (μg/g wet wt)
39) Total Number of Nuclei ($X 10^6$)
40) Number of Nuclei Conc. ($X 10^6/g$)

STATISTICAL ANALYSIS

Comparisons of the dependent variables listed above were done using a two-way analysis of variance (S.P.S.S. ANOVA program) according to Nie et al., 1975. Group means were compared to give main effects for exercise treatment (A), normal muscle development or time (B) and an inter-

action effect (AB) as shown in Table II. Post hoc procedures involved a Newman-Keuls test (APL computer program, Appendix C) for locating significant mean differences as well as simple effects testing (Appendix C) (Steel and Torrie, 1960; Harter, 1970). Significant differences were accepted at the alpha level P is less than 0.05, where P is the probability that no difference exists between means.

TABLE II TWO-WAY ANALYSIS OF VARIANCE TABLE FOR COMPARISON OF GROUP MEANS

	NORMAL MUSCLE DEVELOPMENT-TIME (B)		
EXERCISE TREATMENT (A)	Control 5 wks C5 N=5	Control 15 wks C15 N=10	Control 25 wks C25 N=10
	Sprint Trained 5 wks ST5 N=5	Sprint Trained 15 wks ST15 N=8	Sprint Trained 25 wks ST25 N=9

TISSUE PREPARATION

The trained animals were sacrificed along with their age-matched controls by decapitation (small animal guillotine) 48 to 60 hours after the last exercise session. After exsanguination the right and left gastrocnemius muscles were isolated and excised. A portion of the R. medial gastrocnemius muscle (100-200 mg) was used for histochemical analysis and the whole L. gastrocnemius muscle (1-2 g) was trimmed of connective tissue, blotted and then weighed for biochemical analysis. Both left and

right gastrocnemius muscle samples were then frozen in isopentane (2-methyl-butane), precooled in liquid nitrogen and stored at -60°C for subsequent analysis.

HISTOCHEMICAL PROCEDURES

Serial sections (10μ thick) were cut on an Ames Tissue Tek cryostat, mounted on microscope slides, and air-dried for several hours before staining. The sections were then stained for myosin ATPase activity (pH 9.4) according to the method of Padykula and Herman (1955) as modified by Guth and Samaha (1969), NADH-diaphorase activity as suggested by Dubowitz and Brooke (1973) and alpha-glycerol-phosphate activity according to Wattenberg and Leong (1960). Photomicrographs were taken from the medial head of the gastrocnemius muscle as this region has three distinct fiber types (Ariano et al., 1973). Four to six hundred muscle fibers (mean = 515) were numbered and counted from each photomicrograph and classified as slow-oxidative (SO), fast-glycolytic (FG) or fast-oxidative-glycolytic (FOG) according to the fiber typing system of Peter et al. (1972). Fiber populations were calculated as a percentage of the total number of muscle fibers counted.

Mean muscle fiber areas were estimated for 60 fibers per cross section through the use of planimetry. Microscope slides were projected at a magnification of x 750 and individual fibers were measured with a compensating polar planimeter (Keuffel and Esser, Germany) according to the method of Maxwell et al. (1973). From sections stained for myosin ATPase whole R. medial cross-sectional areas were projected at a magnification of x 12 and measured by planimetry. Calculations were then made estimating the total number of fibers per muscle cross section, total number of fibers per fiber type, and the percentage contribution of each fiber type

to cross-sectional area (Maxwell et al., 1973). This data may be found in Table XXVI, Appendix C.

BIOCHEMICAL PROCEDURES

All preparative operations were made at 0-4°C unless otherwise stated and biochemical tissue concentrations were expressed in mg or µg per g wet wt of tissue. Frozen L. gastrocenmius muscles were crushed and powdered by hand with the use of a porcelain mortar and pestle at the temperature of liquid nitrogen. The muscle powder was then brought up to a volume of 20 ml with ice cold buffer (50 mM tris-HCl and 100mM KCl at pH 7.4) according to Goldberg (1968) and Burešová et al. (1975). The muscle suspension was then homogenized with two strokes of a Broeck glass homogenizer (Fisher Scientific Co.) fitted with a ground glass pestle which had a clearance of 0.005-0.007 mm. This homogenizer was designed to fracture cells while it left nuclei and other cell constituents intact. Differential centrifugation of the homogenate at 600 x gravity (g.) for 20 min followed by washing of the pellet and a further spin of the supernatant at 1500 x g. for 15 min brought down the myofibrillar-nuclear fraction (Winnick and Winnick, 1960; Goldberg, 1968; Kirnos and Vanyoshiu, 1976). The myofibrillar-nuclear pellets were drained of supernatant, resuspended, combined and brought up to a volume of 14 ml with buffer. The sarcoplasmic fraction (supernatant and washings) was then brought up to a volume of 12 ml and spun in a Beckman L2-50 ultracentrifuge using a swing-bucket SW-41 rotor. The mitochondrial pellet was brought down with a spin at 22,000 x g. (R Average) for 20 min at 4°C (Seraydarian and Mommaerts, 1965; Kirnos and Vanyoshiu, 1976). The supernatant was then drawn off the mitochondrial pellet and recentrifuged at 105,000 x g. (R average) for 90 min at 4°C, which yielded the microsomal pellet (Goldberg,

1968; Howarth and Baldwin, 1971). The supernatant from this final centrifugation was then as per usual designated the soluble fraction. The mitochondrial and microsomal pellets were washed once and then rehomogenized in 4 ml of buffer with a tight fitting Brock glass homogenizer. All homogenates and muscle fractions were immediately refrozen in clean glass test tubes and stored at -50°C for no longer than 10 days prior to analysis.

Skeletal muscle fractionation was verified with enzyme determinations as well as electron microscopic examination. Glucose-6-phosphate dehydrogenase (G-6-PDH) was assayed spectrophotometrically (Sigma Chemical Co.) as a marker enzyme for the myofibrillar-nuclear and microsomal fractions according to Tolbert (1974), and there was a 96% recovery of the enzyme in the four subcellular fractions when compared to the total homogenate. No activity was detected in the mitochondrial fraction indicating that this fraction was almost completely free of myofibrillar-nuclear and microsomal fragments. Most of the G-6-PDH activity (86.2%) was recovered in myofibrillar-nuclear and microsomal fractions. The remainder (13.8%) was detected in the soluble fraction. NADPH dependent isocitrate dehydrogenase (ICDH) was assayed according to Ellis and Goldberg (1971) as a marker enzyme for the cytoplasmic components of the cell (ie. myofibrillar-nuclear and soluble fractions). The recovery of enzyme activity in the subcellular fractions was 94.2% when compared to the whole muscle homogenate. No detectable activity was found in the mitochondrial fraction while 3.8% of the activity was observed in the microsomal fraction indicating a slight contamination. The remaining 71.7% and 24.5% of the activity was detected in the myofibrillar-nuclear and soluble fractions respectively. The enzyme verification data may be found in Table

XXVII, Appendix D.

Electron microscopic examination of the mitochondrial (Plate I) and microsomal (Plate II) fractions qualitatively revealed little cross-contamination of these components. These fractions also appeared to be free from myofibrillar-nuclear fragments (Appendix D). It was observed, however, that although most mitochondria remained intact an estimated 20-30% of these organelles had a split in the outer membrane. This seemed to cause a leakage of mitochondrial fluid. It is unlikely that this membrane splitting occurred as a result of the fractionation procedure but was possibly the result of the homogenation technique utilized.

PROTEIN AND NUCLEIC ACID DETERMINATIONS

Duplicate protein determinations were made on 1.0 ml aliquots of the homogenates and the four subcellular fractions of the L. gastrocnemius. Then either 4.0 ml or 1.0 ml of 0.3 N KOH was added to these aliquots to solubilize protein (see Appendix E). After warming this solution at 37°C for 30 min protein was measured by a modified Lowry (1951) method using bovine albumin standards (Howarth and Baldwin, 1971).

RNA and DNA were extracted in screw cap culture tubes by a modified Schmidt-Thaunnhauser method as suggested by Munro and Fleck (1966). A 1.0 ml volume of ice cold 0.6 N perchloric acid (PCA) was added to 2.5 ml aliquots of the muscle fractions. After centrifugation at 1,000 x g. for 20 min the resulting precipitates were washed and recentrifuged twice in cold 0.2 N PCA. The precipitate was then dissolved for 1 hour in 3.0 ml of 0.3 N KOH warmed to 37°C in a metabolic shaker bath. Protein was then precipitated with 3.0 ml of 1.2 N PCA added to the hydrolysate mixture, which was centrifuged at 1,000 x g. for 20 min. The pellet was washed and

recentrifuged twice with 2.0 ml of 0.2 N PCA. The combination of the supernatant and washings contained the ribonucleotide products of RNA hydrolysis. The remaining sample pellets were then treated for 20 min with 2 ml of 5% trichloroacetic acid (TCA) warmed to 70°C in a metabolic shaker bath. This hydrolysate mixture was cooled in an ice bath to stop any DNA degradation after which it was centrifuged for 15 min at 1,000 x g. The supernatant containing the hydrolyzed DNA end-products was drawn off the pellet and transferred to clean culture tubes for subsequent analysis.

RNA was determined spectrophotometrically using a two wavelength method (260 nm and 232 nm) to correct for protein contamination (Hutchinson and Munro, 1961; Fleck and Begg, 1965; Munro and Fleck, 1966). Appropriate blanks and calf liver RNA standards were used. Details of the method used for RNA determinations are outlined in Appendix E.

DNA was measured spectrophotometrically at 490 nm according to the modified Indole procedure suggested by Hubbard et al. (1970). Calf thymus DNA standards and blanks were analyzed with each set of DNA determinations. Details of the Indole procedure for DNA measurement are outlined in Appendix E. Duplicate determinations were made for both RNA and DNA in the homogenates, myofibrillar-nuclear and soluble fractions. However, single readings were recorded for the mitochondrial and microsomal fractions due to the limited size of the rat gastrocnemius muscle.

The total number of nuclei per L. gastrocnemius muscle was estimated from the total homogenate DNA content according to the following equation:

$$\text{Total Number of Nuclei (millions)} = \frac{\text{Total Homogenate DNA (mg)} \times 10^3}{6.2}$$

where 6.2 is the amount of DNA in millimicrograms in a single diploid rat

nucleus (Winick and Noble, 1965). The concentration of nuclei per gram wet wt of tissue was then calculated by dividing the total number of nuclei by the muscle weight (g).

RESULTS

The results are presented under six major headings: Body Weights and Muscle Weights, Total Muscle DNA and Concentrations, Total Number of Nuclei and Concentrations, Total Muscle Protein and Concentrations, Total Muscle RNA and Concentrations, and Fiber Types and Fiber Areas. Group data are summarized in tabular and graphical forms which display means, standard errors of the mean and significant effects. The significant effects have been symbolized with the letters A (main experimental effect for training), B (main effect for time or development) and AB (interaction effect between training and time). Complete data for all experimental animals is presented in Tables VII through XXIV, Appendix B. Analysis of variance tables and post hoc procedures for each dependent variable are located in Appendix C.

BODY WEIGHTS AND MUSCLE WEIGHTS

Group means for body weights and muscle weights are shown in Table III. In each case there was significant growth over time (B) and significant mean differences were observed between 5, 15 and 25 weeks of age in both the training and control groups. Although sprint trained animals in groups ST15 and ST25 were 33 and 12 grams lighter than their age-matched controls these differences were not significant. Similarly, no differences were found in the gastrocnemius muscle weights with training and there were no interaction effects. Complete body and muscle weight data may be found in Table VII, Appendix B.

TOTAL MUSCLE DNA AND CONCENTRATIONS

The data in Table IV is a summary of the total DNA group means (µg)

TABLE III THE EFFECTS OF TIME AND SPRINT TRAINING ON THE MEAN BODY WEIGHTS AND GASTROCNEMIUS MUSCLE WEIGHTS OF THE SIX ANIMAL GROUPS

VARIABLES	ANIMAL GROUPS WITH LEVELS OF TIME								SIGNIFICANT EFFECTS[b]
	C5	ST5		C15	ST15		C25	ST25	
			*			*			
BODY WEIGHT	119[a]	121		401	368		500	489	B
(g)	±1.9	±1.2		±13.3	±15.9		±10.0	±10.4	
			*			*			
MUSCLE WEIGHT	0.435	0.443		1.897	1.824		2.244	2.262	B
(g)	±0.009	±0.013		±0.053	±0.068		±0.072	±0.107	

a Group means with standard errors of the mean (SEM)

b Significant main effect for Time (B) where P is less than 0.001

* Significant differences between levels of time where P is less than 0.05

TABLE IV THE EFFECTS OF TIME AND SPRINT TRAINING ON THE TOTAL DNA IN THE HOMOGENATE AND SUBCELLULAR FRACTIONS OF GASTROCNEMIUS MUSCLE IN THE SIX ANIMAL GROUPS

VARIABLES	ANIMAL GROUPS WITH LEVELS OF TIME							SIGNIFICANT EFFECTS[b]
	C5	ST5		C15	ST15	C25	ST25	
HOMOGENATE DNA (μg)	386[a] ±9.1	386 ±10.9	*	1085 ±33.0	1064 ±44.3	1086 ±36.3	1072 ±42.4	B
MYOFIBRILLAR NUCLEAR DNA (μg)	371 ±10.6	371 ±11.1	*	1069 ±30.5	1032 ±42.8	1013 ±32.2	1019 ±45.0	B
SOLUBLE DNA (μg)	2.4 ±0.1	2.4 ±0.1	*	7.9 ±0.3	7.9 ±0.4	7.9 ±0.5	7.8 ±0.4	B
MITOCHONDRIAL DNA (μg)	2.9 ±0.1	2.9 ±0.1	*	8.6 ±0.5	8.4 ±0.5	8.2 ±0.4	8.1 ±0.2	B
MICROSOMAL DNA (μg)	2.2 ±0.1	2.2 ±0.1	*	7.5 ±0.4	7.1 ±0.5	7.2 ±0.2	7.1 ±0.3	B

[a] Group means with standard errors of the mean (SEM)

[b] Significant main effect for Time (B) where P is less than 0.001

* Significant differences between levels of time where P is less than 0.05

in the homogenate and subcellular fractions of rat gastrocnemius muscle. The mean recovery of DNA in the four fractions was 98.0% when compared with total DNA in the homogenate. A significant increase in total DNA was found in the homogenate and all fractions of the gastrocnemius over time (B). Most of this increase occurred between 5 and 15 weeks of age, while no significant alterations in total DNA were evident between 15 and 25 weeks of age. Training did not have any significant effect on total DNA in any portion of the muscle, and there were no interaction effects. The raw data for total DNA appears in Tables XX-XXIV, Appendix B.

DNA concentration means (μg/g) are recorded in Table V. Developmental alterations were again evident in all parts of the gastrocnemius, and significant decreases were observed in the DNA concentrations at each level of time. As was found with the total DNA group means, sprint training did not alter DNA concentrations nor was there any significant interaction effect. Tables XX-XXIV, Appendix B, contain the individual animal data for DNA concentration.

TOTAL NUMBER OF NUCLEI AND CONCENTRATIONS

The group means for both variables are displayed in Table VI. Significant growth changes in total number of nuclei and concentration of nuclei paralleled the total DNA and DNA concentration results respectively. The total number of nuclei increased significantly from 5 to 15 weeks of age after which time no change occurred. Nevertheless, the concentration of nuclei per gram of tissue dropped significantly between 5, 15 and 25 weeks of age. Neither training nor interaction effects were observed with respect to the total number of nuclei or concentration.

TABLE V THE EFFECTS OF TIME AND SPRINT TRAINING ON THE CONCENTRATION OF DNA IN THE HOMOGENATE AND SUBCELLULAR FRACTIONS OF THE GASTROCNEMIUS MUSCLE IN THE SIX ANIMAL GROUPS

VARIABLES	ANIMAL GROUPS WITH LEVELS OF TIME								SIGNIFICANT EFFECTS[b]
	C5	ST5		C15	ST15		C25	ST25	
HOMOGENATE DNA (μg/g)	913[a] ±17.8	869 ±32.3	*	571 ±13.4	583 ±10.7	*	485 ±10.3	478 ±14.5	B
MYOFIBRILLAR NUCLEAR DNA (μg/g)	877 ±27.2	836 ±31.6	*	563 ±16.0	566 ±12.1	*	452 ±9.9	454 ±15.1	B
SOLUBLE DNA (μg/g)	5.7 ±0.3	5.3 ±0.3	*	4.2 ±0.2	4.3 ±0.2	*	3.6 ±0.3	3.5 ±0.2	B
MITOCHONDRIAL DNA (μg/g)	6.8 ±0.2	6.6 ±0.3	*	4.6 ±0.2	4.6 ±0.3	*	3.7 ±0.2	3.6 ±0.2	B
MICROSOMAL DNA (μg/g)	5.1 ±0.1	4.9 ±0.2	*	4.0 ±0.3	3.9 ±0.2	*	3.2 ±0.1	3.2 ±0.2	B

[a] Group means with standard errors of the mean (SEM)

[b] Significant main effect for Time (B) where P is less than 0.001

* Significant differences between levels of time where P is less than 0.05

TABLE VI THE EFFECTS OF TIME AND SPRINT TRAINING ON THE TOTAL NUMBER AND CONCENTRATION OF NUCLEI OF THE GASTROCNEMIUS MUSCLE IN THE SIX ANIMAL GROUPS

VARIABLES	ANIMAL GROUPS WITH LEVELS OF TIME								SIGNIFICANT EFFECTS[b]
	C5	ST5		C15	ST15		C25	ST25	
TOTAL NUMBER OF NUCLEI (millions)	62.3[a] ±1.5	62.2 ±1.8	*	175.0 ±5.3	171.6 ±7.1		175.2 ±5.9	172.8 ±6.8	B
NUMBER OF NUCLEI (millions/g)	143.3 ±0.5	143.7 ±0.7	*	92.4 ±2.2	94.1 ±1.7	*	78.2 ±1.7	77.0 ±2.3	B

a Group means and standard errors of the means (SEM)

b Significant **main effect** for Time (B) where P is less than 0.0[illegible]

* Significant differences between levels of time where P i[illegible] [illegible]an 0.05

TOTAL MUSCLE PROTEIN AND CONCENTRATIONS

Total protein data (mg) of the homogenates and the four subcellular fractions of the gastrocnemius muscle is illustrated in Figures 1.1 through 1.5. The mean recovery of total protein in the four fractions was 97.6% when compared to the total homogenate protein. Significant increases in total protein over time (B) were prevalent in all parts of the gastrocnemius. These increases proceeded from 5 to 15 to 25 weeks of age with the exception of mitochondrial protein which only changed between 5 and 15 weeks.

Total soluble protein (Figure 1.3) at 25 weeks of age was significantly lower after sprint training, and the interaction effect (AB) was also significant. Although sprint training appeared to elevate total myofibrillar-nuclear (Figure 1.2) and microsomal protein (Figure 1.5) at 15 and 25 weeks respectively, only the former training effect was significant. Complete total protein data is recorded in Tables X through XIV in Appendix B.

Group mean concentrations of protein (mg/g) are presented in Figures 2.1 to 2.5. As was observed with the total protein data, protein concentrations in all portions of the gastrocnemius rose significantly with muscle development. A consistant significant increase in protein concentration occurred between 5 and 15 weeks of age but was met with various changes between 15 and 25 weeks. There was a significant drop in homogenate and mitochondrial protein concentrations, a rise in the soluble protein fraction, and little change in the myofibrillar-nuclear and microsomal fractions.

Sprint training elicited a significant increase in myofibrillar-nuclear protein per gram of tissue at 15 weeks (Figure 2.2), while a sig-

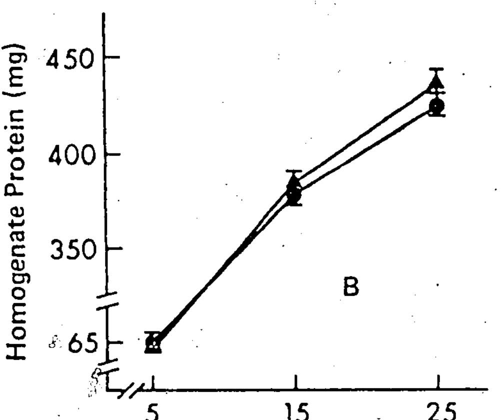

Fig. 1.1

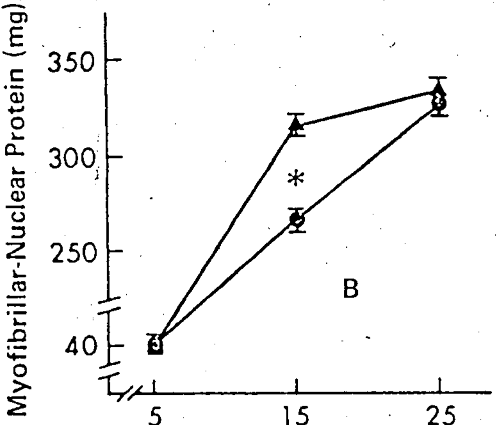

Fig. 1.2

LEGEND

- ▲ Sprint trained
- ● Control
- A Significant experimental effect
- B Significant time effect
- AB Significant interaction effect
- * Significant difference between pairs of means
- I Standard error of the mean

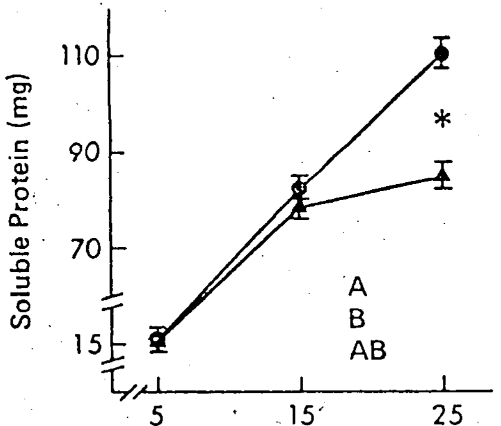

Fig. 1.3

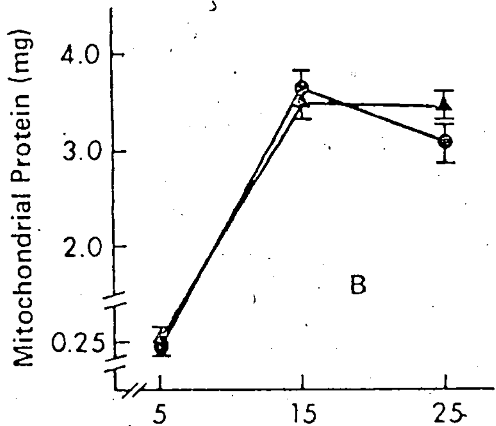

Fig. 1.4

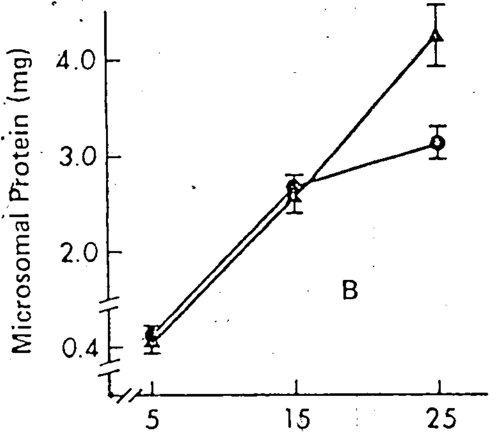

Fig. 1.5

FIGURES 1.1-1.5 THE EFFECTS OF TIME AND SPRINT TRAINING ON TOTAL PROTEIN IN THE HOMOGENATE AND SUBCELLULAR FRACTIONS OF GASTROCNEMIUS MUSCLE

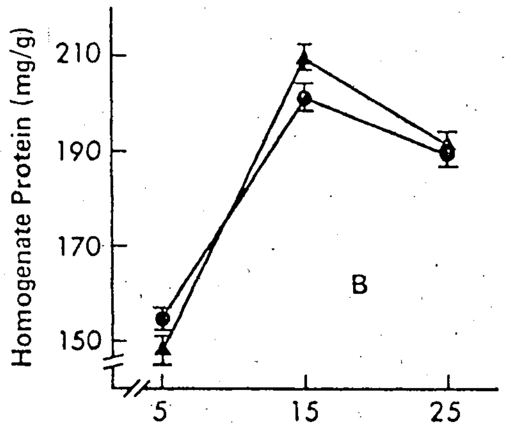

Fig. 2.1

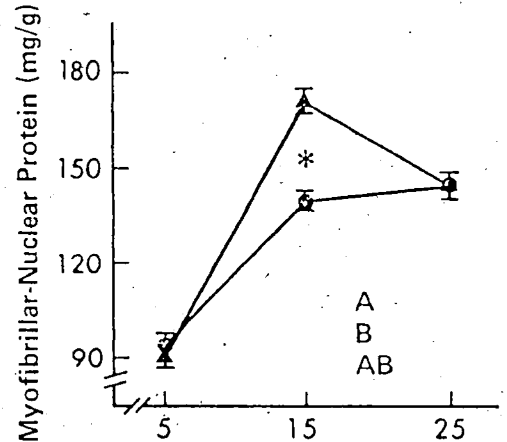

Fig. 2.2

LEGEND

- ▲ Sprint trained
- ● Control
- A Significant experimental effect
- B Significant time effect
- AB Significant interaction effect
- * Significant difference between pairs of means
- I Standard error of the mean

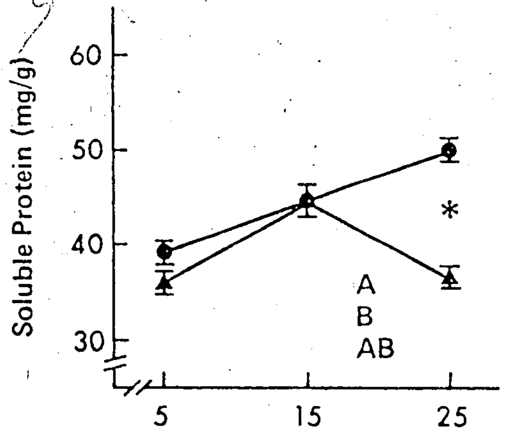

Fig. 2.3

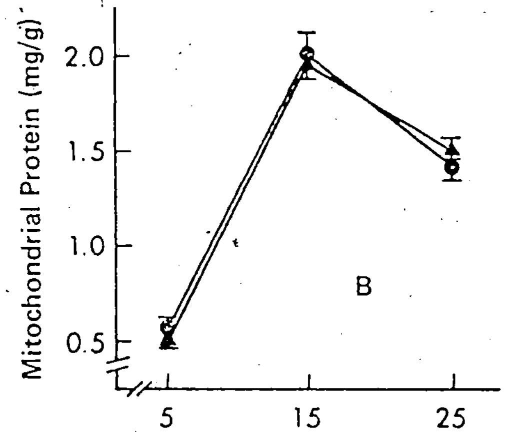

Fig. 2.4

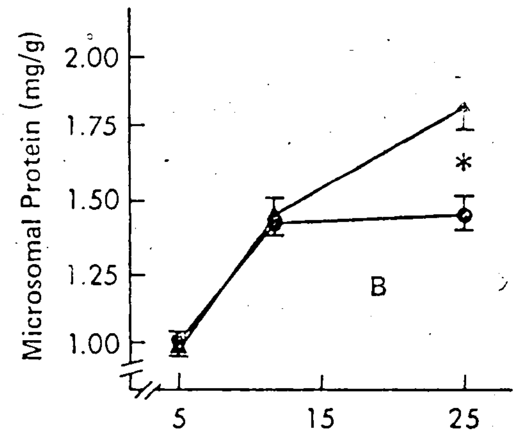

Fig. 2.5

FIGURES 2.1-2.5 THE EFFECTS OF TIME AND SPRINT TRAINING ON PROTEIN CONCENTRATION IN THE HOMOGENATE AND SUBCELLULAR FRACTIONS OF GASTROCNEMIUS MUSCLE

nificant decline in soluble protein concentration (Figure 2.3) was evident in 25 week old runners. These myofibrillar and soluble protein alterations resulted in significant time-training interactions (AB). Training also appeared to elevate the homogenate and microsomal protein concentrations above control values at 15 and 25 weeks respectively, but only the microsomal increase was significant. Mitochondrial protein concentrations were unaffected by sprint training. The raw data for protein concentrations appears in Tables X-XIV, Appendix B.

TOTAL MUSCLE RNA AND CONCENTRATIONS

The data in Figures 3.1 through 3.5 summarize the total RNA values (µg) in the homogenate and subcellular fractions of rat gastrocnemius muscle. The mean recovery of total RNA in the four subcellular fractions was 102.6% when the sum of these values were compared to the total RNA in the homogenate. Significant developmental effects (B) were found in the homogenate and all parts of the muscle. In each case there was a prominent rise in total RNA between 5 and 15 weeks of age. Total homogenate, myofibrillar-nuclear and microsomal RNA continued to increase in control muscles until 25 weeks of age. However, only the myofibrillar-nuclear increase (Figure 4.2) proved significant. Total mitochondrial and soluble RNA values were lower or unchanged in the C25 controls as compared to C15 animals, but these were non-significant occurrences.

Twenty-five week old sprint trained animals had significantly more total RNA in the soluble and microsomal fractions than did their age-matched controls. Due to the elevation in total microsomal RNA at 25 weeks (Figure 4.5) a significant time-training interaction effect was disclosed. Sprint trained animals also had more total myofibrillar-nuclear and mitochondrial RNA than did the age-matched controls at 15

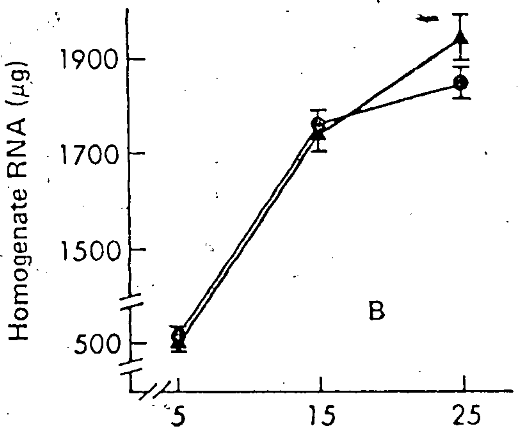

Fig. 3.1

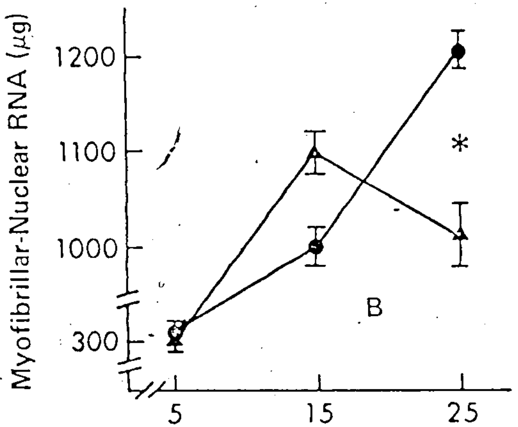

Fig. 3.2

LEGEND

- ▲ Sprint trained
- ● Control
- B Significant time effect
- AB Significant interaction effect
- * Significant difference between pairs of means
- I Standard error of the mean

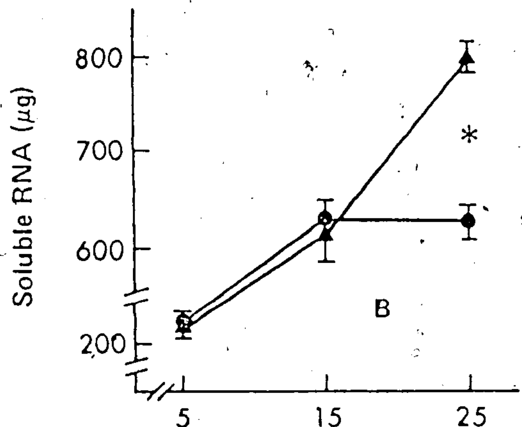

Fig. 3.3

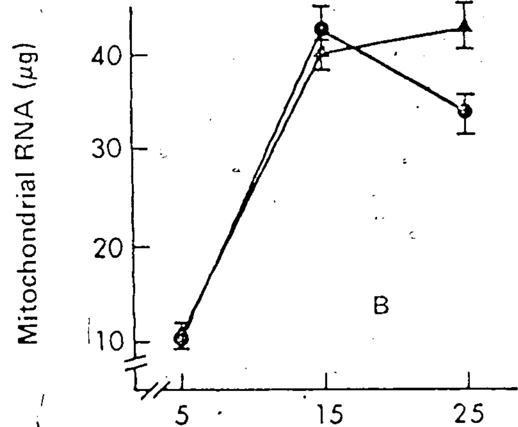

Fig. 3.4

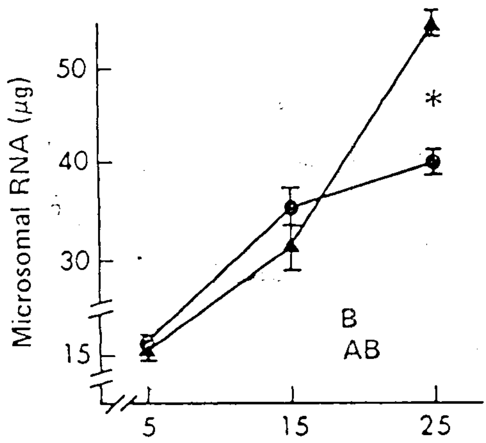

Fig. 3.5

FIGURES 3.1-3.5 THE EFFECTS OF TIME AND SPRINT TRAINING ON TOTAL RNA IN THE HOMOGENATE AND SUBCELLULAR FRACTIONS OF GASTROCNEMIUS MUSCLE

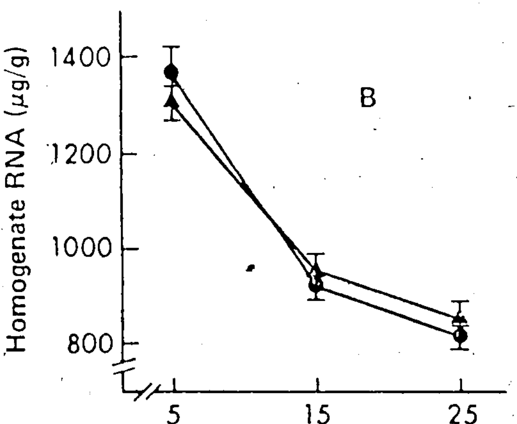

Fig. 4.1

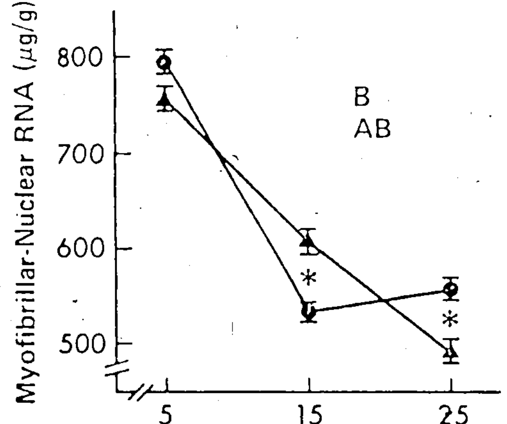

Fig. 4.2

LEGEND

- ▲ Sprint trained
- ● Control
- B Significant time effect
- AB Significant interaction effect
- * Significant difference between pairs of means
- I Standard error of the mean

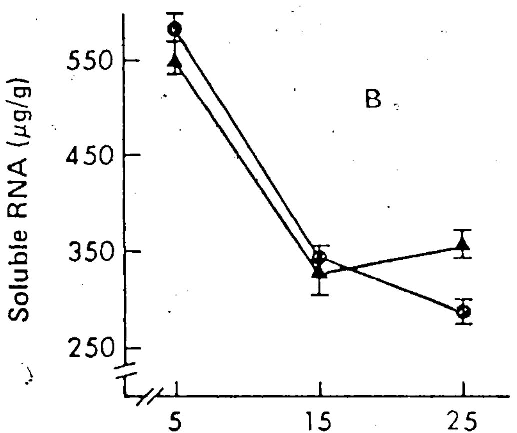

Fig. 4.3

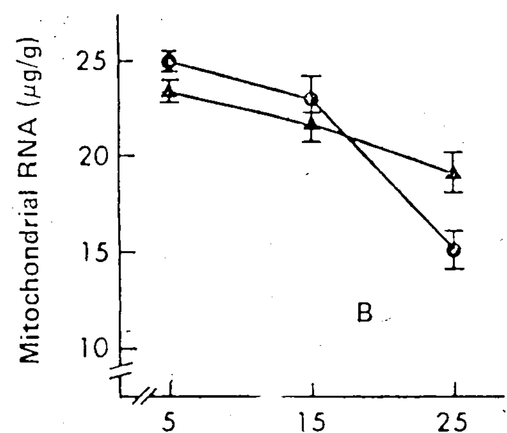

Fig. 4.4

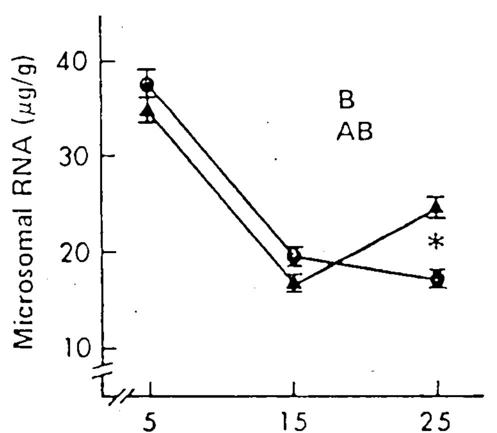

Fig. 4.5

FIGURES 4.1-4.5 THE EFFECTS OF TIME AND SPRINT TRAINING ON RNA CONCENTRATION IN THE HOMOGENATE AND SUBCELLULAR FRACTIONS OF GASTROCNEMIUS MUSCLE

and 25 weeks respectively, but these differences were not significant. Total myofibrillar-nuclear RNA (Figure 4.2) then significantly decreased in the 25 week old runners when compared to C25 controls. The total RNA data for individual animals is presented in Tables XV-XIX, Appendix B.

The mean RNA concentrations (µg/g) of the gastrocnemius muscle are illustrated in Figures 4.1 to 4.5. Although total RNA increased with muscle development, the RNA concentrations in the homogenate and subcellular fractions declined over time (B). A significant drop was observed in the homogenate and myofibrillar-nuclear, soluble and microsomal fractions of the muscle between 5 and 15 weeks of age. Although mitochondrial RNA concentration did fall from 5 to 15 weeks of age, this decline was not significant (Figure 4.4). Decreases in control RNA concentrations were also detected with growth from 15 to 25 weeks, but only the homogenate and mitochondrial changes were significant.

Several RNA concentration training effects were found in the subcellular fractions at various points in time. The ST15 concentration of RNA in the myofibrillar-nuclear fraction was significantly greater (75 µg/g) than that of the C15 group (Figure 4.2). No other significant differences between trained and control animals were observed at 15 weeks of age. The myofibrillar-nuclear concentration of RNA was then reversed at 25 weeks, with the control values (C25) being 75 µg/g higher than those of the runners (ST25). This reversal produced a significant disordinal interaction (AB) in the myofibrillar-nuclear fraction. The RNA concentrations in the soluble, mitochondrial and microsomal fractions of sprint trained animals at 25 weeks of age were greater than sedentary control values. However, only the microsomal RNA (ug/g) was significantly greater (Figure 4.5). The increase in ST25 microsomal RNA was enough to pro-

duce a significant time-training interaction (AB) in this fraction. Tables XV-XIX, Appendix B, provide complete RNA concentration data for individual animals.

FIBER TYPES AND FIBER AREAS

Fast and slow twitch characteristics were easily distinguished from sections stained for myosin ATPase as shown in Plates III, IV and V, Appendix D. NADH diaphorase serial sections had a greater range of staining intensities (Plates VI, VII and VIII, Appendix C), nevertheless they differentiated oxidative properties of the muscle fibers quite well. Alpha-glycerol-phosphate stained sections were not used in fiber classification unless there was some discrepancy in the oxidative stain.

Mean percentages of SO, FOG and FG fiber types in the medial head of the gastrocnemius muscle are illustrated in Figures 5.1, 5.2 and 5.3 respectively. Developmental shifts in fiber composition with aging (B) were significant in all three fiber types. A significant increase in the SO fiber population (Figure 5.1) was observed between 5 and 15 weeks of age in sedentary control animals. FOG fiber percentages of the control groups remained unchanged over time, while a significant decline in the proportion of FG fibers was evident between groups C5 and C15.

Runners in both of the training groups tended to have 3%-4% fewer SO fibers (Figure 5.1), but neither the training effect nor the interaction were significant. Figures 5.2 and 5.3 reveal significant sprint training (A) and interaction effects (AB). Sprint training produced a significant 13% decrease in FOG fibers at 15 weeks of age (Figure 5.2), and this difference was maintained to 25 weeks. A concomitant 15% significant increase was observed in the FG fiber distributions (Figure 1.3) of 15 and 25 week old runners. The significant interaction indicated that

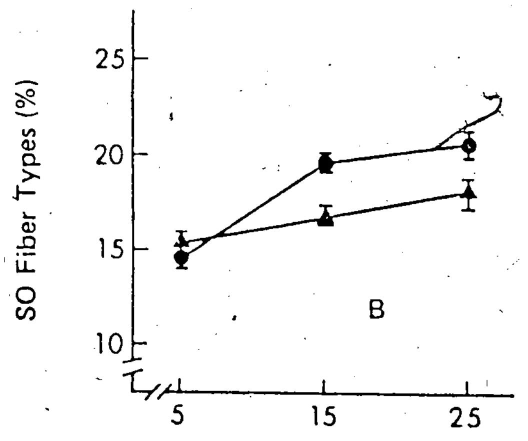

Fig. 5.1

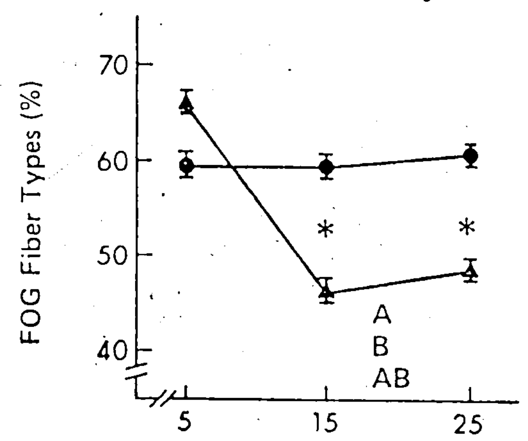

Fig. 5.2

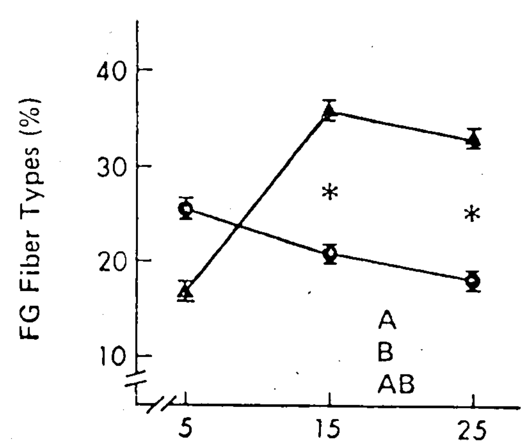

Fig. 5.3

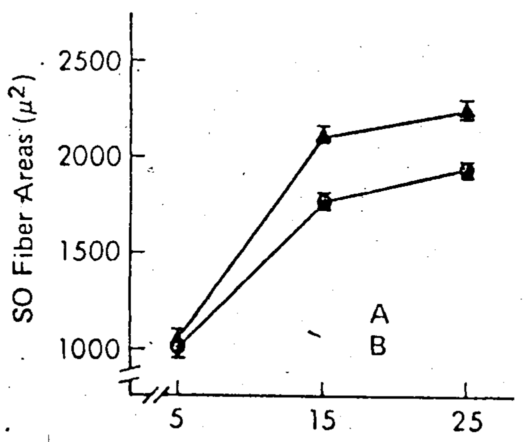

Fig. 5.4

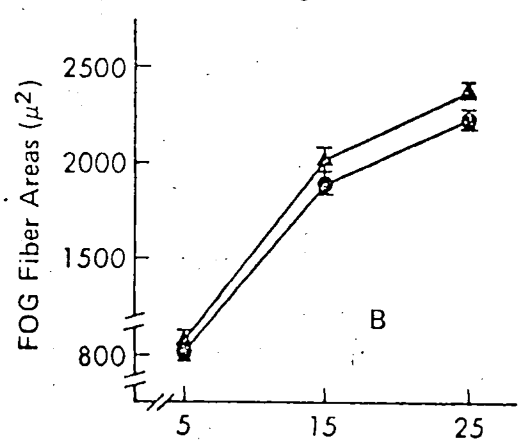

Fig. 5.5

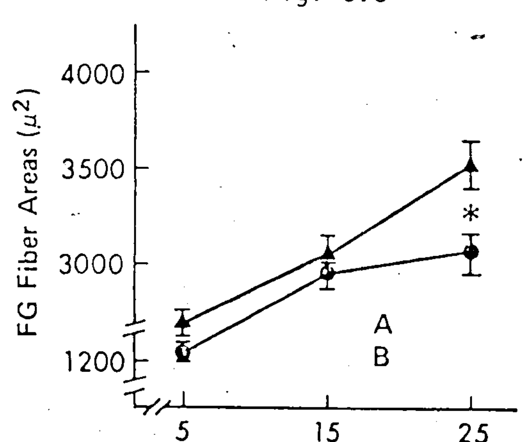

Fig. 5.6

FIGURES 5.1-5.6 THE EFFECTS OF TIME AND SPRINT TRAINING ON FIBER TYPE COMPOSITION AND MEAN FIBER AREAS OF THE MEDIAL GASTROCNEMIUS MUSCLE (see Legend Fig. 4.1-4.5).

sprint training modified normal fiber type development by reversing the relative proportions of FOG and FG muscle fiber types. Complete fiber type data for each animal appears in Table VIII, Appendix B.

The data for mean fiber type areas is displayed in Figures 5.4, 5.5 and 5.6. Significant increases in the SO and FG mean fiber areas were observed with development (B) between 5 and 15 weeks of age, while FOG fiber areas (Figure 1.5) were significantly larger at all three points in time. Sprint training significantly increased the SO mean fiber areas at both 15 and 25 weeks of age while a significant enlargement of the FG mean fiber areas (Figure 1.6) was not evident until 25 weeks of age. Training appeared to have no effect on the mean areas of FOG fibers and no significant interaction effects were prevalent. The mean fiber area data for individual animals is found in Table IX, Appendix B.

On the basis of whole medial gastrocnemius area estimates (Table XXVI, Appendix D) and mean fiber area data (Figures 5.4-5.6), the total number of fibers and the percentage cross-sectional area of a given fiber type were calculated (Table XXVI). The estimated total number of muscle fibers in the medial gastrocnemius declined from approximately 43,800 to 32,300 to 25,600 at 5, 15 and 25 weeks respectively. There did not appear to be any differences in the estimated total fiber numbers per cross section between trained and sedentary control animals. However, sprint trained animals did seem to have a greater total number of FG fibers along with a concomitant decrease in the estimated total number of FOG and SO fibers when compared with the age-matched controls.

Sprint trained groups also had a 15% and 18% enlargement of the FG fiber area of the whole muscle cross section at 15 and 25 weeks of age respectively. A corresponding drop in the total FOG fiber percentage ar-

ea was also found. This data corroborates the changes observed in mean fiber areas found in Figures 5.4 to 5.6. No differences were evident in the total number of SO fibers or the SO fiber percentage area when trained animals were compared to the age-matched controls.

DISCUSSION

The discussion is presented under the same major headings as were used previously: Body Weights and Muscle Weights, Total Muscle DNA and Concentrations, Total Number of Nuclei and Concentrations, Total Muscle Protein and Concentrations, Total Muscle RNA and Concentrations and Fiber Types and Fiber Areas. An attempt has been made to explain and integrate the changes observed with development and sprint training in conjunction with related literature.

BODY WEIGHTS AND MUSCLE WEIGHTS

The increase in body and gastrocnemius muscle weights of developing sedentary control rats found in the present study has been well documented (Howarth and Baldwin, 1971; Bailey et al., 1973; Hubbard et al., 1974; Houston and Green, 1975; Giovannetti and Stothers, 1975; Pitts and Bull, 1976). Although sprint trained rats tended to weigh less than sedentary controls (Table III), the differences were not significant. Similar non-appreciable differences in body weight have been reported by Baldwin et al. (1976) and Jobin (1977), while other researchers have found that sprint training leads to reduced body weights (Staudte et al., 1973; Houston and Green, 1975; Hickson et al., 1976). Houston and Green (1975) have suggested that vigorous training programs have an appetite-suppressing effect while sedentary control rats tend to accumulate large fat deposits. This proposal seems to be justified in the case of moderate interval and endurance training programs (Hubbard et al., 1974; Houston and Green, 1975; Mackie, 1977), but the effects of short duration, high intensity sprint training remain unclear. It appears that body weight changes with training are dependent upon a number of factors including age, sex,

intensity, and duration of the training regimens.

It was originally suspected that high intensity sprint training might result in whole muscle hypertrophy of the gastrocnemius because weight training regimens have produced this adaptation (Gordon et al., 1967b, Jaweed et al., 1974). However, this hypothesis was not substantiated (Table III). Although whole muscle hypertrophy has been reported with endurance running programs (Jaweed et al., 1974), the data from this investigation compares favourably with recent reports that muscle weights are unaffected by sprint training (Baldwin et al., 1977; Hickson et al., 1977; Jobin, 1977). Findings of selective muscle fiber hypertrophy will be discussed in the section dealing with mean fiber areas.

TOTAL MUSCLE DNA AND CONCENTRATIONS

DNA alterations in developing skeletal muscle are marked by an increase in total (µg) DNA with an overall decline in concentration (µg/g) over time (Devi et al., 1963; Winick and Noble, 1965; Gordon et al., 1966; Howarth and Baldwin, 1971; Bailey et al., 1973; Hubbard et al., 1974; Fowler et al., 1977). Comparable results shown in Table V indicated a rapid drop in DNA concentration between 5 and 15 weeks of age followed by a more gradual decrease up to 25 weeks. Devi et al. (1963) and Winick (1965) have both shown that DNA concentrations are highest at birth and undergo an even more dramatic decline between birth and several weeks of age. It appears quite clear from this study and others that initial increases in whole muscle nucleic acids do not match the rapid growth in muscle due to net protein synthesis, and consequently both DNA and RNA muscle concentrations fall with growth.

Inconsistancies still exist concerning the effects of training on skeletal muscle DNA. Bailey et al. (1973) and Hubbard et al. (1974) have

reported that 9 weeks of endurance swimming and treadmill running respectively, maintain DNA concentrations of rat soleus, plantaris and gastrocnemius muscles above normal control levels. This investigation, however, revealed no changes in DNA in any portion of rat gastrocnemius after high intensity sprint training. This finding is in accordance with the work of Gordon et al. (1967a, 1967b).

It may be that the differences in experimental DNA data in this and the four other studies referenced previously are related to the factors of growth and training intensity. It seems that skeletal muscle DNA concentration is not altered with high intensity training, whether it be sprinting or weight lifting, regardless of animal age (Table V, Gordon et al., 1967b). On the other hand, DNA concentrations do appear to be maintained at higher levels in young endurance trained animals (Bailey et al., 1973; Hubbard et al., 1974). This adaptation, however, appears to be age dependent because Gordon et al. (1967a) found no change in adult endurance trained rats.

TOTAL NUMBER OF NUCLEI AND CONCENTRATIONS

Total number of nuclei and concentrations per gram of gastrocnemius muscle (Table VI) necessarily followed the same developmental patterns as were observed in homogenate DNA because this data was calculated from total homogenate DNA values. These calculations are based upon the assumptions that DNA is located almost entirely within the nucleus, and that a single diploid rat nucleus contains a given quantity of DNA (Enesco and Puddy, 1964; Winick and Puddy, 1965). The present results corroborate the findings of Hubbard et al., (1974) who found increased nuclear proliferation in rat gastrocnemius between 7 and 13 weeks of age, while training had no effect on this growth phenomena. It, therefore, appears that nei-

ther endurance nor sprint training can modify the normal growth patterns related to accumulation of muscle nuclei since these developmental changes are likely genetically predetermined.

TOTAL MUSCLE PROTEIN AND CONCENTRATIONS

Normal growth is associated with a large prepubertal rise in whole muscle protein (mg) followed by a more gradual protein increase after puberty (Devi et al., 1963; Winick and Noble, 1965; Srivastava and Chaudhary, 1969; Howarth and Baldwin, 1971; Burleigh, 1974; Hubbard et al., 1974; Giovannetti and Stothers, 1975; Millward et al., 1975; Fowler et al., 1977). Millward and co-workers (1975) have shown that the growth rate of rat gastrocnemius is approximately 4 times higher between 3 and 9 weeks of age than between 9 and 47 weeks. Both whole muscle and total subcellular protein increased in a similar fashion in this investigation. The only departure from this developmental protein increase was the lack of change in the mitochondrial fraction between 15 and 25 weeks of age (Figure 1.4). This finding is in accordance with the work of Srivastava (1968, 1969) and suggests that the increases in mitochondrial protein before puberty are adequate enough to handle the aerobic demands placed upon growing skeletal muscle.

Protein concentrations (mg/g) in all parts of the gastrocnemius also rose between 5 and 15 weeks of age and then showed no change or a slight decrease at 25 weeks. Srivastava and Chaudhary (1969) have reported concomitant protein concentration increases in mouse skeletal muscle and subcellular fractions between 4, 13 and 21 weeks of age. These authors did report somewhat higher protein concentration values in mouse hindlimb muscle, with a 31 mg/g increase in homogenate concentration between 13 and 21 weeks of age. A non-significant 10 mg/g decrease in homogenate

protein was revealed in the present investigation over a comparable time period (Figure 2.1). Nevertheless, it is difficult to make accurate comparisons between experimental data from different animals. Burleigh (1974) has pointed out that growth rate differences are quite apparent between skeletal muscle groups and species of animals.

Protein alterations in response to muscular exercise are dependent on many factors and relatively little research has been done in this area. Hellander (1961) originally reported an increase in myofilamental nitrogen content in the calf muscles of endurance trained guinea pigs, whereas Gordon and co-workers claim an elevation in sarcoplasmic protein content of endurance trained rat quadriceps muscle (Gordon et al., 1967a; Jaweed et al., 1974). Hubbard et al. (1974), on the other hand, could not show any change in rat anterior tibialis myofibrillar, sarcoplasmic and stromal fractions after 9 weeks of endurance running.

There was some reason to believe that myofibrillar protein content of rat skeletal muscle might respond to high intensity sprint training because increases in the protein content of this fraction have been reported with weight training programs (Gordon et al., 1967b; Jaweed et al., 1974). Ten weeks of sprint running did significantly increase the total protein and protein concentration in the myofibrillar-nuclear fraction of gastrocnemius over age-matched controls. However, this adaptation was not maintained over the next 10 weeks of training (Figures 1.2 and 2.2). The homogenate protein concentration reflected a similar yet non-significant training effect. It appears, therefore, that although normal muscle growth may be altered with prepubertal sprint training, these changes in myofibrillar protein content may only be transient. It might also be suggested that the post-puberty sprint training program did not represent a

sufficient overload to older animals to maintain the previously achieved myofibrillar-nuclear protein adaptations.

Although there were significant growth increments in total protein and concentration in gastrocnemius muscle, no differences from this aging pattern were observed in the soluble, mitochondrial and microsomal fractions after 10 weeks of sprint running (Figures 1.3-1.5 and 2.3-2.5). A loss of soluble and an increase in microsomal protein was evident in these fractions with a 20 week sprint training program. Gordon et al. (1967b) have reported a decrease in sarcoplasmic protein content of weight-trained rat quadriceps which may correlate with the soluble protein decrement found in this experiment. Russian exercise scientists have observed a 20% increase in the protein content of scarcoplasmic reticulum after high speed training (Yakovlev, 1975) and similar microsomal protein changes were found in this investigation. These results coupled with reports of increased calcium uptake of sarcoplasmic reticulum (Bonner et al., 1977; Sembrowich and Gollnick, 1977) suggest that the skeletal muscle sarcotubular system can be modified in both function and structure with training. This potential for adaptation is also supported by findings of greater RNA concentrations in the microsomal fraction after training (Figure 4.5 and Rogozkin, 1976).

Although endurance training programs seem to increase skeletal muscle mitochondrial protein content by as much as 30% to 60% (Holloszy, 1967; Edington, 1969; Yakovlev, 1975), high intensity sprint training did not appear to affect the quantity of protein in this fraction (Figures 1.4 and 2.4). The anaerobic nature of this activity, therefore, does not appear to necessitate alterations in the oxidative potential of the gastrocnemius muscle.

TOTAL MUSCLE RNA AND CONCENTRATIONS

A consistent rise in the total RNA matched with a decline in RNA concentration has been reported in homogenates and subcellular fractions of growing rat and mouse skeletal muscle (Devi et al., 1963; Srivastava et al., 1968, 1969; Howarth and Baldwin, 1971; Giovannetti and Stothers, 1975; Fowler et al., 1977). The RNA results of this investigation (Figures 3.1 to 4.5) showed similar developmental patterns and were consistent with the rat gastrocnemius data recorded by Howarth (1971) and Giovannetti (1975). Even though total skeletal muscle RNA increases with age these increments are not of the same magnitude as the accumulation of muscle protein and weight. Therefore, when RNA is expressed per gram of muscle tissue the concentration of RNA actually drops.

Few work physiologists have examined the effects of exercise on net RNA synthesis in skeletal muscle, but increases in RNA polymerase activity and the incorporation of radioactively tagged amino acids into nuclear, microsomal and ribosomal RNA following exhaustive exercise have been documented (Bostrom et al., 1974; Rogozkin, 1976a, 1976b). The present investigation revealed a slight but non-significant increase in total homogenate RNA after training, while whole muscle RNA concentration was not different [illegible] the normal control data. Sprint training did appear to produce a redistribution of RNA in the gastrocnemius subcellular fractions. A significantly smaller decrement in myofibrillar-nuclear RNA concentration was found in the ST15 group when compared to age-matched controls (Figure 4.2). This coincided with the greater increase in protein concentration observed over the same time period. No other RNA alterations were evident in sprint trained animals up to 15 weeks of age. This implies that myofibrillar-nuclear RNA is most likely responsible for the

increases in net protein synthesis in this fraction in developing sprint trained muscle. Burleigh (1974) has reported that growing myo- ls have rows of ribosomes on the surfaces between fibers which suggests that myofibrillar protein development may be dependent upon ribosomal RNA. It, therefore, remains unclear whether training induced RNA concentration increases at 15 weeks are due to enhanced production of nuclear RNA or myofibrillar associated ribosomal RNA.

Ten more weeks of sprint training seemed to reverse the subcellular distribution of gastrocnemius muscle RNA. In control animals myofibrillar-nuclear and microsomal RNA concentrations were unchanged between 15 and 25 weeks of age, while soluble and mitochondrial levels continued to decline. Sprint training, however, produced a significant decrease in the myofibrillar-nuclear RNA concentration while soluble, mitochondrial, and microsomal fractions of RNA were appreciably higher than age-matched controls. Most of the RNA found in sarcoplasmic fractions of skeletal muscle is of the ribosomal type (Young, 1970, 1974; Millward et al., 1973). Soluble fractions contain free ribosomes, while sarcoplasmic reticulum bound polyribosomes are located in the microsomal portion (Burleigh, 1974; Rogers, 1976). Rogozkin (1976b) has proposed that training results in an increased nuclear-cytoplasmic transport of RNA. He also reports a rise in free amino acid concentrations in exercise adapted muscle. He suggests that this adaptation, coupled with the increased translocation of ribosomal RNA, produces an optimal condition for enhanced protein synthetic activity. In this study no evidence was found for increased net protein synthesis in whole muscle. It is possible that exercise greatly increases skeletal muscle protein synthetic activity, but accelerated degradation rates preclude a net gain in whole muscle protein. The data from the present investiga-

tion suggested a shift in RNA from muscle nuclei to the sarcoplasm with training between 15 and 25 weeks of age. It also provided evidence for net synthesis of microsomal protein in conjunction with a rise in the RNA content of this fraction.

There has been increasing evidence over the last few years that the regulation of nucleic acid and protein synthesis under conditions of overload is influenced by endogenous regulators such as creatine, amino acids, ATP, ADP and cyclic AMP (Meerson, 1975; Rogers, 1976; Rogozkin, 1976a, 1976b). Although these variables were not measured in this investigation, changes in these compounds during exercise may implicate them as regulating factors (Meerson, 1975). Neural and hormonal influences are also likely candidates for the control of protein and nucleic acid metabolism in exercise adapting skeletal muscle (Il'in et al., 1970; Young, 1970, 1974; Edgerton, 1973; Trenkle, 1974; Buresova et al., 1975).

FIBER TYPES AND FIBER AREAS

Developmental shifts in the fiber type populations of various skeletal muscles have been reported by a number of investigators (Cooper et al., 1970; Faulkner et al., 1972; Maxwell et al., 1973; Gutmann et al., 1974; Curless and Nelson, 1976). The present research revealed alterations in SO and FG fiber distributions of the medial gastrocnemius between 5 and 15 weeks of age, while FOG percentages remained constant (Figures 5.1-5.3). No significant differences were found in any of the fiber types between the C15 and C25 groups. These findings differ with those of Mackie (1977) who showed no change in SO fiber percentages and a slight increase in the number of FG fibers with development to 15 weeks. However, fiber population estimates are prone to large variations depending upon the selection of counting sites and the criteria used to count

NADH-diaphorase stained sections.

A marked reversal in the distribution of FOG and FG fibers in medial gastrocnemius after 10 and 20 weeks of sprint training is shown in Figures 5.2 and 5.3. These results corroborate earlier findings of increased FG fiber percentages with sprint training of young animals (Gaboriault, 1977; Jobin, 1977; Mackie, 1977). However, this exercise adaptation may be age dependent because Fitts et al. (1973) and Saubert et al. (1973) were unable to demonstrate FG fiber type changes with sprint training of adult miniature pigs and rats respectively.

The slight decrease in SO fiber percentages with training was not significant (Figure 5.1), and thus the fiber typing data does not provide evidence for a conversion of contractile characteristics in muscle fibers. Nevertheless, a shift in the metabolic properties of the fast-twitch fibers does seem to have occurred in the sprint trained medial gastrocnemius. The increase in the proportion of FG fibers with a concomitant loss of FOG percentages infers that fast-oxidative-glycolytic fibers have lost some of their oxidative potential. On the other hand, total fiber number estimates (Table XXVI, Appendix D) provide another explanation. A substantial drop was observed in the estimated total number of fibers per medial gastrocnemius of control animals from 5 to 15 and 15 to 25 weeks (Table XXVI) in all three fiber types. Sprint trained animals, however, seemed to maintain FG fiber numbers over time. These findings imply that the increase in FG fiber percentages found in sprint runne[illegible] may only reflect a maintenance of total fast-glycolytic fiber numbers [illegible]her than a metabolic shift.

Rea[illegible]on (1975) has reported blood lactate values in excess of 180 mg% in [illegible]s [illegible]ined with the same program used in this investigation. A

similar training regimen represents 160% of the workload elicit $\dot{V}O_2$ max. (Saubert *et al.*, 1973). This leads to the conclusion that sprint running represents a highly anaerobic activity for these animals requiring rapid energy production from glycolytic metabolic pathways. The glycolytic enzyme training adaptations reported by other investigators support this conclusion (Staudte *et al.*, 1973; Fitts *et al.*, 1974; Hickson *et al.*, 1976; Baldwin *et al.*, 1977; Jobin, 1977). A maintenance and/or shift in fast-twitch fiber populations toward FG fibers would, therefore, be an adaptation specific to the metabolic demands of sprint trained skeletal muscle.

Hyperplasia has been suggested as another response to exercise and surgical overloads (Edgerton, 1970; Reitsma, 1970; Hall-Craggs, 1972; Gonyea and Ericson, 1977). The estimates of medial gastrocnemius fiber numbers (Table XXVI) in this study gave no evidence for this theory. However, it was not within the scope of this investigation to accurately determine whether fiber splitting occurred. Therefore, hyperplasia remains as another possible mechanism of adaption in sprint trained muscle.

Most muscle fiber area hypertrophy occurs during the rapid growth period between birth and puberty (Cooper *et al.*, 1970; Faulkner *et al.*, 1972; Maxwell *et al.*, 1973; Burleigh, 1974; Muller, 1975; Curless and Nelson, 1976; Hickson *et al.*, 1976b). Many of these researchers have also found that the increase in fiber areas is less prominent after puberty. Medial gastrocnemius fiber areas of all three fiber types in this investigation were substantially enlarged at 15 weeks of age (Figures 5.4-5.6), and the mean values were almost identical with those reported by Muller (1975). These fiber area changes during development parallel the increases found in muscle weights of growing rats and provide a firm base

from which exercise specific alterations can be studied.

Selective hypertrophy of skeletal muscle fibers with various modes of training has also been reported in the literature (Gordon et al., 1967a, 1967b; Faulkner et al., 1972; Maxwell et al., 1973; Howells and Goldspink, 1974; Gonyea and Ericson, 1976). These studies generally reveal hypertrophy of SO and FOG fiber types with endurance running, while sprint and weight training programs normally lead to FG fiber enlargement. Sprint training gave rise to both SO and FG fiber hypertrophy in this investigation (Figures 5.4 and 5.6) and did not seem to affect the normal development of FOG fibers (Figure 5.5). Although sprint training seemed to decrease the percentage of SO muscle fibers, these fibers appear to have responded to training via hypertrophy. This implies sufficient recruitment of slow-oxidative fibers to cause a training adaptation. On the other hand hypertrophy of FG fibers in response to sprint training appears to be time dependent. Initially an increase in the percentage of FG fibers was observed in the ST15 group, and this adaptation was then further attenuated with hypertrophy of the FG fibers after 10 more weeks of sprint training. It should be noted that the histochemical data presented in this study is only representative of the medial portion of the gastrocnemius. If similar sprint training adaptations occurred in the whole muscle, which is composed of 95% fast-twitch fibers (Ariano et al., 1973), the overall shift to more and then larger FG fibers would have been further amplified.

The decrease in the estimated total number of medial gastrocnemius muscle fibers in control animals (Table XXVI, Appendix C) was analogous to the changes in guinea pig plantaris and soleus reported by Maxwell et al. (1973). Sprint training did not seem to alter the estimated total

number of muscle fibers, but a maintenance of the total number of FG fibers was observed instead of the decline seen with development in sedentary controls. The percentage contribution of FG fibers to the whole muscle cross-sectional area was also much higher in both of the sprint training groups. This data supported both the FG muscle fiber hypertrophy results and the contention that a greater overload is placed on these motor units during sprint training.

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

1. Large developmental changes in body weights, muscle weights, muscle proteins, nucleic acids and fiber composition are found with normal growth of sedentary rats. The rate of change in these parameters is usually greater during early development (5 to 15 weeks of age) than at later stages (15 to 25 weeks). Examination of these developmental effects seems necessary for the complete understanding of exercise specific adaptations in growing skeletal muscle.

2. Body weights, muscle weights, homogenate DNA, subcellular fractions of DNA and estimated numbers of nuclei do not seem to be affected by sprint training of young animals. Exercise of this type does not appear to have a differential effect on normal growth patterns in these variables.

3. Whole muscle protein and RNA content changes are not evident after sprint training. However, redistribution of protein and RNA in selected subcellular fractions does seem to occur. The most pertinent changes were the increase in myofibrillar-nuclear protein and RNA after 10 weeks of training and the shift of RNA to sarcoplasmic fractions after 20 weeks of sprint running. These changes suggest that protein synthetic and RNA adaptations are taking place in response to high intensity inter-

val exercise, even though they are not manifested in whole muscle preparations.

4. Fiber composition changes resulting from sprint training reflect a maintenance and/or a shift in fast-twitch fiber populations from FOG to FG fibers. Enlargement or hypertrophy of FG fibers between 15 and 25 weeks of age further amplifies the training effect. These findings appear to be an adaptation specific to the metabolic demands of high intensity intermittant work, and imply a greater overload on FG motor units during sprint exercise.

5. There is a need for further research on the effects of training in developing skeletal muscle. Future investigations might be aimed at examining protein and nucleic acid contents of sprint or endurance trained muscles which are homogeneous in fiber composition. In this way protein and nucleic acid synthesis could be related to specific motor units. Determinations of protein turnover in skeletal muscle would also be valuable for the assessment of protein adaptations in response to exercise. The regulation of protein and nucleic acid metabolism in response to exercise is another area of investigation which is wide open to future research endeavors.

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APPENDIX A

REVIEW OF LITERATURE

SKELETAL MUSCLE FIBER TYPES, GROWTH[1] AND EXERCISE

Mammalian skeletal muscle from a variety of species including guinea pigs (Barnard et al., 1970; Maxwell et al., 1973), cats (Burke et al., 1973), horses (Lindholm et al., 1975), rats (Edgerton et al., 1969) and humans (Edgerton et al., 1975; Prince et al., 1975) is composed of three muscle fiber types (Ariano et al., 1973). The fiber typing system of Peter et al. (1972) classifies muscle fibers as slow-oxidative (SO), fast-glycolytic (FG) and fast-oxidative-glycolytic (FOG) according to contractile speed and metabolic properties. A number of other classification systems have been used (Close, 1972), but Peter's system seems to be the most functional.

These three muscle fiber types may be partially differentiated during prenatal growth. When a muscle cell first becomes recognizable in the fetus it is called a myotube which is formed by the fusion of mononucleate cells called myoblasts. Two types of myotubes have been observed and classified as primary and secondary. In certain mixed muscles the primary myotubes are the first to develop and the secondary ones then grow around them. Primary myotubes acquire the histochemical properties of SO and FOG muscle fibers and these characteristics tend to be maintained during growth. Secondary myotubes develop into FG fibers although they may react transiently to oxidative histochemical stains during their development (Burleigh, 1974). This explanation gives credence to the

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[1] Much of the information on skeletal muscle growth is contained in Burleigh (1974).

findings of Close (1964), Maxwell et al. (1973) and Wilkinson et al. (1976) that rat muscle fibers are not completely differentiated until approximately 100 days after birth. Also during muscle fiber differentiation primary myotubes are initially larger than secondary ones. It has been suggested that a greater hypertrophy of secondary (FG) fibers is promoted in early growth due to more forceful contractions of these fibers. Young (1970), Burke et al. (1973) and Maxwell et al. (1973) have confirmed that FG fibers have larger diameters than both SO and FOG fibers several weeks after birth. However, there is enlargement of all three muscle fiber types after birth, and most of this hypertrophy is evident during the rapid growth period between birth and puberty (Cooper et al., 1970; Faulkner et al., 1972; Maxwell et al., 1973; Burleigh, 1974; Muller, 1975; Curless and Nelson, 1976; Hickson et al., 1976b). These researchers have also found that skeletal muscle fibers continue to increase in size after puberty, but this growth phase is somewhat slower. These fiber hypertrophy changes during muscle development parallel the increases found in muscle weights of growing rats (Howarth and Baldwin, 1971; Bailey et al., 1973; Hubbard et al., 1974; Giovannetti and Stothers, 1975; Pitts and Bull, 1976).

The size of skeletal muscles is initially largely determined by the rate and duration of cellular and nuclear divisions before muscle fibers form. It has also been suggested that the skeletal muscle mass for a given species is determined largely by the total number of muscle nuclei. There is a good correlation between estimated number of muscle nuclei and animal body size. For example, it has been proposed that humans have more muscle nuclei than rats or mice because their cells continue to divide for a longer period of time before birth due to a longer gestation period.

This hypothesis appears generally true, but several investigators have reported increases in nuclear proliferation of skeletal muscle during pubertal growth, although cell division may have ceased (Enesco and Puddy, 1964; MacConnachie et al., 1964; Young, 1970; Hubbard et al., 1974).

There is another way in which muscle fiber growth can be regulated other than by cell division with the addition of nuclei. There is reasonably good evidence to suggest that overloaded muscle fibers can undergo hyperplasia or longitudinal splitting (Edgerton, 1973; Burleigh, 1974). Goldspink (1970, 1971) has postulated that protein of growing myofibrils is synthesized in the cytoplasm and that myofibrils replicate by splitting once they have reached a certain size. Burleigh (1974) has qualified these findings by suggesting that on the basis of electron micrographs, myofibrils do not appear to split completely, but that muscle fiber mass increases as a result of myofibrillar segmentation. Edgerton (1973) has also suggested that normal muscle growth may be accompanied by increased development of muscle spindles or intrafusal fibers several days after birth.

The effects of exercise on histochemically determined fiber populations of mammalian skeletal muscle has been studied extensively in the last decade. A number of investigators have reported fiber composition alterations in selected muscles with endurance training (Edgerton et al., 1969; Barnard et al., 1970; Faulkner et al., 1971; Maxwell et al., 1971, 1973; Syrovy et al., 1972; Wilkinson et al., 1976). Maxwell et al. (1973) has hypothesized that selective adaptations in working muscle with endurance training may reflect different patterns of motor unit recruitment. Similar histochemical data has been published on the effects of high intensity sprint training. Fiber type changes in gastrocnemius, soleus

and plantaris muscles have been observed with sprint training (Saubert et al., 1973; Mackie, 1976; Wilkinson et al., 1976). Comparable sprint training and isometric exercise programs have resulted in no change in muscle fiber populations (Bagby et al., 1972; Exner et al., 1973a, 1973b; Fitts et al., 1973, 1974). In most cases fiber type changes have been reported in animals trained before puberty, while few investigators have found changes in trained adult muscle. The proposal that fiber compositional changes in developing trained muscles reflect a maintenance of preexisting composition rather than a conversion of fiber types (Mackie, 1976; Wilkinson et al., 1976) is indirectly supported by the published data of others (Edgerton et al., 1969; Faulkner et al., 1971; Bagby et al., 1973; Syrovy et al., 1972; Fitts et al., 1973; Maxwell et al., 1973). This data generally supports the contention that exercise induced compositional changes in muscle may be confounded by normal developmental changes (Mackie, 1976; Wilkinson et al., 1976).

SKELETAL MUSCLE GROWTH AND PROTEIN SYNTHESIS

Muscle growth must be accompanied by protein synthesis because its essential components such as contractile filaments, metabolic enzymes, and membranes are made up of proteins. DNA is the genetic substance which controls the synthesis of specific cellular proteins. As shown in Figure 6., DNA found in the cell nucleus serves to direct the formation of messenger RNA in a process called transcription. RNA which has been formed in the nucleus then migrates (translocation) to the cytoplasm by way of the endoplasmic reticulum where, in association with ribosomes, it acts as a template for the synthesis of specific cellular proteins (translation) (Lehninger, 1970; Edgerton, 1973; Harper, 1975).

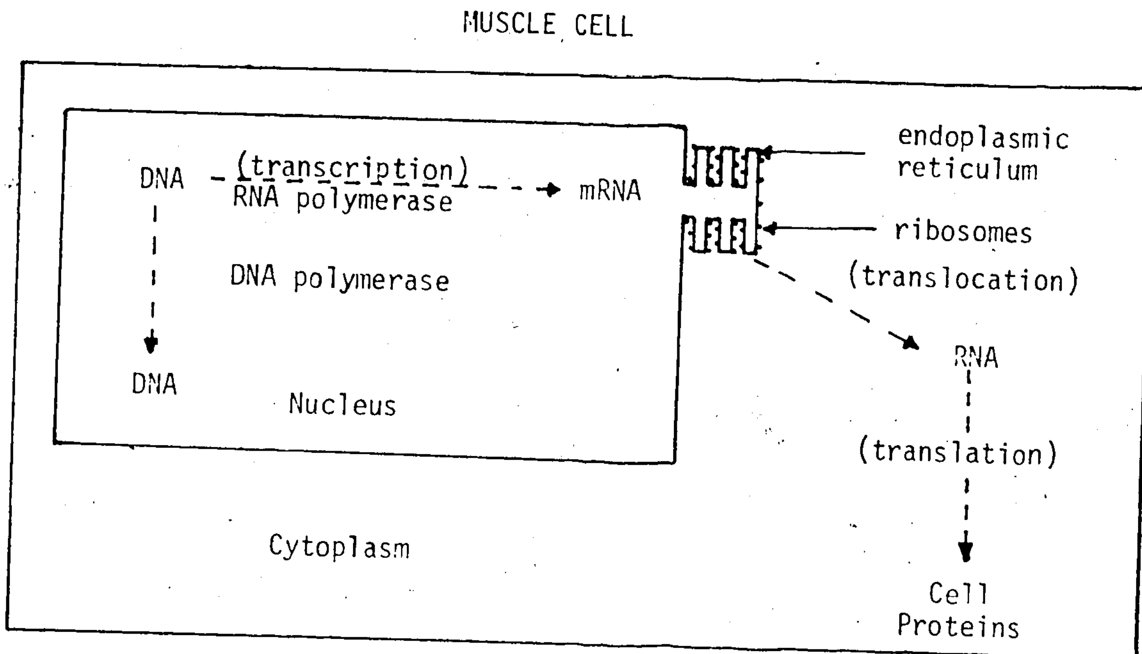


FIGURE 6. SIMPLIFIED SCHEMATIC DIAGRAM OF PROTEIN SYNTHESIS SHOWING THE TRANSCRIPTION, TRANSLOCATION AND TRANSLATION PROCESSES. (Modified from Edgerton, 1973).

The total amount of DNA in a cell is proportional to the number of chromosomes and genes in the nuclei. When cell division occurs, nuclear DNA is replicated by a reaction catalyzed by DNA polymerase. The constancy of DNA per cell nucleus has therefore been used as a tool to estimate the number of nuclei in skeletal muscle tissue (Enesco and Puddy, 1963; Winick and Noble, 1965). As mentioned previously several investigators have reported nuclear proliferation both prenatally (Burleigh, 1974) and postnatally (Enesco and Puddy, 1964; MacConnachie et al., 1964; Hubbard et al., 1974). It remains unclear in the literature whether with cessation of nuclear divisions there is yet an increase in total muscle DNA. Hubbard et al. (1974) reported an increase in both total number of muscle nuclei and the protein to DNA ratio (Bailey et al., 1973) in

growing rat gastrocnemius muscle. Increases in total muscle DNA and number of nuclei have been observed by others (Enesco and Puddy, 1964; Winick and Noble, 1965; Gordon et al., 1966; Bailey et al., 1973; Burleigh, 1974). Gordon et al. (1966) reported an increase of total DNA in growing rat quadriceps muscle up to 90 days of age. They suggested that muscle growth was due to both a proliferation of nuclei and hypertrophy up until this age, while total DNA per unit weight of tissue declines (Devi et al., Young, 1970; Bailey et al., 1973; Hubbard et al., 1974; Giovannetti and Stothers, 1975). This implies that although there is a rise in total muscle DNA it does not match the increase in whole muscle mass.

The increases in protein to DNA ratio which have been shown in developing muscle (Bailey et al., 1973; Hubbard et al., 1974; Millward et al., 1975) also reflect a greater increase in total muscle protein in comparison to DNA. Edgerton (1973) has suggested that increases in skeletal muscle DNA and nuclear numbers may be confounded by changes in connective tissue and satellite cells rather than muscle fibers. However, Young (1974) and Millward et al. (1975) have reported that non-contractile cells account for no more than a few per cent of observed changes. It should be remembered though, that nucleic acid content and growth of skeletal muscle can be obscured by several factors: 1. the variance in the number of muscle nuclei, 2. the extent of elongation in growing muscle fibers within species and 3. the units in which nucleic acids and protein content are measured with respect to muscle tissue concentration or whole muscle estimates (Burleigh, 1974).

Messenger RNA functions primarily in the cytoplasm of the cell as a template from which cellular proteins can be synthesized. However, more than 80% of skeletal muscle RNA is associated with ribosomes, so

that changes in ribosomal RNA content of skeletal muscle also reflect alterations in ribosome composition (Young, 1970). Ribosomes are not found within the actomyosin filaments of the muscle scarcomere but have been observed in the cytoplasm between myofibrils (Burleigh, 1974). Growing myofibrils possess rows of ribosomes on their surface which suggests that the protein needed for myofibrillar growth is synthesized in the sarcoplasm (Goldspink, 1970, 1971; Burleigh, 1974). The number of ribosomes associated with developing myofibrils declines in proportion to the increase in myofibrils. It is, therefore, unclear whether growing muscle fibers synthesize new ribosomes or merely produce more contractile and sarcoplasmic protein (Burleigh, 1974).

The total RNA content of growing skeletal muscle rises with increased muscle weight and fiber size, but, as is found with DNA, the concentration of muscle RNA, which is highest at birth, shows a steady decline with age (Devi et al., 1963; Winick and Noble, 1965; Srivastava and Chandhary, 1969; Young, 1970; Burleigh, 1974; Giovannetti and Stothers, 1975; Millward et al., 1975). Burleigh (1974) has proposed that neither the total amount of RNA or the concentration of RNA in muscle seems to show an obvious relationship to the rate of whole muscle growth. This proposal is corroborated by the findings of Winick and Noble (1965). Nevertheless, Srivastava and Chandhary (1969) have reported decreased precursor incorporation into RNA with aging. Changes in the rate and efficiency of RNA synthesis in skeletal muscle have also been demonstrated with diet manipulations (Millward et al., 1973). Hamosh et al. (1967) have also shown an increased RNA concentration in microsomes prepared from hypertrophied muscle. It may be that during normal growth the efficiency of RNA protein synthesis is a regulatory factor, while with

other influences on the muscle both efficiency and concentration of RNA affect protein synthesis and growth (Millward et al., 1975).

Protein breakdown as well as synthesis is also of importance when studying skeletal muscle growth (Young, 1970; Burleigh, 1974; Millward et al., 1970a, 1970b, 1975). Millward and co-workers (1975) did a comprehensive study of diet and protein turnover rates in growing skeletal muscle. They reported a sixfold increase in cell size, a fourfold increase in cell number, and a rise in total DNA content in growing rats maintained on a normal high protein diet. The rate of protein synthesis fell from 28.6% in young rats to 4.9%/day in rats 330 days old, and this was attributed to a decreased concentration of RNA [illegible] well as a decreased activity of RNA (g of protein synthesis/[illegible]/g of RNA). The protein breakdown rates coincided with the decreases [illegible] synthesis from young to old animals. A direct negative relationship was observed between rapid growth rates and a high rate of protein catabolism (Millward et al., 1975). Similar results have been reported by others (Srivastava and Chaudhary, 1969; Young, 1970; Burleigh, 1974). It could, therefore, be postulated that when the limits of protein synthesis have been reached in growing animals protein breakdown may control muscle growth.

Protein synthesis in growing skeletal muscle is influenced by other regulatory factors (Young, 1974). Diet or the nutritional state of a growing animal seems to have a pronounced effect on muscle proteins (Young, 1970, 1974; Millward et al., 1970b, 1973, 1975; Beecher, 1974; Burleigh, 1974; Trenkle, 1974; Giovannetti and Stothers, 1975). Changes in the concentrations of low molecular weight compounds (Rogozkin, 1976; Rogers et al., 1976), and the levels of amino acids (Goldberg, 1969; Millward et al., 1970a; Pain and Manchester, 1970; Young, 1970, 1974;

Giovannetti and Stothers, 1975) also appear to control protein metabolism in muscle. There is reasonable evidence for neurotrophic regulation of muscle development and this has been investigated by various research groups (Gutmann and Zak, 1961a, 1961b; Il'in et al., 1970; Edgerton, 1973; Young, 1974; Burešová et al., 1975). Lastly, the role of hormones in regulating skeletal muscle protein turnover and growth has been reviewed (Cheek et al., 1968a, 1968b; Goldberg, [illegible]; Young, 1970, 1974; Edgerton, 1973; Trenkle, 1974).

The factors affecting normal skeletal growth have been summarized in Figure 7.

PROTEIN METABOLISM AND EXERCISE

Few researchers have evaluated the effects of exercise on protein metabolism and skeletal muscle growth. Helander (1961) was the first investigator to study the protein compositional adaptations of working muscle. He reported that guinea pigs exposed to 4 months of mild endurance running had more myofibrillar protein than control animals or animals whose activity had been restricted. Hypertrophy was observed in both exercised and restricted animals, but exercise increased myofilamental size whereas restricted activity increased sarcoplasmic protein content. Opposite training effects of endurance running and swimming programs were observed by Gordon et al. (1967a) and Jaweed et al. (1974). They demonstrated muscle fiber hypertrophy and an increased concentration of sarcoplasmic protein which was commensurate with a reduction in myofibrillar protein of rat quadriceps and gastrocnemius muscles. The results of Gordon et al. (1967a) are similar to other findings reported in the literature (Yakovlev et al., 1963; Jaweed et al., 1974). Hubbard et al. (1974) con-

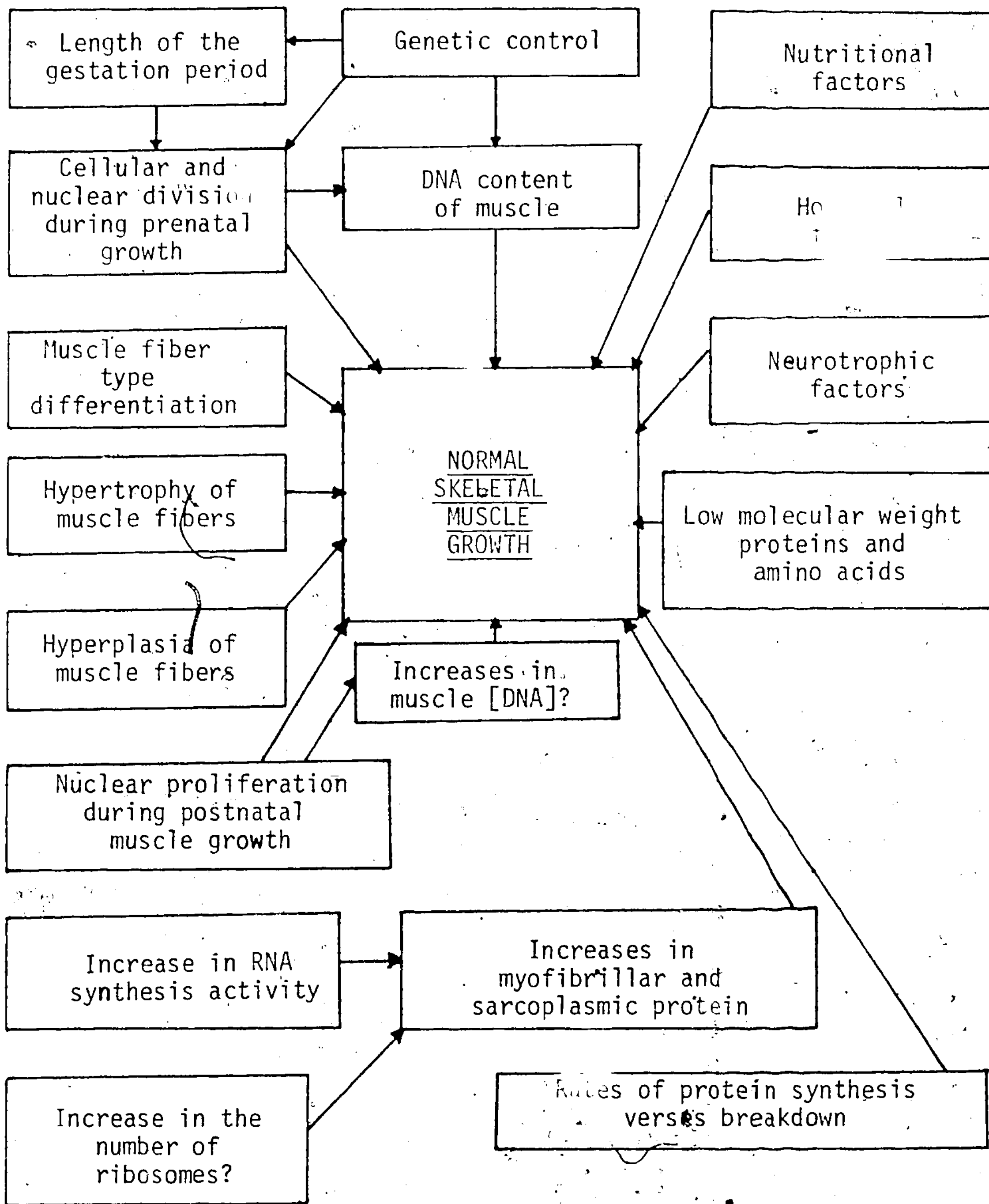


FIGURE 7. SUMMARY OF FACTORS AFFECTING MUSCLE GROWTH

fused the issue again when they found that endurance training resulted in non-selective hypertrophy of sarcoplasmic, myofibrillar and stromal protein. It is conceivable that Helander's (1961) and Hubbard's (1974) data could reflect an interaction effect between early muscle development and exercise. They used prepubertal animals and trained them between 6 and 22 weeks of age while the other researchers cited trained mature rats between 18 and 30 weeks of age (Gordon et al., 1967a; Jaweed et al., 1974).

In trying to elucidate differential training effects of endurance versus strengthening exercises Gordon et al. (1967b) and Jaweed et al. (1974) studied the muscle protein composition of rats exposed to weight lifting exercises. They found that weight lifting produced myofibrillar protein hypertrophy with little change [illegible] sarcoplasmic protein concentration. Hypertrophy of different muscl[illegible] types also appeared to be exercise specific. It was shown that strength training increased the mean area of white (FG) fibers and there was little change in red fiber size (Gordon et al., 1967b). These investigators also found that endurance training caused no change in whole muscle size but increases in the mean areas of both red and white muscle fibers may occur (Gordon et al., 1967a). It was concluded that myofibrillar hypertrophy stems from brief forceful exercises which increase strength and endurance training and leads to sarcoplasmic hypertrophy commensurate with an increased capacity for energy production (Gordon et al., 1967a, 1967b).

Similarly, muscle fiber hypertrophy changes have been reported with growth and endurance training of guinea pigs (Faulkner et al., 1971; Maxwell et al., 1973). Training produced increases in the mean area of SO fibers in soleus muscles along with hypertrophy of all three fiber

types in plantaris (Maxwell et al., 1973). Faulkner et al. (1971) found an increased proportion of red to white muscle fibers in plantaris after endurance training. Trained muscle also contained 37% more fibers than controls. Both investigators observed muscle fiber hypertrophy with normal growth between 6 and 14 weeks of age.

Bailey et al. (1973) and Hubbard et al. (1974) have recently studied the chronic effect of endurance training on growth and protein synthesis in young rats. Both research groups reported increases in body and muscle weights with age in both control and exercised groups. They also found no significant differences in muscle weights between control and exercised animals. Hubbard et al. (1974) observed lower total body weights in running animals compared with their age-matched controls (Gordon et al., 1967a, 1967b; Fitts et al., 1973, 1974; Wilkinson et al., 1976). Bailey et al. (1973), however, showed no significant weight differences in swimming animals which is also in accordance with the work of Gordon et al. (1967a). However, changes in both body weight and muscle weights can be misleading. Control animals may increase body weight largely due to fat deposition while exercising animals may have a higher ratio of total body protein to fat. Muscle weights are also more clearly expressed in terms of whole body weight (Hubbard et al., 1974).

Nucleic acid content of trained skeletal muscle has been investigated by several of the previously mentioned authors, but again there are inconsistancies in the findings. Gordon et al. (1967a, 1967b) found no change in the total muscle DNA content of mature rats trained with endurance running, swimming and weight lifting programs. These findings have been confirmed in young animals exercised between the ages of 3 and 12 weeks (Bailey et al., 1973). The concentration of DNA (μg/mg wet wt) in

developing rat muscle decreases with age while chronic exercise effects are still unclear (Bailey et al., 1973; Hubbard et al., 1974). Young swim-trained rats had significantly increased concentrations of DNA in gastrocnemius muscles when compared to age-matched controls (Bailey et al., 1973). In contrast, Hubbard et al. (1974) showed no changes in trained gastrocnemius muscles while significant decreases in DNA concentrations of soleus and plantaris muscles were found in rats exercised up to 18 weeks of age. Similarly, these investigators reported larger ratios of protein per mg DNA in both control and exercised skeletal muscle with age. They also demonstrated a significantly increased protein to DNA ratio resulting from endurance training. However, there was some disagreement concerning the age at which these training adaptations occurred (Bailey et al., 1973; Hubbard et al., 1974). These discrepancies may again reflect an interaction effect between normal muscle growth and exercise alterations, since these researchers began training their animals at different ages (Bailey et al., 1973; Hubbard et al., 1974).

RNA concentration of rat gastrocnemius and cardiac muscles has been studied after acute exhaustive swimming (Bostrom et al., 1974). Cardiac tissue RNA concentration significantly decreased immediately after acute exercise while there was no change in the gastrocnemius. One to three days after exercise a significant rise in RNA was observed in both cardiac and gastrocnemius muscle. It was suggested that the decline in cardiac mustle RNA following exercise reflected an increase in protein catabolism. Further evidence for rises in protein catabolism following prolonged endurance exercise has been reviewed by Poortmans (1975). Then 24 to 72 hours after exercise there is an increase in RNA mediated protein synthesis (Bostrom et al., 1974). These findings have been con-

firmed in a review of Soviet research (Rogozkin, 1976a). Rogozkin (1976b) has also reported increases in myofibrillar, scarcoplasmic and stromal protein content of endurance trained rat skeletal muscle. He has proposed that an increase in free amino acid levels along with an enhanced nuclear-cytoplasmic transport of RNA produces an increase in protein synthetic activity of endurance trained skeletal muscle.

Consolazio et al. (1975) investigated urinary nitrogen excretion levels of eight human male subjects during a five week training program. Training was very intense including treadmill running, bicycle exercise, calesthenics, and isometric training regimens. All subjects showed significant increases in protein intake during training matched with weight gains after approximately 10 days of training. There was no evidence of increased protein catabolism with exercise when urinary nitrogen excretion levels were monitored. These findings provided indirect evidence for enhanced protein storage and synthesis with strenuous training accompanied by little change in protein breakdown (Consolazio et al., 1975).

The incorporation of radioactively tagged amino acids into electrically stimulated rat skeletal muscle has also been studied. Pain and Manchester (1970) found that incorporation was decreased during and immediately following stimulation. An increase in amino acid incorporation was then observed several hours after stimulation. They explained this change as a compensatory response to the initial decline which resulted from exercise. Similar results involving tritiated uridine uptake have been reported recently when rat soleus muscles were electrically stimulated (Muchnick and Kotsian, 1975).

A considerable body of knowledge has developed concerning the adaptation of skeletal muscle to surgically induced compensatory hypertrophy.

Goldberg et al. (1975) recently did a comprehensive review of this subject. He discussed increases in muscle weights, fiber areas, protein content, protein turnover, DNA, RNA and amino acid uptake which have been observed with compensatory hypertrophy. However, the magnitude of these changes are not necessarily analogous to the changes one might expect in normal exercise hypertrophy. Maximal electrical stimulation and surgically induced hypertrophy places an abnormally large overload on skeletal muscle, above and beyond in vivo exercise stress.

The chronic effects of exercise on muscle growth and protein metabolism are summarized in Figure 8.

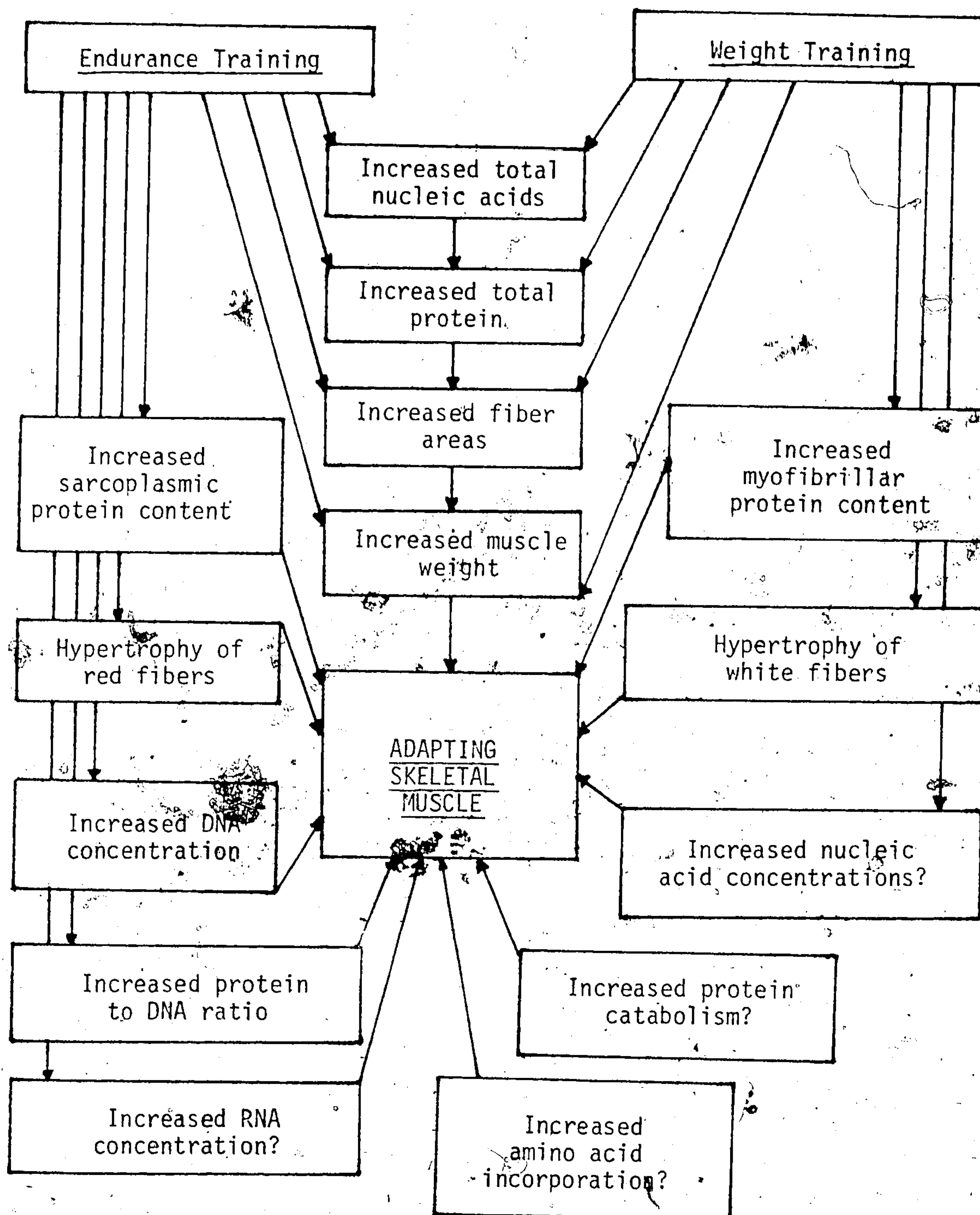


FIGURE 8. SUMMARY OF THE EFFECTS OF EXERCISE ON MUSCLE GROWTH AND PROTEIN SYNTHESIS.

APPENDIX B

TABLE VII BODY WEIGHTS AND MUSCLE WEIGHTS FOR THE SIX GROUPS OF ANIMALS

ANIMAL GROUP and NUMBER		BODY WEIGHT (g)	MUSCLE WEIGHT (g)	ANIMAL GROUP and NUMBER		BODY WEIGHT (g)	MUSCLE WEIGHT (g)
C5	112	124	0.436	ST5	117	123	0.459
	113	119	0.422		118	119	0.445
	114	123	0.447		119	122	0.423
	115	114	0.418		120	117	0.390
	116	117	0.423		121	123	0.450
MEAN SEM		119 ±2	0.435 ±0.009			121 ±1	0.450 ±0.013
C15	59	395	1.975	ST15	39	436	2.116
	60	371	1.718		40	354	1.741
	61	408	1.731		41	362	1.795
	62	429	2.108		42	337	1.727
	63	463	2.013		43	391	1.974
	64	435	2.140		44	371	1.784
	65	420	1.873		47	406	1.967
	66	[illegible]	1.857		48	288	1.492
	67	311	1.642		—	—	—
	68	402	1.911		—	—	—
MEAN SEM		401 ±13	1.897 ±0.053			368 ±16	1.82[illegible] ±0.068
C25	25	494	2.062	ST25	13	525	2.550
	26	500	2.360		15	454	2.277
	27	495	2.291		16	534	2.535
	28	468	2.047		17	500	2.216
	29	480	2.5[illegible]3		18	454	2.276
	30	568	2.663		19	500	2.292
	31	482	2.176		20	470	2.306
	32	465	1.898		21	506	2.420
	33	518	2.300		24	456	1.473
	34	532	2.132		—	—	—
MEAN SEM		500 ±10	2.244 ±0.072			389 ±10	2.262 ±0.107

TABLE VIII FIBER TYPE POPULATIONS IN THE MEDIAL GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		FIBER TYPING (%) SO	FOG	FG	ANIMAL GROUP and NUMBER		FIBER TYPING (%) SO	FOG	FG
	112	13.4	68.6	18.0		117	13.0	67.6	19.4
	113	20.1	50.5	29.4		118	15.1	63.7	21.2
C5	114	13.1	65.1	21.8	ST5	119	19.6	67.0	13.[illegible]
	115	12.2	51.7	36.1		120	16.3	66.1	17[illegible]
	116	13.6	60.3	26.1		121	14.6	71.3	14.[illegible]
MEAN SEM		14.5 ±1.4	59.2 ±3.6	26.3 ±3.1			15.7 ±1.1	67.1 ±1.2	17.1 ±1.5
	59	22.8	49.2	28.0		39	22.0	41.0	37.0
	60	23.2	60.5	16.3		40	13.4	48.0	38.6
	61	13.8	67.0	19.2		41	16.4	46.6	37.0
	62	23.4	55.3	21.3		42	16.9	41.3	41.8
C15	63	22.2	57.7	20.0	ST15	43	10.9	55.2	33.8
	64	—	—	—		44	21.4	44.4	34.2
	65	17.7	61.0	21.3		47	17.7	47.3	35.0
	66	20.4	60.8	18.8		48	15.9	53.5	30.6
	67	16.4	62.6	21.0		—	—	—	—
	68	14.3	61.6	24.1		—	—	—	—
MEAN SEM		19.4 ±1.3	59.5 ±1.7	21.1 ±1.1			16.8 ±1.3	47.2 ±1.8	36.0 ±1.2
	25	23.3	56.7	20.0		13	16.7	53.0	30.3
	26	21.8	56.2	22.0		15	15.0	54,6	30.4
	27	21.2	59.0	19.8		16	16.1	51.1	32.8
	28	18.5	61.5	20.1		17	9.2	45.7	35.1
C25	29	23.3	52.7	14.0	ST25	18	18.9	46.9	34.2
	30	14.2	69.5	16.3		19	18.6	49.8	31.6
	31	—	—	—		20	—	—	—
	32	21.4	62.9	15.7		21	18.9	43.3	37.8
	33	—	—	—		24	19.2	48.9	31.9
	34	25.1	53.8	21.1		—	—	—	—
MEAN SEM		21.1 ±1.2	60.3 ±1.8	18.6 ±1.0			17.8 ±0.6	49.2 ±1.3	33.0 ±0.9

TABLE IX FIBER AREAS IN THE MEDIAL GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		FIBER AREAS (μ^2) SO	FOG	FG	ANIMAL GROUP and NUMBER		FIBER AREAS (μ^2) SO	FOG	FG
	112	——	——	——		117	912	791	1317
	113	1048	806	1254		118	1317	1088	1706
C5	114	1138	745	1197	ST5	119	1077	805	1202
	115	894	867	1302		120	1110	847	1525
	116	980	812	1269		121	919	858	1375
MEAN SEM		1015 ±52	808 ±25	1256 ±23			1067 ±63	878 ±54	1425 ±87
	59	——	——	——		39	2980	2502	3546
	60	1780	1999	2864		40	——	——	——
	61	1853	1871	3057		41	1990	1849	3111
	62	1764	1639	2531		42	1859	1770	2670
C15	63	2142	2562	3505	ST15	43	2217	3105	3562
	64	——	——	——		44	1821	2179	3058
	65	2009	1884	2786		47	1846	2133	3037
	66	1521	1604	262[illegible]		48	2245	2107	3134
	67	2063	2033	3150		—	——	——	——
	68	1623	1800	2894		—	——	——	——
MEAN SEM		1844 ±76	1924 ±106	2926 ±110			2137 ±155	2092 ±90	3160 ±117
	25	1898	2157	2897		13	2980	3115	4410
	26	1988	2137	3180		15	2209	2622	4199
	27	2096	2681	3647		16	1848	1917	3589
	28	1892	2041	2682		17	1963	2225	3492
C25	29	1514	1823	2399	ST25	18	2136	2006	3148
	30	2266	2663	4095		19	1953	1934	2927
	31	——	——	——		20	——	——	——
	32	1838	2052	2744		21	2364	2661	3377
	33	——	——	——		24	2610	2361	3423
	34	2070	2344	3171		—	——	——	——
MEAN SEM		1945 ±79	2237 ±108	3102 ±195			2258 ±135	2355 ±149	3571 ±177

TABLE X TOTAL HOMOGENATE PROTEIN AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER	HOMOGENATE (mg)	PROTEIN (mg/g)	ANIMAL GROUP and NUMBER	HOMOGENATE (mg)	PROTEIN (mg/g)
112	70.5	152.3	117	69.9	165.6
113	61.5	147.1	118	64.8	141.2
C5 114	69.5	164.3	ST5 119	61.6	138.4
115	60.9	144.0	120	59.4	132.9
116	65.8	168.7	121	70.0	155.6
MEAN SEM	65.6 ±2.0	155.3 ±4.8		65.1 ±2.1	146.7 ±6.0
59	394.2	199.6	39	429.3	202.9
60	386.1	244.7	40	369.9	212.5
61	321.3	185.6	41	380.7	212.1
62	413.1	196.0	42	375.3	217.3
C15 63	377.4	187.5	ST15 43	432.0	218.8
64	456.3	213.2	44	370.0	207.3
65	421.2	224.9	47	396.9	201.8
66	315.9	170.1	48	319.7	214.3
67	318.6	194.0	—	—	—
68	405.0	211.9	—	—	—
MEAN SEM	380.9 ±15.2	202.8 ±6.8		384.2 ±12.8	210.9 ±2.2
25	399.6	193.8	13	459.5	180.2
26	437.4	185.3	15	450.4	197.8
27	450.9	196.8	16	459.0	181.1
28	402.3	196.5	17	469.8	212.0
C25 29	472.5	188.0	ST25 18	412.3	181.2
30	491.4	184.5	19	469.8	205.0
31	396.9	182.4	20	434.7	188.5
32	394.2	207.7	21	479.5	198.1
33	409.3	178.0	24	253.8	172.3
34	396.9	186.2	—	—	—
MEAN SEM	425.1 ±11.3	189.9 ±2.8		432.1 ±23.3	190.7 ±4.4

TABLE XI TOTAL MYOFIBRILLAR-NUCLEAR PROTEIN AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		MYOFIBRILLAR-NUCLEAR PROTEIN (mg)	(mg/g)	ANIMAL GROUP and NUMBER		MYOFIBRILLAR-NUCLEAR PROTEIN (mg)	(mg/g)
	112	41.9	90.5		117	41.6	98.6
	113	41.4	99.0		118	43.6	95.0
C5	114	40.6	96.0	ST5	119	41.5	93.3
	115	41.0	96.9		120	35.3	79.0
	116	38.4	98.5		121	40.9	90.[illegible]
MEAN SEM		40.7 ±0.6	96.2 ±1.5			40.6 ±1.4	91.4 ±3.3
	59	291.6	147.6		39	391.5	185.0
	60	221.4	128.9		40	237.6	136.5
	61	234.9	135.7		41	321.3	179.0
	62	270.0	128.1		42	272.7	157.9
C15	63	261.9	130.1	ST15	43	372.6	188.8
	64	268.9	125.6		44	346.7	194.3
	65	294.3	157.1		47	307.8	156.5
	66	268.9	144.8		48	268.9	180.2
	67	202.6	123.4		—	—	—
	68	325.1	170.1		—	—	—
MEAN SEM		264.0 ±11.5	139.1 ±4.9			314.9 ±19.0	172.3 ±7.0
	25	315.9	153.0		13	380.7	149.3
	26	341.3	144.6		15	294.3	129.2
	27	378.0	165.0		16	380.7	150.2
	28	267.3	130.6		17	396.9	179.1
C25	29	364.5	145.0	ST25	18	284.4	124.9
	30	402.3	151.1		19	334.8	146.1
	31	325.1	149.4		20	302.4	131.1
	32	306.7	161.6		21	402.3	166.2
	33	322.9	140.4		24	199.8	135.6
	34	247.3	116.0		—	—	—
MEAN SEM		327.1 ±15.0	145.7 ±4.6			330.7 ±22.3	145.7 ±6.0

TABLE XII TOTAL SOLUBLE PROTEIN AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		SOLUBLE PROTEIN (mg)	(mg/g)	ANIMAL GROUP and NUMBER		SOLUBLE PROTEIN (mg)	(mg/g)
	112	18.9	40.8		117	17.8	42.2
	113	16.6	39.7		118	17.5	38.1
C5	114	16.5	39.0	ST5	119	16.6	37.3
	115	16.4	38.8		120	15.1	33.8
	116	15.6	40.0		121	16.6	36.9
MEAN SEM		16.8 ±0.6	39.7 ±0.4			16.7 ±0.5	37.7 ±1.4
	59	100.9	51.1		39	87.9	41.7
	60	84.2	49.0		40	60.2	34.7
	61	90.2	52.1		41	85.6	47.7
	62	81.0	33.4		42	83.3	48.2
C15	63	84.2	41.8	ST15	43	83.3	42.2
	64	70.4	32.9		44	69.4	38.9
	65	74.1	39.6		47	84.2	42.8
	66	81.0	43.6		48	83.3	55.8
	67	76.4	46.5		—	—	—
	68	90.3	47.2		—	—	—
MEAN SEM		83.3 ±2.8	44.2 ±1.9			79.6 ±3.4	44.0 ±2.3
	25	106.5	51.6		13	92.6	36.3
	26	143.5	60.8		15	90.3	39.7
	27	96.3	42.0		16	83.3	32.9
	28	108.8	53.1		17	84.2	38.0
C25	29	134.2	53.4	ST25	18	94.9	41.7
	30	102.6	38.5		19	96.3	42.0
	31	122.7	56.4		20	87.9	38.1
	32	87.9	46.3		21	90.3	37.3
	33	99.5	43.3		24	48.6	33.0
	34	101.8	47.8		—	—	—
MEAN SEM		110.4 ±5.6	49.3 ±2.2			85.4 ±4.8	37.7 ±1.1

TABLE XIII TOTAL MITOCHONDRIAL PROTEIN AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		MITOCHONDRIAL PROTEIN (mg)	(mg/g)	ANIMAL GROUP and NUMBER		MITOCHONDRIAL PROTEIN (mg)	(mg/g)
	112	0.26	0.56		117	0.26	0.62
	113	0.23	0.55		118	0.24	0.52
C5	114	0.21	0.50	ST5	119	0.23	0.52
	115	0.23	0.54		120	0.22	0.49
	116	0.20	0.51		121	0.21	0.47
MEAN SEM		0.23 ±0.01	0.53 ±0.12			0.23 ±0.01	0.52 ±0.03
	59	1.60	0.18		39	4.75	2.25
	60	1.73	1.01		40	4.75	2.73
	61	1.73	1.00		41	—	—
	62	1.05	0.50		42	4.63	2.68
C15	63	5.92	2.94	ST15	43	2.72	1.38
	64	2.59	1.21		44	3.21	1.80
	65	1.73	0.92		47	3.09	1.57
	66	8.33	4.49		48	1.85	1.24
	67	9.87	6.01		—	—	—
	68	2.16	1.13		—	—	—
MEAN SEM		3.67 ±1.01	2.00 ±0.59			3.57 ±0.43	1.95 ±0.23
	25	0.93	0.45		13	5.55	2.18
	26	2.60	1.10		15	4.69	2.06
	27	1.67	0.73		16	—	—
	28	1.36	0.66		17	3.58	1.62
C25	29	2.47	0.98	ST25	18	3.76	1.65
	30	2.16	0.81		19	3.83	1.67
	31	8.33	3.83		20	4.20	1.82
	32	3.09	1.62		21	1.36	0.56
	33	6.79	2.95		24	0.68	0.46
	34	1.97	0.93		—	—	—
MEAN SEM		3.14 ±0.77	1.41 ±0.35			3.46 ±0.58	1.50 ±0.23

TABLE XIV TOTAL MICROSOMAL PROTEIN AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		MICROSOMAL PROTEIN (mg)	MICROSOMAL PROTEIN (mg/g)	ANIMAL GROUP and NUMBER		MICROSOMAL PROTEIN (mg)	MICROSOMAL PROTEIN (mg/g)
C5	112	0.43	0.93	ST5	117	0.43	1.02
	113	0.41	0.98		118	0.44	0.96
	114	0.46	1.09		119	0.42	0.94
	115	0.41	0.97		120	0.37	0.83
	116	0.44	1.13		121	0.46	1.02
MEAN SEM		0.43 ±0.01	1.02 ±0.04			0.42 ±0.02	0.95 ±0.04
C15	59	3.09	1.56	ST15	39	4.69	2.22
	60	2.65	1.54		40	1.85	1.06
	61	1.85	1.07		41	1.97	1.10
	62	1.85	0.88		42	2.22	1.29
	63	2.72	1.35		43	2.35	1.19
	64	5.25	2.45		44	2.53	1.42
	65	2.41	1.29		47	2.96	1.51
	66	2.47	1.33		48	2.47	1.66
	67	2.28	1.39		—	—	—
	68	2.47	1.29		—	—	—
MEAN SEM		2.70 ±0.31	1.42 ±0.13			2.63 ±0.32	1.43 ±0.13
C25	25	3.47	1.68	ST25	13	8.95	3.51
	26	2.59	1.10		15	2.96	1.30
	27	1.60	0.70		16	4.63	1.83
	28	2.04	0.99		17	2.90	1.31
	29	5.00	1.99		18	—	—
	30	4.01	1.51		19	4.94	2.15
	31	—	—		20	5.25	2.27
	32	3.52	1.86		21	2.47	1.02
	33	[illegible]	1.89		24	2.10	1.42
	34	2.47	1.16		—	—	—
MEAN SEM		3.22 ±0.37	1.43 ±0.15			4.28 ±0.79	1.85 ±0.28

TABLE XV TOTAL HOMOGENATE RNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		HOMOGENATE RNA (µg)	HOMOGENATE RNA (µg/g)	ANIMAL GROUP and NUMBER		HOMOGENATE RNA (µg)	HOMOGENATE RNA (µg/g)
C5	112	660	1425	ST5	117	654	1549
	113	509	1218		118	537	1170
	114	650	1536		119	510	1146
	115	504	1191		120	556	1243
	116	615	1578		121	656	1458
MEAN SEM		587.6 ±34.0	1389.6 ±79.7			582.6 ±30.4	1313.2 ±80.6
C15	59	1920	972	ST15		2040	964
	60	1680	979		40	1900	1093
	61	1320	763		41	1440	802
	62	2160	1025		42	1480	856
	63	1820	906		43	2200	1114
	64	1920	897		44	1740	978
	65	1560	833		47	1980	1009
	66	1720	922		48	1170	782
	67	1480	901		—	—	—
	68	1980	1038		—	—	—
MEAN SEM		1756.0 ±80.5	923.6 ±26.7			1743.8 ±124.4	949.7 ±44.5
C25	25	1780	861	ST25	13	2050	803
	26	2000	847		15	2080	913
	27	1790	782		16	2040	805
	28	1920	940		17	2280	1028
	29	2040	812		18	1760	773
	30	2160	811		19	2020	880
	31	1840	846		20	2260	978
	32	1660	877		21	2040	843
	33	1700	737		24	992	673
	34	1680	788		—	—	—
MEAN SEM		1857.0 ±53.3	830.1 ±18.0			1946.9 ±137.3	855.1 ±36.2

TABLE XVI TOTAL MYOFIBRILLAR-NUCLEAR RNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		MYOFIBRILLAR-NUCLEAR RNA (µg)	MYOFIBRILLAR-NUCLEAR RNA (µg/g)	ANIMAL GROUP and NUMBER		MYOFIBRILLAR-NUCLEAR RNA (µg)	MYOFIBRILLAR-NUCLEAR RNA (µg/g)
C5	112	356	769	ST5	117	353	836
	113	326	780		118	344	749
	114	349	825		119	327	735
	115	323	764		120	300	671
	116	331	849		121	352	782
MEAN SEM		337.0 ±6.5	797.4 ±16.8			335.2 ±10.0	754.6 ±27.2
C15	59	1360	689	ST15	39	1410	665
	60	1040	605		40	1180	680
	61	880	508		41	1040	579
	62	1040	493		42	1060	611
	63	860	429		43	1200	608
	64	1080	505		44	1020	574
	65	1040	555		47	1280	651
	66	920	495		48	780	525
	67	880	536		—	—	—
	68	1020	532		—	—	—
MEAN SEM		1012.0 ±46.5	534.7 ±22.4			1121.3 ±67.5	611.6 ±18.5
C25	25	1240	601	ST25	13	1360	533
	26	1120	475		15	1060	464
	27	1300	566		16	1120	442
	28	1200	586		17	1060	476
	29	1320	525		18	984	432
	30	1480	556		19	1120	489
	31	1140	522		20	1180	513
	32	1120	590		21	1420	588
	33	1240	539		24	520	353
	34	1180	555		—	—	—
MEAN SEM		1234.0 ±35.2	551.5 ±12.0			1091.6 ±85.8	476.7 ±22.3

TABLE XVII TOTAL SOLUBLE RNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		SOLUBLE RNA (µg)	SOLUBLE RNA (µg/g)	ANIMAL GROUP and NUMBER		SOLUBLE RNA (µg)	SOLUBLE RNA (µg/g)
	112	278	600		117	286	678
	113	213	510		118	225	490
C5	114	269	636	ST5	119	214	481
	115	211	499		120	235	526
	116	255	654		121	271	602
MEAN		245.2	579.8			246.2	555.4
SEM		±14.0	±32.0			±13.8	±37.3
	59	631	319		39	706	334
	60	720	419		40	446	256
	61	569	329		41	343	191
	62	624	286		42	645	373
C15	63	686	341	ST15	43	925	469
	64	645	301		44	720	403
	65	590	315		47	768	390
	66	693	373		48	473	317
	67	617	375		—	—	—
	68	754	395		—	—	—
MEAN		652.9	345.3			628.3	341.6
SEM		±18.6	±13.7			±68.3	±31.1
	25	535	259		13	816	320
	26	891	378		15	782	343
	27	699	305		16	802	316
	28	542	265		17	939	424
C25	29	713	284	ST25	18	795	349
	30	720	270		19	823	359
	31	727	334		20	891	387
	32	494	260		21	837	346
	33	665	289		24	569	386
	34	514	241		—	—	—
MEAN		650.0	288.5			806.0	358.9
SEM		±39.9	±13.0			±34.0	±11.6

TABLE XVIII TOTAL MITOCHONDRIAL RNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		MITOCHONDRIAL RNA (μg)	MITOCHONDRIAL RNA (μg/g)	ANIMAL GROUP and NUMBER		MITOCHONDRIAL RNA (μg)	MITOCHONDRIAL RNA (μg/g)
	112	10.9	23.5		117	10.8	25.6
	113	10.7	25.6		118	11.3	24.6
C5	114	10.1	23.9	ST5	119	10.7	25.0
	115	10.6	25.1		120	9.6	21.5
	116	9.5	24.4		121	10.1	22.4
MEAN SEM		10.4 ±0.3	24.5 ±0.4			10.5 ±0.3	23.6 ±0.7
	59	34.3	19.6		39	51.4	24.3
	60	—	—		40	46.9	26.6
	61	24.0	13.9		41	32.0	17.8
	62	30.8	14.6		42	54.9	31.8
C15	63	61.7	30.6	ST15	43	41.1	20.8
	64	49.1	22.9		44	30.8	17.3
	65	34.3	18.3		47	43.4	22.1
	66	68.6	36.9		48	24.0	16.1
	67	46.9	28.3		—	—	—
	68	40.0	20.9		—	—	—
MEAN SEM		43.3 ±4.9	22.9 ±2.6			40.6 ±3.8	22.1 ±1.9
	25	18.3	8.9		13	59.4	23.3
	26	34.3	14.5		15	26.3	11.6
	27	24.0	10.4		16	40.0	15.8
	28	18.3	8.9		17	54.9	24.8
C25	29	34.3	13.6	ST25	18	60.6	26.6
	30	54.9	20.6		19	53.7	23.4
	31	45.7	21.0		20	57.1	24.8
	32	49.1	25.8		21	20.6	8.5
	33	45.7	19.9		24	19.4	13.2
	34	16.0	7.5		—	—	—
MEAN SEM		34.1 ±4.5	15.1 ±2.0			43.6 ±5.7	19.1 ±2.3

TABLE XIX TOTAL MICROSOMAL RNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		MICROSOMAL RNA (µg)	MICROSOMAL RNA (µg/g)	ANIMAL GROUP and NUMBER		MICROSOMAL RNA (µg)	MICROSOMAL RNA (µg/g)
	112	14.5	31.3		117	14.4	34.1
	113	18.0	43.1		118	19.0	41.4
C5	114	14.7	34.8	ST5	119	18.1	40.7
	115	17.9	42.3		120	12.2	27.3
	116	13.9	35.6		121	14.8	32.9
MEAN SEM		15.8 ±0.9	37.4 ±2.3			15.7 ±1.3	35.3 ±2.6
	59	46.9	23.7		39	36.6	17.3
	60	36.6	21.3		40	20.6	11.8
	61	41.1	23.7		41	18.3	10.2
	62	41.1	19.5		42	20.6	11.9
C15	63	45.7	22.7	ST15	43	53.7	27.2
	64	29.7	13.9		44	45.7	25.6
	65	34.3	20.5		47	43.4	22.1
	66	24.0	12.9		48	18.3	12.3
	67	30.8	18.8		—	—	—
	68	26.3	13.8		—	—	—
MEAN SEM		35.7 ±2.5	19.1 ±1.3			32.2 ±5.1	17.3 ±2.4
	25	49.1	23.8		13	61.7	24.2
	26	36.6	15.5		15	—	—
	27	26.3	11.5		16	49.1	19.2
	28	34.3	16.8		17	51.4	23.2
C25	29	43.4	17.2	ST25	18	64.0	28.1
	30	54.9	20.6		19	59.4	25.9
	31	45.7	21.0		20	57.1	24.8
	32	41.1	21.7		21	61.7	25.5
	33	29.7	12.9		24	41.1	27.9
	34	40.0	18.7		—	—	—
MEAN SEM		40.1 ±2.8	18.0 ±1.2			55.7 ±2.8	24.8 ±1.0

TABLE XX — TOTAL HOMOGENATE DNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		HOMOGENATE DNA (μg)	HOMOGENATE DNA (μg/g)	ANIMAL GROUP and NUMBER		HOMOGENATE DNA (μg)	HOMOGENATE DNA (μg/g)
	112	416	898		117	413	979
	113	372	890		118	392	854
C5	114	398	941	ST5	119	373	838
	115	368	870		120	351	785
	116	377	967		121	401	891
MEAN		386.2	913.2			386.0	869.4
SEM		±[illegible]	±17.8			±10.9	±32.3
	59	1180	572		39	1280	605
	60	1000	583		40	992	570
	61	960	556		41	1020	568
	62	1112	528		42	1008	584
C15	63	1288	640	ST15	43	1080	547
	64	1080	505		44	1152	646
	65	1060	566		47	1120	569
	66	1168	629		48	860	576
	67	968	590		—	—	—
	68	1032	540		—	—	—
MEAN		1084.8	570.9			1064.0	583.1
SEM		±33.0	±13.4			±44.3	±10.7
	25	1080	572		13	1072	420
	26	1232	583		15	1088	478
	27	1152	556		16	1240	489
	28	920	528		17	952	430
C25	29	1088	640	ST25	18	1180	518
	30	1240	505		19	1120	489
	31	980	566		20	1020	442
	32	928	629		21	1152	476
	33	1160	590		24	820	557
	34	1080	540		—	—	—
MEAN		1086.0	484.6			1071.6	477.7
SEM		±36.3	±10.3			±42.4	±14.5

TABLE XXI TOTAL MYOFIBRILLAR-NUCLEAR DNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		MYOFIBRILLAR-NUCLEAR DNA (µg)	MYOFIBRILLAR-NUCLEAR DNA (µg/g)	ANIMAL GROUP and NUMBER		MYOFIBRILLAR-NUCLEAR DNA (µg)	MYOFIBRILLAR-NUCLEAR DNA (µg/g)
C5	112	395	853	ST5	117	392	929
	113	348	833		118	368	802
	114	393	929		119	367	825
	115	345	817		120	333	745
	116	372	954		121	395	878
MEAN SEM		370.6 ±10.6	877.2 ±[illegible]7.2			371.0 ±11.1	835.8 ±31.6
C15	59	1120	543	ST15	39	1240	586
	60	980	570		40	952	547
	61	1072	619		41	960	535
	62	1088	516		42	1020	591
	63	1232	612		43	1032	523
	64	1048	490		44	1120	628
	65	1040	555		47	1088	553
	66	1200	646		48	840	563
	67	928	565		—	—	—
	68	980	512		—	—	—
MEAN SEM		1068.8 ±30.5	562.8 ±16.0			1031.5 ±42.8	565.7 ±12.1
C25	25	1000	485	ST25	13	1040	408
	26	1120	475		15	1032	453
	27	960	419		16	1180	465
	28	860	420		17	848	383
	29	1060	422		18	1160	510
	30	1128	424		19	1100	480
	31	960	441		20	1000	434
	32	860	453		21	1042	433
	33	1120	487		24	768	521
	34	1060	497		—	—	—
MEAN SEM		1012.8 ±32.2	452.3 ±9.9			1018.9 ±45.0	454.1 ±15.1

TABLE XXII TOTAL SOLUBLE DNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		SOLUBLE DNA (µg)	SOLUBLE DNA (µg/g)	ANIMAL GROUP and NUMBER		SOLUBLE DNA (µg)	SOLUBLE DNA (µg/g)
	112	2.2	4.8		117	2.2	5.2
	113	2.4	5.8		118	2.6	5.6
C5	114	2.6	6.1	ST5	119	2.6	5.8
	115	2.4	5.7		120	1.9	4.2
	116	2.4	6.3		121	2.6	5.8
MEAN SEM		2.4 ±0.1	5.7 ±0.3			2.4 ±0.1	5.3 ±0.3
	59	8.6	4.4		39	[illegible].3	3.0
	60	8.4	4.9		40	8.0	4.6
	61	7.4	4.3		41	6.8	3.8
	62	8.6	4.1		42	8.0	4.6
C15	63	8.0	4.0	ST15	43	9.4	4.8
	64	7.8	3.6		44	7.8	4.4
	65	8.4	4.5		47	8.8	4.5
	66	5.7	3.1		48	7.8	5.2
	67	7.4	4.5		—	—	—
	68	8.8	4.6		—	—	—
MEAN SEM		7.9 ±0.3	4.2 ±0.2			7.9 ±0.4	4.4 ±0.2
	25	8.6	4.2		13	8.6	3.4
	26	7.4	3.1		15	7.4	3.3
	27	9.1	4.0		16	7.4	2.9
	28	5.7	2.8		17	9.1	4.1
C25	29	8.8	3.5	ST25	18	6.8	3.0
	30	6.3	2.4		19	6.8	3.0
	31	6.8	3.1		20	10.3	4.5
	32	10.9	5.7		21	7.8	3.2
	33	7.4	3.2		24	6.3	4.3
	34	7.6	3.6		—	—	—
MEAN SEM		7.9 ±0.5	3.6 ±0.3			7.8 ±0.4	3.5 ±0.2

TABLE XXIII TOTAL MITOCHONDRIAL DNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		MITOCHONDRIAL DNA (μg)	MITOCHONDRIAL DNA (μg/g)	ANIMAL GROUP and NUMBER		MITOCHONDRIAL DNA (μg)	MITOCHONDRIAL DNA (μg/g)
	112	3.2	6.9		117	3.2	7.5
	113	2.7	6.5		118	2.8	6.2
C5	114	3.0	7.0	ST5	119	2.8	6.4
	115	2.7	6.3		120	2.7	6.0
	116	2.8	7.2		121	3.0	6.7
MEAN SEM		2.9 ±0.1	6.8 ±0.2			2.9 ±0.1	6.6 ±0.3
	59	—	—		39	7.0	3.3
	60	8.4	4.9		40	8.6	4.9
	61	7.6	4.4		41	8.8	4.9
	62	8.4	4.0		42	11.2	6.5
C15	63	11.8	5.9	ST15	43	7.4	3.8
	64	7.0	3.3		44	8.8	4.9
	65	8.0	4.3		47	9.1	4.6
	66	9.1	4.9		48	6.3	4.2
	67	7.8	4.8		—	—	—
	68	9.1	4.8		—	—	—
MEAN SEM		8.6 ±0.5	4.6 ±0.2			8.4 ±0.5	4.6 ±0.3
	25	6.3	3.1		13	8.8	3.5
	26	9.1	3.9		15	—	—
	27	9.7	4.2		16	8.6	3.4
	28	8.4	4.1		17	8.0	3.6
C25	29	9.6	3.8	ST25	18	8.0	3.5
	30	10.0	3.8		19	7.2	3.1
	31	6.5	3.0		20	8.0	3.5
	32	8.4	4.4		21	8.6	3.6
	33	7.0	3.0		24	7.4	5.0
	34	6.8	3.2		—	—	—
MEAN SEM		8.2 ±0.5	3.7 ±0.2			8.1 ±0.2	3.6 ±0.2

TABLE XXIV TOTAL MICROSOMAL DNA AND CONCENTRATION IN THE GASTROCNEMIUS MUSCLES OF THE SIX ANIMAL GROUPS

ANIMAL GROUP and NUMBER		MICROSOMAL DNA (µg)	MICROSOMAL DNA (µg/g)	ANIMAL GROUP and NUMBER		MICROSOMAL DNA (µg)	MICROSOMAL DNA (µg/g)
	112	2.4	5.2		117	2.4	5.6
	113	2.0	4.9		118	2.1	4.7
	114	2.2	5.3	ST5	119	2.1	4.8
	115	2.0	4.7		120	2.0	4.5
	116	2.1	5.4		121	2.3	5.0
MEAN		2.2	5.1			2.2	4.9
SEM		±0.1	±0.1			±0.1	±0.2
	59	7.6	3.9		39	9.7	4.6
	60	8.2	4.8		40	7.4	4.3
	61	10.5	6.1		41	6.3	3.5
	62	6.8	3.2		42	5.7	3.3
C15	63	7.8	3.9	ST15	43	8.0	4.1
	64	7.4	3.5		44	6.8	3.8
	65	6.2	3.3		47	6.8	3.5
	66	7.4	4.0		48	5.8	3.9
	67	7.4	4.5		—	—	—
	68	5.8	3.0		—	—	—
MEAN		7.5	4.0			7.1	3.9
SEM		±0.4	±0.3			±0.5	±0.2
	25	7.6	3.7		13	6.3	2.5
	26	8.0	3.4		15	8.8	3.9
	27	5.7	2.5		16	6.3	2.5
	28	7.0	3.4		17	6.8	3.1
C25	29	6.8	2.7	ST25	18	7.0	3.1
	30	7.6	2.9		19	7.4	3.2
	31	6.8	3.1		20	6.8	2.9
	32	7.8	4.1		21	7.8	3.2
	33	7.2	3.1		24	7.0	4.8
	34	7.4	3.5		—	—	—
MEAN		7.2	3.2			7.1	3.2
SEM		±0.2	±0.2			±0.3	±0.2

APPENDIX C

TABLE XXV

TWO-WAY ANALYSIS OF VARIANCE SUMMARY
TABLES AND NEWMAN-KEULS POST HOC TESTS[a]
ON SIGNIFICANT EFFECTS FOR EACH DEPENDENT VARIABLE

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
BODY WEIGHT (g)	A	2221.4	1	2221.4	1.945	0.171
	B	1063122.0	2	531561.0	465.525	0.001
	AB	2178.8	2	1089.4	0.954	0.394
	S-within	46816.0	41	1141.9[b]		
	MEANS OF B:	120.1 384.7	494.3	1 2 3[c]		
				--- --- ---		
MUSCLE WEIGHT (g)	A	0.1	1	0.1	0.086	0.770
	B	26.3	2	13.2	299.918	0.001
	AB	0.1	2	0.1	0.190	0.827
	S-within	1.8	41	0.1		
	MEANS OF B:	0.434 1.861	[illegible].253	1 2 3		
				--- --- ---		

[a] Newman-Keuls test results are reported when the F ratios were significant

[b] Error mean square value

[c] Source means (B) for 5, 15 and 25 weeks are ordered 12 and 3 respectively while source means (A) for control and training are ordered 1 and 2 respectively. The lines (---) show significant differences when *the numbers are underlined* with separate lines.

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
SO FIBER TYPES (%)	A	23.5	1	23.5	2.284	0.139
	B	134.8	2	67.4	6.544	0.004
	AB	39.7	2	19.8	1:926	0.160
	within	381.0	37	10.3		
	MEANS OF B:	15.2 18.1	19.5	1 2 3		
FOG FIBER TYPES (%)	A	274.1	1	274.1	10.715	0.002
	B	769.9	2	384.9	15.048	0.001
	AB	873.4	2	436.7	17.071	0.001
	S-within	946.5	37	25.6		
	MEANS OF A:	54.5 59.7	2 1			
	MEANS OF B:	53.4 54.8	63.2	2 3 1		
FG FIBER TYPES (%)	A	457.6	1	457.6	32.890	0.001
	B	321.6	2	160.8	11.556	0.001
	AB	1276.6	2	638.3	45.877	0.001
	S-within	514.8	37	13.9		
	MEANS OF A:	22.0 28.7	1 2			
	MEANS OF B:	21.7 25.8	28.6	1 3 2		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
SO FIBER AREAS (μ^2)	A	446148.4	1	446148.4	5.323	0.027
	B	8426334.0	2	4213167.0	50.267	0.001
	AB	130333.2	2	65166.6	0.778	0.468
	S-within	2849728.0	34	83815.5		
	MEANS OF A:	1601 1821	1 2			
	MEANS OF B:	1041 1991 2102		1 2 3		
FOG FIBER AREAS (μ^2)	A	131225.9	1	131225.9	1.520	0.226
	B	14688084.0	2	7344042.0	85.074	0.001
	AB	14878.2	2	7439.1	0.086	0.918
	S-within	2935072.0	34	86325.6		
	MEANS OF B:	843 2008 2296		1 2 3		
FG FIBER AREAS (μ^2)	A	785372.0	1	785372.0	5.033	0.031
	B	28802352.0	2	14401176.0	92.283	0.001
	AB	153840.9	2	76920.4	0.493	0.615
	S-within	5305856.0	34	156054.6		
	MEANS OF A:	2428 2719	1 2			
	MEANS OF B:	1341 3043 3337		1 2 3		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
HOMOGENATE PROTEIN (mg)	A	113.9	1	113.9	0.058	0.811
	B	1122595.0	2	561297.5	285.481	0.001
	AB	99.6	2	49.8	0.025	0.975
	S-within	80612.0	41	1966.1		
	MEANS OF B:	65.4 382.6	428.6	1 2 3		
HOMOGENATE PROTEIN (mg/g)	A	0.4	1	0.4	0.002	0.961
	B	23587.8	2	11793.9	62.636	0.001
	AB	500.1	2	250.0	1.328	0.276
	S-within	7720.0	41	188.3		
	MEANS OF B:	151 190.3	206.9	1 3 2		
MYOFIBRILLAR-NUCLEAR PROTEIN (mg)	A	3541.8	1	3541.8	1.642	0.2[illegible]7
	B	701231.3	2	350615.6	162.568	0.001
	AB	5809.9	2	2905.0	1.347	0.271
	S-within	88426.0	41	2156.7		
	MEANS OF B:	40.7 289.5	328.9	1 2 3		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
MYOFIBRILLAR-NUCLEAR PROTEIN (mg/g)	A	963.837	1	963.837	4.109	0.049
	B	31736.211	2	15868.105	67.643	0.001
	AB	3058.804	2	1529.402	6.520	0.003
	S-within	9618.000	41	234.585		
	MEANS OF A:	127.0	136.5	1 2 (1 underlined)		
	MEANS OF B:	93.8	145.7 155.7	1 3 2 (3 2 underlined)		
SOLUBLE PROTEIN (mg)	A	985.309	1	985.309	6.918	0.012
	B	52796.547	2	26398.273	185.334	0.001
	AB	1304.645	2	652.322	4.580	0.016
	S-within	5839.875	41	142.436		
	MEANS OF A:	70.2	60.6	2 1 (each underlined separately)		
	MEANS OF B:	16.8	81.5 97.9	1 2 3 (each underlined separately)		
SOLUBLE PROTEIN (mg/g)	A	230.195	1	230.195	7.930	0.007
	B	255.676	2	127.838	4.404	0.019
	AB	271.598	2	135.799	4.678	0.015
	S-within	1190.125	41	29.027		
	MEANS OF A:	44.4	39.8	2 1 (each underlined separately)		
	MEANS OF B:	38.7	43.5 44.1	1 3 2 (3 2 underlined)		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
MITOCHONDRIAL PROTEIN (mg)	A	0.059	1	0.059	0.013	0.909
	B	96.895	2	48.448	11.023	0.001
	AB	0.328	2	0.164	0.037	0.963
	S-within	171.408	39	4.395		
	MEANS OF B:	0.23 3.30 3.62		1 3 2		
MITOCHONDRIAL PROTEIN (mg/g)	A	0.001	1	0.001	0.0[illegible]	0.974
	B	14.873	2	7.436	6.093	0.005
	AB	0.041	2	0.020	0.017	0.984
	S-within	47.599	39	1.220		
	MEANS OF B:	0.53 1.45 1.98		1 3 2		
MICROSOMAL PROTEIN (mg)	A	1.094	1	1.094	0.720	0.401
	B	80.038	2	40.019	26.334	0.001
	AB	2.770	2	1.385	0.912	0.410
	S-within	59.267	39	1.520		
	MEANS OF B:	0.43 2.67 3.75		1 2 3		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES		DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
MICROSOMAL PROTEIN (mg/g)	A	0.159		1	0.159	0.707	0.406
	B	3.092		2	1.546	6.857	0.003
	AB	0.472		2	0.236	1.046	0.361
	S-within	8.794		39	0.225		
	MEANS OF B:	0.99	1.42	1.64	1 2 3		
HOMOGENATE RNA (µg)	A	6307.773		1	6307.773	0.088	0.769
	B	14897089.000		2	7448544.000	103.387	0.001
	AB	23307.906		2	11653.953	0.162	0.851
	S-within	2953856.000		41	72045.250		
	MEANS OF B:	586	1750	1902	1 2 3		
HOMOGENATE RNA (µg/g)	A	760.664		1	760.664	0.056	0.814
	B	2103631.000		2	1051815.000	77.638	0.001
	AB	24879.465		2	12439.730	0.918	0.407
	S-within	555456.000		41	13547.707		
	MEANS OF B:	843	937	1352	3 2 1		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
MYOFI[illegible] NUCLEAR [illegible]	A	1463.920	1	1463.920	0.055	0.816
	B	5866725.000	2	2933362.000	109.977	0.001
	AB	114178.563	2	57089.281	2.140	0.131
	S-within	1093568.000	41	26672.387		
	MEANS OF B:	336	1067 1163	1 2 3		

MYOFIBRILLAR-NUCLEAR RNA (µg/g)	A	1980.598	1	1980.598	0.609	0.440
	B	541715.063	2	270857.500	83.242	0.001
	AB	45919.730	2	22959.863	7.056	0.002
	S-within	133408.000	41	3253.854		
	MEANS OF B:	514	574 776	3 2 1		
				--- --- ---		
SOLUBLE RNA (µg)	A	20954.152	1	20954.152	1.633	0.209
	B	1895360.000	2	947680.000	73.839	0.001
	AB	68545.875	2	34272.938	2.670	0.081
	S-within	526208.000	41	12834.340		
	MEANS OF B:	246	641 728	1 2 3		
				--- --- ---		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
SOLUBLE RNA (µg/g)	A	2141.611	1	2141.611	0.609	0.440
	B	526807.188	2	263403.563	74.935	0.001
	AB	17822.242	2	8911.121	2.535	0.092
	S-within	144118.000	41	3515.073		
	MEANS OF B:	324 343	568	3 2 1 (2 1 underlined)		
MITOCHONDRIAL RNA (µg)	A	56.158	1	56.158	0.331	0.568
	B	8533.215	2	4266.605	25.181	0.001
	AB	289.724	2	144.862	0.855	0.433
	S-within	6777.367	40	169.434		
	MEANS OF B:	10.4 42.0	38.9	1 3 2 (3 2 underlined)		
MITOCHONDRIAL RNA (µg/g)	A	6.420	1	6.420	0.181	0.673
	B	376.439	2	188.219	5.316	0.009
	AB	55.214	2	27.607	0.780	0.465
	S-within	1416.277	40	35.407		
	MEANS OF B:	17.1 22.5	24.1	3 2 1 (3 2 underlined)		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
MICROSOMAL RNA (μg)	A	168.778	1	168.778	2.121	0.153
	B	7336.129	2	3668.064	46.093	0.001
	AB	730.947	2	365.473	4.592	0.016
	S-within	3183.219	40	79.580		
	MEANS OF B:	15.8	33.9	47.9	1 2 3	
MICROSOMAL RNA (μg/g)	A	10.309	1	10.309	0.449	0.507
	B	2651.071	2	1325.535	57.727	0.001
	AB	184.112	2	92.056	4.009	0.026
	S-within	918.492	40	22.962		
	MEANS OF B:	18.2	21.4	36.3	2 3 1	
HOMOGENATE DNA (μg)	A	1499.801	1	1499.801	0.134	0.716
	B	4561970.000	2	2280985.000	203.440	0.001
	AB	796.545	2	398.272	0.036	0.965
	S-within	459696.000	41	11212.094		
	MEANS FO B:	386	1075	1079	1 2 3	

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
HOMOGENATE DNA (µg/g)	A	1772.491	1	1772.491	0.979	0.328
	B	1321381.000	2	660690.500	365.032	0.001
	AB	5819.797	2	2909.898	1.608	0.213
	S-within	74208.000	41	1809.951		
	MEANS OF B:	482	577	891	3 2 1	
MYOFIBRILLAR-NUCLEAR DNA (µg)	A	1133.821	1	1133.821	0.108	0.744
	B	4204113.000	2	2102056.000	200.213	0.001
	AB	3989.900	2	1994.950	0.190	0.828
	S-within	430464.000	41	10499.121		
	MEANS OF B:	371	1016	1050	1 3 2	
MYOFIBRILLAR-NUCLEAR DNA (µg/g)	A	1600.266	1	1600.266	0.719	0.401
	B	1245659.000	2	622829.500	279.902	0.001
	AB	4592.688	2	2296.344	1.032	0.365
	S-within	91232.000	41	2225.171		
	MEANS OF B:	453	565	857	3 2 1	

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
SOLUBLE DNA (μg)	A	0.020	1	0.020	0.016	0.899
	B	286.867	2	143.434	119.901	0.001
	AB	0.003	2	0.002	0.001	0.999
	S-within	49.047	41	1.196		
	MEANS OF B:	2.4 7.9	7.8	1 3 2		
SOLUBLE DNA (μg/g)	A	0.113	1	0.113	0.236	0.630
	B	29.196	2	14.598	30.438	0.001
	AB	0.648	2	0.324	0.675	0.515
	S-within	19.663	41	0.480		
	MEANS OF B:	3.5 4.3	5.5	3 2 1		
MITOCHONDRIAL DNA (μg)	A	0.070	1	0.070	0.052	0.821
	B	273.341	2	136.671	101.870	0.001
	AB	0.085	2	0.043	0.032	0.969
	S-within	52.323	39	1.342		
	MEANS OF B:	2.9 8.1	8.5	1 3 2		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
MITOCHONDRIAL DNA (μg/g)	A	0.026	1	0.026	0.058	0.810
	B	66.926	2	33.463	74.925	0.001
	AB	0.175	2	0.088	0.196	0.823
	S-within	17.418	39	0.447		
	MEANS OF B:	3.6 4.6	6.7	3 2 1		
MICROSOMAL DNA (μg)	A	0.269	1	0.269	0.307	0.583
	B	244.408	2	122.204	139.582	0.001
	AB	0.464	2	0.232	0.265	0.768
	S-within	35.896	41	0.876		
	MEANS OF B:	2.2 7.1	7.2	1 2 3		
MICROSOMAL DNA (μg/g)	A	0.128	1	0.128	0.330	0.569
	B	22.955	2	11.477	29.565	0.001
	AB	0.061	2	0.030	0.078	0.925
	S-within	15.917	41	0.388		
	MEANS OF B:	3.2 3.9	5.0	3 2 1		

cont'd

VARIABLE	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO	PROBABILITY
NUMBER OF NUCLEI ($x 10^6$)	A	39.020	1	39.020	0.134	0.716
	B	118677.500	2	59338.750	203.470	0.001
	AB	21.080	2	10.540	0.036	0.965
	S-within	11957.000	41	291.634		
	MEANS OF B:	62.3	173.3 / 174.0	1 2 3		
NUMBER OF NUCLEI ($x 10^6/g$)	A	0.897	1	0.897	0.029	0.866
	B	34007.441	2	17003.719	549.886	0.001
	AB	14.801	2	7.400	0.239	0.788
	S-within	1267.813	41	30.922		
	MEANS OF B:	77.6	93.3 / 143.5	3 2 1		

APL COMPUTER PROGRAM FOR NEWMAN-KEULS AND SIMPLE EFFECTS TESTING

```
      ∇ DUNLN[⎕]∇
       ∇ DUNLN
[1]    M←M
[2]    VM←MBS
[3]   BR1:MM1←M-1
[4]    N←CNUM
[5]    EMS←SEMS
[6]    DV←SNKC
[7]    →BR2×⍳MM1=(⍴(DV,1))-1
[8]    'WRONG NUMBER OF VALUES.   TRY AGAIN'
[9]    →BR1
[10]  BR2:MULT←(EMS÷N)*0.5
[11]   DV←DV×MULT
[12]   RAND←⍋VM
[13]   ' '
[14]   (⍕'SEQUENCE OF MEANS: ' ),⍕ RAND
[15]   NEW←VM[RAND]
[16]   ' '
[17]   NEW
[18]   (3×M) ' 1  2  3  4  5  6  7  8  9  10  11  12  13  14  15
        16  17  18  19  20  21  22  23  24  25  26  27  28  29  30'
[19]   DV←DV[⍋DV],1
[20]   JSAVE←M
[21]   MP1←M+1
[22]   MAX←(⌈NEW[1])⌈⌈NEW[M]
[23]   I←0
[24]  BR3:I←I+1
[25]   COMP←MP1-I
[26]   DIFF←COMP↑NEW[COMP]-NEW
[27]   DV←(-COMP)↑DV
[28]   JC←COMP-J←(⌈(DIFF-DV)÷10000+MAX)⍳10
[29]   →BR4×(J≥JSAVE)∨J=COMP
[30]   JSAVE←J
[31]   V1←(3×J-1)⍴' '
[32]   V2←(3×JC+1)⍴'-'
[33]   V3←V1,V2
[34]   V3
[35]  BR4:→BR3×⍳I<M-1
[36]   →0×⍳JSAVE≠ M
[37]   '***  ALL MEANS ARE SIGNIFICANTLY DIFFERENT.'
       ∇
```

INPUT PORTION OF APL PROGRAM

```
      ∇INPUT[⎕]∇
      ∇ INPUT
[1]   'CELL MEANS'
[2]   CM←⎕
[3]   M←3
[4]   'CASES'
[5]   CAS←⎕
[6]   'ERROR MS'
[7]   SEMS←⎕
[8]   SEMSS←SEMS
[9]   MATR←(2,3)⍴CM
[10]  ' '
[11]  'MEANS OF A'
[12]  MAS←⎕(+/MATR)÷3
[13]  ' '
[14]  'MEANS OF B'
[15]  MBS←⎕(+⌿MATR)÷2
[16]  CNUM←(QQ←+/CAS)÷3
[17]  EDF←QQ-6
[18]  SNKC←SNK+(40-EDF)×0.004
[19]  ' '
[20]  'TIME RANGES (Y OR N)'
[21]  Y←⎕
[22]  →BR1×Y='N'
[23]  DUNLN
[24] BR1:' '
[25]  'INTERACTIONS (Y OR N)'
[26]  Y←⎕
[27]  →BR2×Y='N'
[28]  SEMS←SEMS×2
[29]  MBS←MATR[1;]-MATR[2;]
[30]  DUNLN
[31] BR2:' '
[32]  'SIMPLE EFFECTS (Y OR N)'
[33]  Y←⎕
[34] →0×⍳Y='N'
[35]  SEMS←SEMSS
[36]  MBS←CM
[37]  CNUM←CNUM÷2
[38]  SNKC←SNK5+(40-EDF)×0,005
[39]  M←6
[40]  DUNLN
[41]  ' '
      ∇
```

APPENDIX D

TABLE XXVI

THE EFFECTS OF TIME AND SPRINT TRAINING ON ESTIMATED MEAN TOTAL CROSS SECTIONAL AREAS, TOTAL NUMBER OF FIBERS PER TYPE AND PERCENTAGE CONTRIBUTION TO WHOLE MUSCLE AREA BY EACH FIBER TYPE IN RAT MEDIAL GASTROCNEMIUS MUSCLE

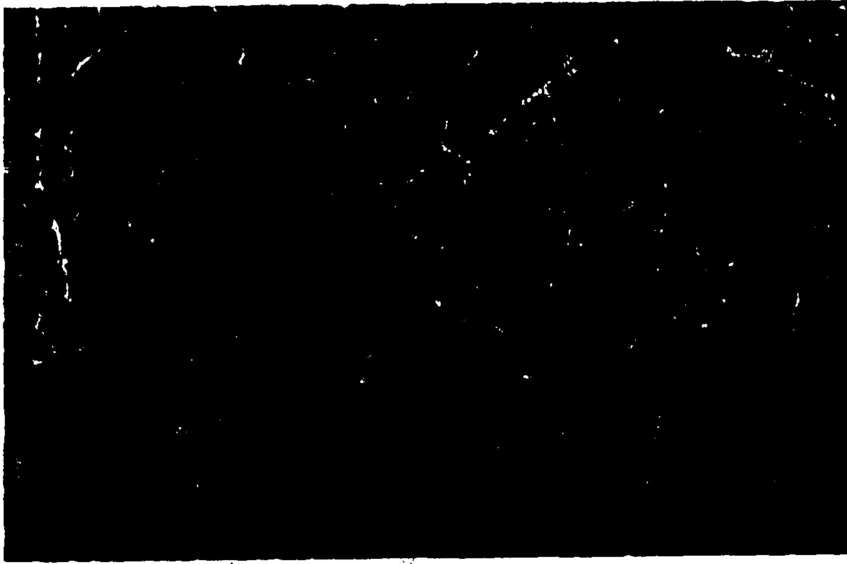
VARIABLES	ANIMAL GROUPS WITH LEVELS OF TIME					
	C5	ST5	C15	ST15	C25	ST25
TOTAL CROSS SECTIONAL AREAS ($u^2 \times 10^{-6}$)	41.6	44.2	74.4	72.9	62.7	65.3
TOTAL NUMBER OF FIBERS	43,545	44,121	35,198	29,495	27,160	24,132
NUMBER OF SO FIBERS	6,312	6,918	6,819	5,288	5,996	4,358
NUMBER OF FOG FIBERS	25,774	29,635	20,881	13,869	16,172	11,729
NUMBER OF FG FIBERS	11,459	7,568	7,399	10,338	4,992	7,865
[a]PERCENT AREA SO FIBERS (%)	15.4	16.7	16.9	15.5	18.6	15.7
PERCENT AREA FOG FIBERS (%)	50.0	58.8	54.0	39.8	57.7	42.3
PERCENT AREA FG FIBERS (%)	34.6	24.4	29.1	44.8	24.7	43.0

[a] Percent contribution to whole muscle area for individual fiber types

PLATE III PHOTOMICROGRAPH OF C5 MEDIAL GASTROCNEMIUS MUSCLE STAINED FOR MYOSIN ATPase (pH 9.4), MAGNIFICATION x 480

PLATE IV PHOTOMICROGRAPH OF C15 MEDIAL GASTROCNEMIUS MUSCLE STAINED FOR MYOSIN ATPase (pH 9.4), MAGNIFICATION x 480

PLATE V PHOTOMICROGRAPH OF C25 MEDIAL GASTROCNEMIUS MUSCLE STAINED FOR MYOSIN ATPase (pH 9.4), MAGNIFICATION x 480



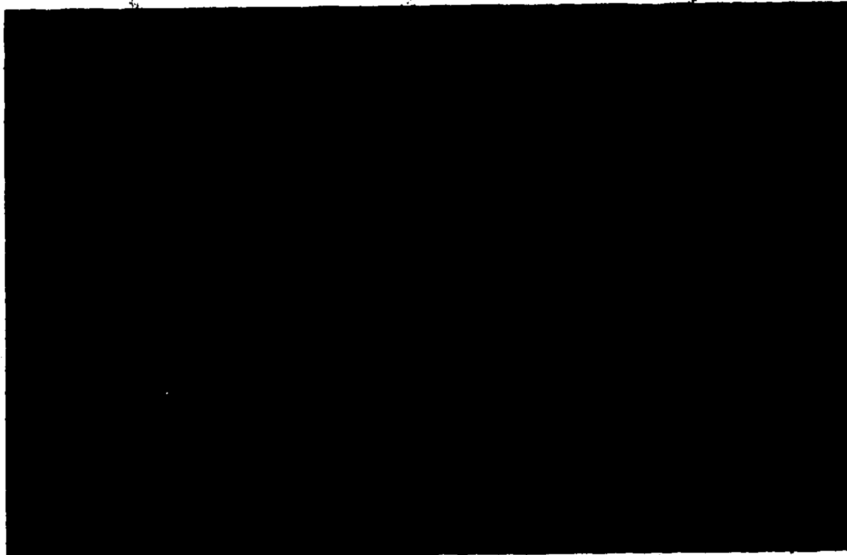


PLATE VI PHOTOMICROGRAPH OF C5 MEDIAL GASTROCNEMIUS MUSCLE STAINED FOR NADH DIAPHORASE, MAGNIFICATION x 480

PLATE VII PHOTOMICROGRAPH OF C15 MEDIAL GASTROCNEMIUS MUSCLE STAINED FOR NADH DIAPHORASE, MAGNIFICATION x 480

PLATE VIII PHOTOMICROGRAPH OF C25 MEDIAL GASTROCNEMIUS MUSCLE STAINED FOR NADH DIAPHORASE, MAGNIFICATION x 480

APPENDIX E

TABLE XXVII GLUCOSE-6-PHOSPHATE AND ISOCITRATE DEHYDROGENASE DATA FOR THE HOMOGENATE AND SUBCELLULAR FRACTIONS OF GASTROCNEMIUS MUSCLE

FRACTION	G6PDH (IU^a/g)	RECOVERY (%)	ICDH (IU/g)	RECOVERY (%)
HOMOGENATE	1.236	100	1.489	100
MYOFIBRILLAR-NUCLEAR	0.721	60.5	1.006	71.7
MITOCHONDRIAL	——	——	——	——
MICROSOMAL	0.306	25.7	0.053	3.8
SOLUBLE	0.165	13.8	0.344	24.5
TOTAL FRACTIONS	1.192	96.4	1.403	94.2

[a]International Units = µM NADPH/min/ml expressed as a concentration per g wet wt of tissue

PLATE I ELECTRON PHOTOMICROGRAPH OF MITOCHONDRIAL FRACTION NEGATIVE-STAINED WITH PHOSPHOTUNGSTIC ACID, MAGNIFICATION x 46,300

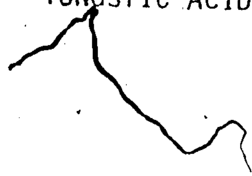
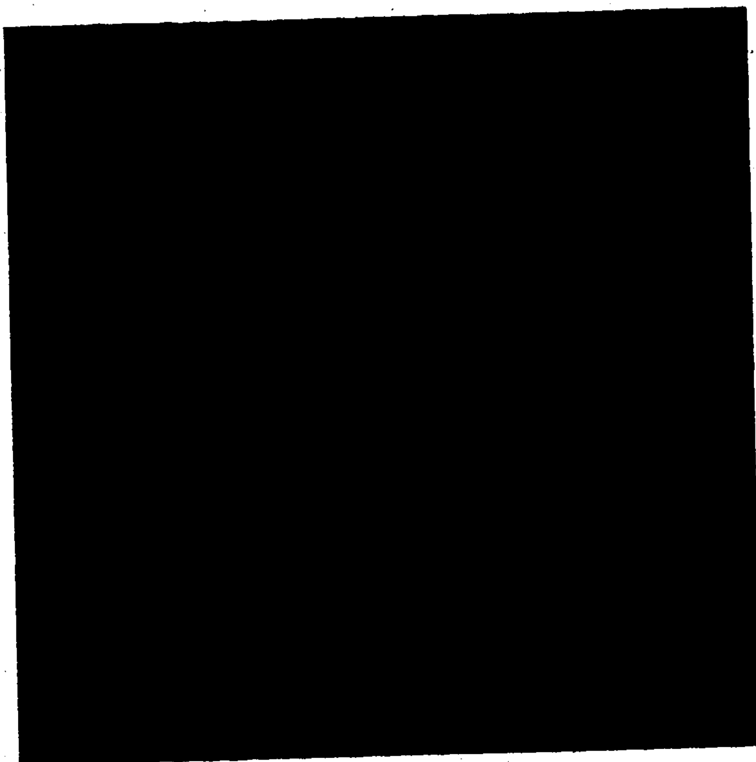


PLATE II ELECTRON PHOTOMICROGRAPH OF MICROSOMAL FRACTION NEGATIVE-STAINED WITH PHOSPHOTUNGSTIC ACID, MAGNIFICATION x 44,900



APPENDIX F

1) MODIFIED LOWRY PROTEIN PROCEDURE (Lowry et al., 1951)

Reagents
- 0.5% $CuSO_4 \cdot H_2O$ in distilled water (D. H_2O)
- 1.0% $NaKC_4H_4O_6$ (sodium potassium tartrate)
- 2.0% Na_2CO_3 in D. H_2O to pH 12.5 with 10 N NaOH
- 2 N Folin reagent 1:1 with D. H_2O
- Lowry C solution

61.7%	Na_2CO_3 sol'n above
35.7%	D. H_2O
1.3%	$CuSO_4$ sol'n above
1.3%	$NaKC_4H_4O_6$ sol'n above
100.0%	Lowry C sol'n

Reaction Mixture

0.1 ml sample
5.0 ml Lowry C
0.3 ml Folin reagent

Procedure

1. Add 0.1 ml sample to a 15 ml test tube.
2. Add 0.5 ml Lowry C and let stand at least 10 min.
3. Add 0.3 ml Folin reagent while mixing on Vortex and let stand 2 hours to 2 days.
4. Read spectrophotometrically at 750 nm.

Blank

Substitute 0.1 ml of buffer for sample.

Standards

Use 0.1 of Bovine Serum Albumin (dissolved in the same buffer) instead of the samples. Cut a stock protein solution to the appropriate values.

Note: Protein Solubilization

One ml samples of the homogenate, myofibrillar-nuclear and soluble fractions were dissolved in 3 ml 0.3 N NaOH for 30 min at 37°C to solubilize protein. Only 1 ml of 0.3 N naOH was added to 1 ml samples of the mitochondrial

and microsomal fractions. Thereafter, the Lowry procedure was followed as previously described.

2) TWO WAVELENGTH METHOD FOR RNA DETERMINATIONS (Fleck and Begg, 1965)

Ribonucleotide products of RNA hydrolysis found in the supernatant and washings following RNA extraction procedures were read spectrophotometrically at the wavelengths of 260 and 232 nm. RNA concentrations were then calculated according to the following equation (Fleck and Begg, 1965):

$$C_{RNA} = 3.79\ (A_{260\ nm}) - 1.50\ (A_{232nm})$$

Where C_{RNA} is the concentration of RNA and $A_{260\ nm}$ and A_{232nm} are the absorbance values at the respective wavelengths. Standard curves were made using calf liver RNA standards (Sigma Chemical Co.), and these values were corrected for polypeptide contamination using the same equation.

3) DETERMINATION OF DNA WITH INDOLE (Hubbard *et al.*, 1970)

Reagents - 4 N HCl
- 0.08% indole in D. H_2O
- Chloroform, spectroanalyzed
- Color reagent
  0.08% indole 1:1 with 4 N HCl (This reagent must be made up just prior to usage.)

Procedure

1. Add 2.0 ml of sample from DNA hydrolysis to a screw-cap culture tube.
2. Add 2.0 ml color reagent (0.08% indole 1:1 4 N HCl), seal caps, and heat for 15 min at 100°C in a metabolic shaker bath.
3. Cool mixture for 5 min in an ice bath to stop color product formation.
4. Add 4.0 ml chloroform and mix well.

5. Shake mixture mechanically for 5 min.
6. Centrifuge at 1000 X g. for 5 min.
7. Aspirate the chloroform layer (bottom) from tube and discard.
8. Repeat chloroform wash, steps 4, 5 and 6.
9. Draw off DNA color product (top layer) and read spectrophotometrically at 490 nm.

Blank

Substitute 2.0 ml of 5% TCA for the sample.

Standards

Use 2.0 ml of calf thymus DNA (Sigma Chemical Co.) dissolved in 0.001 N NaOH instead of samples. Cut stock DNA solution to the appropriate values.