THE EFFECTS OF TWO EXERCISE REGIMENS AND SUPPLEMENTAL VITAMIN C INTAKE UPON BONE GROWTH IN ALBINO RATS

By

Lesley Ann Sive

A THESIS

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

MASTER OF SCIENCE

Department of Health, Physical Education and Recreation

1978

ABSTRACT

THE EFFECTS OF TWO EXERCISE REGIMENS
AND SUPPLEMENTAL VITAMIN C INTAKE
UPON BONE GROWTH IN ALBINO RATS

By

Lesley Ann Sive

This investigation was undertaken to determine the effects of eight weeks of sprint (SPT) or endurance (END) training and vitamin C supplementation on the weight of the tibia-fibula complex and the length of the tibia in normal male albino rats.

Animals from each training group were sacrificed 72 to 96 hours after their last exercise sessions. Selection criteria developed for training performance resulted in a final cell frequency of ten animals per treatment group.

Absolute tibia lengths of the END and SPT animals were significantly shorter than those of the sedentary control (CON) group. No differences in bone length were found between the two training groups. The SPT group had significantly lower absolute and relative tibia-fibula weights than did the END and CON animals.

The vitamin C supplementation did not appear to have any effect on training performance or long bone growth under the high-intensity training conditions imposed in this investigation.

To my parents

ACKNOWLEDGEMENTS

My sincerest appreciation belongs to my family, for their patience, understanding and encouragement throughout my graduate program.

A very special thank you is extended to Dr. W. W. Heusner for the continued guidance, counseling and assistance he provided me as my graduate advisor and committee chairman.

Deep appreciation is given to the members of my committee, Dr. W. W. Heusner, Dr. W. D. Van Huss and Dr. K. W. Ho for making the writing of this thesis a worthwhile experience.

A special thank you is extended to Dr. W. D. Van Huss for his continued support and guidance during my years as a graduate student. A very special thank you is offered to my friends for their concern and understanding, and for helping to maintain my morale in times of difficulty and frustration.

TABLE OF CONTENTS

TABLE OF CONTENTS--continued

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

THE PROBLEM

Many studies have been conducted to determine the effects of exercise on various measures of body growth and development. However, limited and sometimes contradictory evidence has been presented concerning the growth response of bone to physical activity. Some data have indicated that the pressure effect of low-intensity exercise on the epiphyses increases both the length and the weight of long bones (14, 40). Other data have shown that retardation of linear long bone growth occurs in response to strenuous exercise programs during the developmental period of an organism (19, 36, 40, 65, 78, 80). The adaptation of bone to physical training has been hypothesized to be a function of the intensity of training (7). Despite the serious implications of the observed negative effects of exercise on bone growth for the well-being of children involved in competitive athletics at the Elementary and Junior High school levels, little attempt has been made to determine the possible physiological factors involved in growth impairment or the steps which might be taken to prevent its occurrence.

Current literature has indicated that exercise consists of a continuum of specific activities each of which elicits

a specific response within the organism (17, 54, 68). However, other than a small amount of data on intensity of exercise, there is a dearth of information regarding the effects of different types of exercise programs on long bone growth.

Overall body growth and normal bone formation have been found to be dependent on an adequate supply of vitamin C (16, 64, 67, 77). In particular, the vitamin may play a role in the synthesis and/or metabolism of the adrenal steroid hormones (29, 42, 74) which are related to growth. Heavy stress is known to deplete the supply of vitamin C in the adrenals (57, 69). This information has resulted in recommendations of increased vitamin C intake for athletes who participate in strenuous and/or prolonged physical activity (56, 66). A search of the literature, however, revealed no study which has assessed the effect of supplementary vitamin C intake as a means of preventing the bone-growth retardation that is induced by stressful exercise.

Need for the Study

Data were needed to determine the effects of high-intensity sprint and endurance exercise regimens on long bone growth. In addition, an investigation was required to identify the role that vitamin C may play in altering bone-growth changes induced by exercise. Expectations were that a study of bone growth, using specific exercise routines

with and without the administration of supplementary vitamin C, would furnish useful information about the relationships under consideration.

Statement of the Problem

The purpose of this study was to determine the effects of two different types of prolonged physical activity, plus daily vitamin C supplementation, on long bone growth in the male albino rat. The length of the left tibia and the weight of the left tibia-fibula complex were used as criterion measures of bone growth to facilitate comparisons between treatment groups. The exercise programs that were used were designed with the expectation that one would produce primarily aerobic and the other more anaerobic metabolic adaptations in the animals.

Research Hypotheses

The hypotheses that were tested in this study are as follows:

1. The tibial bones of exercised animals should be significantly shorter and heavier than the tibial bones of sedentary animals.
2. The two exercise regimens should produce differential long bone growth in the exercised animals.
3. Significantly less impairment of long bone growth should be found in exercised animals that receive vitamin C supplementation than in exercised animals that do not receive vitamin C supplementation.

Research Plan

Eighty four male albino rats (Sprague-Dawley strain) were randomly assigned to one of the following three activity groups: (a) Sedentary Control (CON), (b) Sprint Running (SPT), and (c) Endurance Running (END). One-half of the animals in each of the activity groups received 2 mg of vitamin C in a .1 cc 5% sugar solution per 100 gm of body weight daily. The remaining animals received similar amounts of a sugar solution as a placebo according to body weight.

The SPT and END training regimens were implemented using electronically controlled running wheels (82). The two programs were more intensive than any exercise routines previously used in this laboratory (see Appendix A). The animals in the SPT group were subjected to an interval training program of high-intensity sprint running. During the final 14 days, they were expected to run at speeds of 108 m/min. Six bouts of exercise were used with 2.5 min between bouts. Each bout consisted of five 15-sec work periods alternated with four 30-sec rest periods. The SPT program was expected to tax the anaerobic capacity of the experimental animals. The END animals were subjected to a rigorous program of distance running. During the final eleven days of the training period, these animals were expected to complete a 60-min continuous run at a speed of 36 m/min. The END program was designed to overload aerobic metabolic capacity.

Ten animals in each of the six activity-diet subgroups were sacrificed at the conclusion of eight weeks of treatments. Health and training performances throughout the treatment period were used as selection criteria for these animals. The left tibia-fibula complex was removed from each animal. The wet weight of the complex and the length of the tibia were determined. Analysis of variance procedures were used to analyze the data.

Rationale

The SPT and END training regimens were designed to simulate high-intensity exercise programs for humans. The intensity of the programs was expected to induce bone growth changes in the exercised animals. Differential long bone growth in the experimental animals was anticipated in response to the differences between the SPT and END training programs.

Vitamin C (ascorbic acid) supplementation was included for study as a possible preventative of the expected decrement in bone growth of the exercised animals. Vitamin C is important in the normal formation of bone matrix (64, 77).

The rat synthesizes its own supply of vitamin C. However, there are several reasons why the rat was used in this study rather than the guinea pig which, like man, does not synthesize the vitamin. The rat is easily trained to run in a wheel whereas the guinea pig is not. In addition,

the rat tibia had been used in earlier studies concerned with the effects of exercise on long bone growth. Treatments were initiated when the animals were 84 days old. Although the animals were postpubertal at this age, rat skeletal growth continues until the animals are over 400 days of age (15).

The techniques that were used for data collection were based on those of a previous bone-growth study completed at the Human Energy Research Laboratory (80).

Limitations

1. The results of animal studies cannot be used to make direct inferences to human beings.
2. The small subgroup size may limit the power of the statistical analyses.
3. Optimal durations of treatments for the achievement of significant results between groups have not been clearly established.
4. Optimal types of training to show differential growth effects on bones may not have been selected.
5. The rat synthesizes its own supply of vitamin C. Therefore, the significance of the supplemental vitamin C effect on bone growth may not be fully revealed in this study.
6. The results of this study are specific to the tibial bones of male albino rats. The data apply only to the SPT and END training regimens used in the investigation.

7. Due to limitations of personnel and facilities, the animals for this investigation had to be received in three separate shipments. The activity treatments were not randomized across shipments. This lack of randomization could have introduced a bias in the exercise-related data. The probability of a genetic bias was small because the supplier (Hormone Assay, Inc., Chicago, Illinois) had a well-controlled substrain of Sprague-Dawley rats. Every possible effort was made to control unique external factors in the laboratory. The diet treatments were randomized across shipments.

CHAPTER II

REVIEW OF LITERATURE

The following review of literature is divided into four sections. The first section will focus on the nature of the effects of exercise on bone growth. The role that vitamin C plays in the formation and maintenance of bone will be covered in the second section. The third section will be devoted to the relationship between ascorbic acid and stress. Theories on the function of ascorbic acid in the adrenal cortex also will be presented. The final section will deal with requirements of vitamin C particularly under conditions of stress.

Exercise and Growth

Mechanical stress is believed to play an important role in the structure and growth of bone (10, 44, 84). However, a review of literature indicates there is little agreement among researchers concerning the nature of the effects of exercise on bone growth.

Steinhaus (75), in his review of the effects of chronic exercise, reported evidence suggesting that the pressure effect of exercise on the epiphyses stimulates bone growth. Donaldson (14) found that voluntary running for a three-month period or longer produces gains in weight

and length of rat leg bones. A comparison of dominant and non-dominant forearms of tennis players, carried out by Buskirk et al. (9), indicated that tennis produces increased length of the radius and ulna in the forearm used to swing the racket.

Recently, Saville and Whyte (71) ran a group of thirty-day-old rats 2000 m/day, five days a week, for periods of up to 10 weeks. They noted increased wet weight and volume of the long bones in the exercised animals. In another study by Saville and Smith on male rats (72), bone responded to isometric exercise by increases in density and breaking strength.

In contrast to these findings, other evidence indicates that excessive and prolonged pressure on the epiphyses may retard bone growth. In 1926 Price-Jones (65) subjected newly-weaned rats to a forced-exercise running program and reported that control animals had longer, heavier bones than did the exercised animals.

Working with two-week-old mice, Kiiskinen and Heikkinen (40) found slight increases in femoral length and weight under low-intensity training conditions (80 m/day at 18 m/min for 12 weeks). However, when the duration of running was progressively increased to 180 m/day, the mean femur length was significantly shorter in the exercised group than in a control group.

In a study by Van Huss et al. (80), a group of 30-day-old albino rats was forced to swim 30 min daily for 35 days.

An impairment of body size was indicated by the finding of significantly lower body weights and shorter tibias in the animals forced to exercise during prepuberty than in control animals.

These observations were reinforced in an investigation conducted by Tipton et al. (78). Statistically significant differences in length, width and mineral percentage of long bones were found between a group of growing rats subjected to strenuous exercise and a group of normal controls.

The mechanisms of these growth changes are yet to be determined. Weinmann and Sicher (81) believe that an "increase of pressure or tension beyond the limits of tolerance leads to destruction of bone by resorption." Evans and Hughes (16) cited Gelbke's study (19) which was designed to determine the effect of tension and pressure on the growth of endochondral bone in young dogs. Continuous prolonged tension and compression were found to result in the replacement of epiphyseal material by a spongy bone resistant to mechanical forces. The conclusion, therefore, was that bone may sacrifice its growth zone to retain its configuration and stability.

Vitamin C and Bone Growth

Vitamins play a major role in the normal metabolic processes of the body. Although some of the specific physiological functions of vitamin C (ascorbic acid) have not been ascertained as yet, the fact that a deficiency manifests itself in numerous symptoms of defective body

functioning is well known.

One of the most important roles of ascorbic acid involves the formation of collagen, a fibrous protein which constitutes the major component of bone matrix. The primary effect of ascorbic acid recently was shown to be related to the hydroxylation of proline and lysine after the formation of protocollagen by peptide binding (5, 12, 33, 79). Hydroxyproline and hydroxylysine are rare amino acids and an integral constituent of collagen. Evidence indicates that the formation of hydroxyproline and hydroxylysine takes place by means of analogous reactions (5). However, studies in vitro suggest that lysine hydroxylation may be less readily affected by ascorbate deficiency than proline hydroxylation (6).

The precise mode of action of the vitamin in the hydroxylation reactions has yet to be determined. Ascorbic acid may function as an essential cofactor participating directly in the hydroxylation mechanism (5, 31, 46) or as an enzyme activator responsible for the conversion of an inactive precursor to an active enzyme (5). However, the two activities of ascorbic acid may really be two aspects of a single phenomenon (5). Jeffrey and Martin (33) demonstrated that ascorbic acid can neutralize the inhibitory actions of puromycin on new collagen formation. Likewise, Liakakos et al. (47) found that large doses of ascorbic acid can supress the inhibitory actions of the corticosteroids on new collagen formation.

Some of the changes that occur in bone as a result of varying degrees of vitamin C deficiency were determined by Poal-Manresa et al. (64). They found that six-week-old guinea pigs, when placed on a scorbutogenic diet for three weeks, had thinner than normal epiphyseal bone plates in the tibia, decreased trabeculae, and a scanty matrix. Administration of 5 mg/day of ascorbic acid to the scorbutic animals resulted in improved quality of the matrix and the formation of new bone in areas which were not severely damaged.

Thornton (77) demonstrated that ascorbic acid deficiency also affects bone salt deposition and stability. Scorbutic guinea pigs were found to have decreased bone salt depositions which paralleled the length of time of the deficiency. Decreased stability of bone salts was significantly greater in ascorbic acid deficient animals than in control animals. Lability of bone salts increased as deposition of the salts decreased which suggested that the two factors were inversely related. Osteoblastic activity and the quantity and quality of the bone matrix appeared to have been influenced by lack of vitamin C.

Vitamin C and Stress

High concentrations of ascorbic acid are present in most metabolically active tissues in the body. It has been well established that the adrenal cortex contains great amounts of this vitamin. During stressful situations, the release of adrenocorticotropin (ACTH) from the

adenohypophysis stimulates the adrenals and results in marked depletion of the gland's ascorbic acid content. Namyslowski (57) found that the adrenals of a group of white rats subjected to prolonged exhaustive swimming contained an average of 242 mg % of ascorbic acid which was less than half the value measured in control animals. In addition, it appears that adrenal ascorbic acid levels in both the guinea pig, which cannot synthesize vitamin C, and the rat, which does synthesize the vitamin, are not fully restored for some hours after cessation of adrenal stimulation (50, 59, 69).

A more economical utilization of adrenal ascorbic acid in trained animals has been indicated by Namyslowski (58). Following exhaustive exercise, the decrease of ascorbic acid in trained rats was less than in untrained rats; furthermore, normal adrenal levels were restored much faster in the trained animals after exercise (59, 60). Namyslowski also noted an increased concentration of adrenal ascorbic acid in trained animals that were rested (60). These findings suggest that training results in the adaptation of the adrenal gland to strenuous exercise.

The body is able to resist different types of stresses with the help of the corticosteroid hormones, in particular the glucocorticoids, which are released from the adrenal cortex. The high concentration of ascorbic acid in the adrenals has led to the concept that this vitamin is related to steroid biosynthesis. A number of conflicting hypotheses

have arisen to attempt to explain the role it plays.

Enhancement of *in vitro* steroid formation in the presence of ascorbic acid has been reported by some investigators (21, 37, 38). However, no such direct *in vivo* evidence appears to be available at this time. In fact, observations have pointed to increased pituitary-adrenocorticotrophic activity with increased corticosteroid production in guinea pigs on a diet which is ascorbic acid deficient (11, 28). More recently, the high concentration of ascorbate in the adrenals has been hypothesized to prevent steroidogenesis (24, 35, 42, 43). Experimentation has shown that ascorbic acid release precedes corticoid output (49). Kitabchi (42) believes that this ascorbate release reverses hydroxylase inhibition in the adrenal with the resulting facilitation of steroid production and release. Liakakos *et al.* (48) have demonstrated that an excess of ingested ascorbic acid may exert an inhibitory effect on the secretion of the major glucocorticoid, cortisol, following adrenal stimulation with ACTH. Finally, the results of studies by Hodges and Hotston (28, 29) have led to doubts that adrenal ascorbic acid has any influence on steroidogenesis. However, these authors do suggest that the vitamin may be important in the metabolism of corticosteroids.

Requirements of Vitamin C

A review of the literature on ascorbic acid shows that there are wide variations from one study to another in the

estimated vitamin C requirements for most population groups. This difference of opinion appears to be the result of insufficient knowledge concerning the metabolic role of ascorbic acid and differences in methods by which ascorbic acid status is evaluated (32).

Evidence does suggest that the minimum daily intake of vitamin C needed to insure prevention of scurvy is 10 mg (4, 22, 53), although amounts of less than 10 mg/day may provide adequate protection for some people (4, 27, 30). In 1938 the League of Nations Technical Commission on Nutrition (45) recommended an allowance of 30 mg/day for the human adult. This value appears to be sufficient to replenish the quantity of ascorbic acid metabolized daily (3). In the U.S., the Food and Nutrition Board of the National Research Council (61) has advocated dietary allowances of 40 mg/day for children up to 11 years of age and 45 mg/day for adults. The Recommended Dietary Allowances (RDA) have been described as:

> ... the levels of intake of essential nutrients considered, in the judgement of the Food and Nutrition Board on the basis of available scientific knowledge, to be adequate to meet the known nutritional needs of practically all healthy persons (61).

In order to cover the needs of most people, the RDA are estimated to exceed average requirements.

The increased metabolic demands placed on the body during exercise would seem to suggest that the need may exist for an additional daily intake of vitamins, particularly vitamin C. Physiological parameters such as

muscular endurance, pulse rate, vital capacity, respiratory quotient, blood pressure, and recovery time have been used to evaluate the need for increased amounts of vitamin C during physical exertion. The results of these studies have indicated both negative and positive effects of vitamin C administration. No beneficial effects of vitamin C supplementation on physiological performance were shown by Keys and Henschel (39), Fox et al. (18), Johnson et al. (34), Bailey et al. (2), Gey et al. (20) and Kirchhoff (41). Studies indicating positive effects of additional vitamin C ingestion have been conducted by Brunner (8), Matthes (52), Wiebel (83), Yakovlev (85) and Harper et al. (23).

When biochemical indices such as urinary ascorbic acid and blood ascorbic acid levels are used, researchers usually interpret the data as being indicative of an increased requirement for vitamin C during vigorous physical activity (32). Namyslowski (55) studied healthy young athletes while they were engaged in normal activities at a physical culture academy and during physical training at ski camp. In both instances he noted decreased urine and serum ascorbic acid levels when the athletes had a vitamin C intake of 100 mg/day. A rise in intake to 300 mg/day produced increases in both urine and blood ascorbic acid levels. Based on these observations Namyslowski recommended daily vitamin C intakes of 100 to 150 mg during normal activities, 200 to 250 mg during ski-training camps, and 200 to 400 mg for long ski runs (56). Bachinsky (1) found

that physically active people have lower amounts of ascorbic acid in the urine than do less active subjects. He interpreted this observation to be evidence of a need for vitamin C supplements in athletes.

Maksjutinskaya et al. (51) studied 20 school boys at summer camp who swam from 1,000 to 5,000 m daily. Ten of the boys were given 75 mg of ascorbic acid per day along with supplements of vitamin A and thiamin. The control subjects received only the samp diet. The group on vitamin supplements excreted slightly higher amounts of ascorbic acid in their urine than did the control group. The results were assumed to be support for an increased intake of vitamins by active subjects.

CHAPTER III

METHODS AND MATERIALS

Gross measurements of total-body oxygen debt and oxygen uptake have been used to reflect human metabolic responses to physical activity. Exhaustive sprint running leads to an increased tolerance of oxygen debt which presumably reflects a greater capacity for the generation of muscular energy via anaerobic metabolism. Training regimens based on this type of running are characterized by maximal workloads and relatively short bouts of repeated exercise. In contrast, distance running is thought to be dependent chiefly upon aerobic muscle metabolism and tends to increase total-body oxygen uptake capacity. Moderate or light workloads and relatively long bouts of continuous exercise are typical of endurance training programs. This study was designed to investigate the effects of eight weeks of sprint and endurance training on the weight of the tibia-fibula complex and the length of the tibia in the male albino rat. In addition, the effects of daily vitamin C supplementation on tibial growth were determined.

Sample

Eighty four normal male albino rats (Sprague-Dawley strain) were obtained from Hormone Assay, Inc., Chicago,

Illinois. They were received at weekly intervals in three shipments of 30, 24 and 30 animals respectively. Each shipment was designated as a separate activity group which was then divided into two diet subgroups. A standard period of 12 days was allowed for adjustment to laboratory conditions. The treatments were initiated when the animals were 84 days old.

Activity Groups

The duration of the experimental period was eight weeks. The three activity treatments were as follows:

Sedentary Group

The 24 animals in the second shipment constituted the sedentary control (CON) group. These animals were not forced to exercise and were housed in individual sedentary cages (24 cm x 18 cm x 18 cm) during both the adjustment period and the treatment period.

Sprint Group

The sprint running (SPT) group was comprised of the 30 animals in the first shipment. Each of these animals was housed in an individual voluntary-activity cage (sedentary cage with access to a freely revolving activity wheel) during the treatment period. The SPT animals were subjected to an interval training program of high-intensity sprint running. The workload of the SPT program was increased gradually until on the 27th day of training, and thereafter, the animals were expected to complete six bouts of exercise with 2.5 min of inactivity between bouts. Each bout included five 15-sec

work periods alternated with four 30-sec rest periods. During the work periods, the animals were required to run at a speed of 108 m/min.

Endurance Group

The endurance running (END) group was composed of the 30 animals in the third shipment. These animals were housed under the same conditions as the SPT animals. The END animals were subjected to a demanding program of distance running. The workload was progressively increased so that on the 30th day of training, and thereafter, the animals were expected to complete 60 min of continuous running at 36 m/min.

Diet Subgroups

In addition to the three activity regimens, two diet supplements were administered. Both supplements were given by oral syringe, seven days a week, between 7 p.m. and 9 p.m. Administration of the diet treatments was begun on the day prior to the initiation of the activity treatments and was terminated on the day prior to sacrifice.

Vitamin C Group

One half of the animals in each activity group were given approximately .1 cc of a 5% sugar solution with 2 mg ascorbic acid/100 gm of body weight.[1]

---

[1]Vitamin C crystals (30-80 mesh) were obtained from the J. T. Baker Chemical Co.

Placebo Group

The remaining animals in the three activity groups received approximately .1 cc of a 5% sugar solution/100 gm of body weight as a placebo.

Training Procedures

The animals were trained in a battery of individual controlled-running wheels (CRW). This apparatus has been described as:

> ... a unique animal-powered wheel which is capable of inducing small laboratory animals to participate in highly specific programs of controlled reproducible exercise. (82)

Animals learn to run in the CRW by avoidance-response operant conditioning. A controlled low-intensity electrical current, applied through the running surface, provides motivation for the animals to run. A light above the wheel signals the start of each work period. The animal is given a predetermined amount of time (acceleration time) to attain a prescribed running speed. If the animal does not reach the prescribed speed by the end of the acceleration time, the light remains on and shock is applied. As soon as the animal reaches the prescribed speed, the light is extinguished and the shock is discontinued. When the animal responds to the light and attains the prescribed running speed during the acceleration time, the light is extinguished immediately and shock is avoided. If the animal fails to maintain the prescribed speed throughout the work period, the light-shock sequence is repeated. Most animals learn to

react to the light stimulus after only a few days of training.

A typical training session consists of alternated work and rest periods. The wheel is braked automatically during all rest periods to prevent spontaneous activity. The brake is released and the wheel is free to turn during work periods.

Performance data are displayed for each animal in terms of the total meters run (TMR) and the cumulative duration of shock (CDS). The TMR and the total expected meters (TEM) are used to calculate the percentage of expected meters (PEM):

$$PEM = 100\ (TMR/TEM)$$

PEM values are the chief criterion used to evaluate and compare training performances. A secondary criterion is provided by the percentage of shock-free time (PSF) which is calculated from the CDS and the total work time (TWT):

$$PSF = 100 - 100\ (CDS/TWT)$$

Animal Care

All housing cages were steam-cleaned every two weeks. Standard procedures for daily CRW cleaning and maintenance were observed.

The animals received food (Wayne Laboratory Blox) and water ad libitum. A relatively constant environment was maintained for the animals by daily handling as well as by temperature and humidity control.

The animals were exposed to an automatically regulated sequence of twelve hours of light (1:00 a.m. to 1:00 p.m.) followed by twelve hours without light (1:00 p.m. to 1:00 a.m.). Since the rat normally is a nocturnal animal, this lighting pattern altered the normal day-night schedule for the animals so that they were trained during the active phase of their diurnal cycle.

Body weights of the SPT and END animals were recorded before and after each training session. The CON animals were weighed weekly.

Sacrifice Procedures

Anticipated limitations of time and personnel restricted the number of animals that could be handled at sacrifice to 10 in each activity-diet subgroup. Since one of the inherent purposes of the study was to compare various parameters in two groups of highly trained animals and a group of untrained animals, five extra rats originally were included in each of the four subgroups that were subjected to regimens of forced exercise. At the end of the investigation, the ten animals to be sacrificed from each of these four subgroups were selected on the basis of their health and their training performance throughout the treatment period. Only animals subjectively determined to be in good health were chosen. Because the training requirements were extremely vigorous, no absolute minimal performance criteria were established. However, individual daily records of PEM and PSF were examined, and healthy animals making the best adaptations

to the training regimens were selected for sacrifice.

Two extra animals were included originally in each of the two sedentary subgroups to allow for the unlikely possibility that some of the unexercised animals might have become ill during the course of the study. Again, only animals subjectively determined to be in good health were chosen for sacrifice. If there were more than 10 healthy animals in either subgroup, the animals to be sacrificed were chosen by lot.

Three sacrifice periods of two-days duration (Monday and Tuesday) were established. All 20 animals within an activity group were killed during a single sacrifice period (i.e., five animals from each of the two diet subgroups each day). The trained animals were killed either 72 or 96 hours after their last exercise bouts were completed. This procedure was followed to eliminate any transient acute effects of exercise. The animals were either 140 or 141 days old at sacrifice.

Final body weights were recorded immediately prior to sacrifice. Each animal was anesthetized by an interperitoneal injection (4 mg/100 gm body weight) of a 6.48% sodium pentobarbital (Halatal) solution. The lower part of the left hind limb was stripped of superficial muscles. The tibia-fibula complex was removed and cleaned of all remaining muscle and epiphyseal cartilage. The bones were placed in Ringer's solution for several minutes to allow additional cleansing. After removal from the solution, they were

blotted dry and weighed to the nearest milligram. Vernier calipers (Craftsman, West Germany) were used to measure the length of the tibia to the learest .1 mm. The length measurement was taken from the most lateral point of the lateral condyle of the proximal tibial epiphysis to the most lateral point of the inferior malleolus. Bone length was expressed in absolute terms (millimeters) and bone weight was expressed in both absolute terms (milligrams) and relative terms (percentage of body weight).

Analysis of Data

This study was conducted as a two-way (3 x 2) factorial design with three levels of activity and two levels of diet. The bone lengths and the absolute and relative bone weights were analyzed using a fixed-effects analysis of variance routine on the Michigan State University Control Data 6500 Computer. Newman-Keuls tests were used to evaluate differences between pairs of means whenever a significant ($p \leq .05$) F-ratio was obtained.

CHAPTER IV

RESULTS AND DISCUSSION

The material in this chapter is organized into five main sections. The first part covers the training results from the Controlled-Running Wheel (CRW) programs and includes the percentage of body weight lost during the daily exercise periods, the environmental factors that operated during training and the training response as reflected by the performance criteria. Body weight results at sacrifice are given in the second section. The effects of vitamin C supplementation on performance and on tibial length and weight are presented next. The fourth major section deals with the tibia data for each training group. Finally, a discussion is offered that attempts to relate the present findings to those of other investigations reported in the literature.

Training Results[1]

The sprint (SPT) and endurance (END) Controlled-Running Wheel (CRW) training programs are presented in

---

[1]Some of the material in this section has been adapted, in part, from the unpublished Ph. D. dissertation of Roland R. Roy (70).

Appendix A. These programs are modified versions of standard regimens routinely used in the Human Energy Research Laboratory, Michigan State University, East Lansing, Michigan. The modifications were incorporated in an attempt to design strenuous exercise programs which would primarily stimulate anaerobic or aerobic metabolic processes in the animals. The performances of the animals were evaluated using the percentage of expected meters (PEM) and the percentage of shock-free time (PSF) as criterion measures.

The performance data for the SPT-C and the SPT-No C groups are presented in Figure 1. Progressive increases in the required running velocity were made rapidly. From the beginning of the fourth week of training to the end of the program, the animals were expected to run at velocities ranging from 90 to 108 m/min (see Figure 1 and Appendix A, Table A-1). No comparable exercise programs for small animals have been found in the literature. The results indicate that the animals could not maintain the program requirements. PEM values fell to approximately 45% during the last three weeks of training as contrasted with the usual criteria of 75% for satisfactory completion of an exercise regimen.

The training data for the END-C and END-No C groups are shown in Figure 2. PEM values were 70 or higher each day of training in both the C and the No C animals. These results indicate that the animals were able to maintain the daily requirements of the END program relatively well.

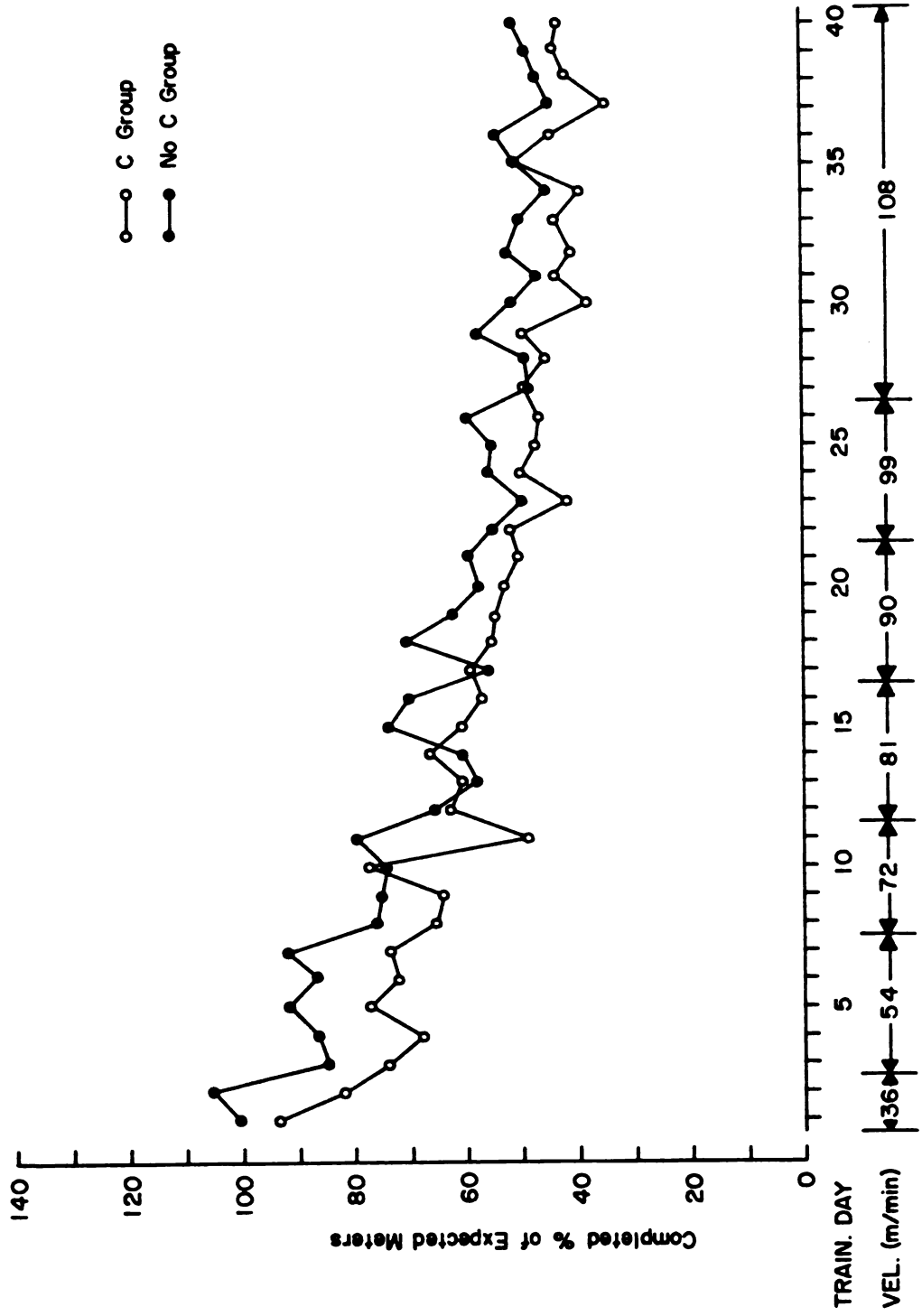

Fig. 1. Mean Daily Percent Expected Meters Run for SPRINT Animals

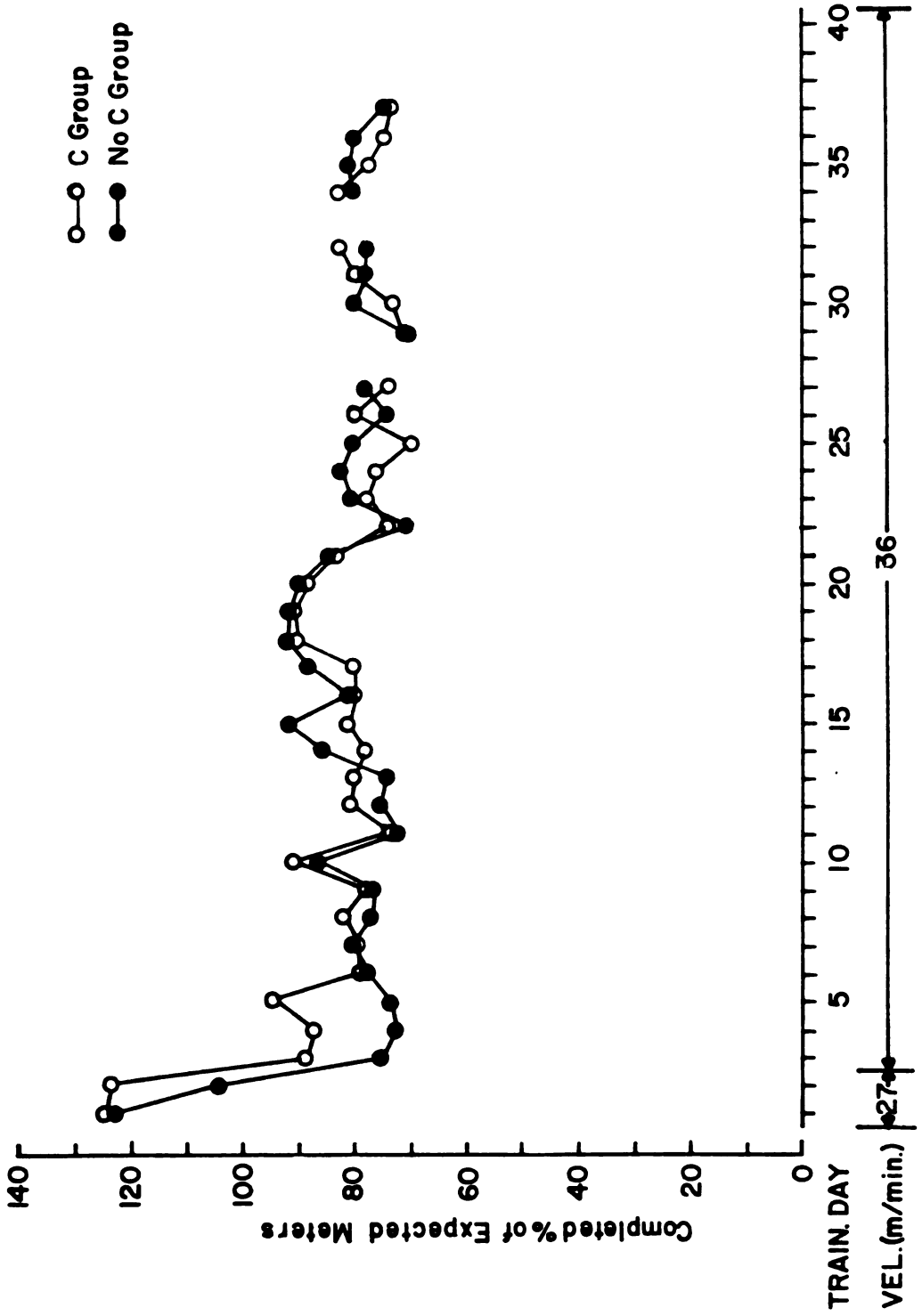

Fig.2. Mean Daily Percent Expected Meters Run for ENDURANCE Animals

The END animals ran at the relatively slow speed of 36 m/min. Periods of continuous running were progressively increased to 60 min at the end of five weeks of training and were maintained at this level for the remainder of the eight week program (see Figure 2 and Appendix A, Table A-2). The single bout of exercise was determined subjectively to result in daily physical exhaustion of the animals. On the average, the rats lost 2.55% of their body weight during each training session (see Appendix B). Body weight data were used to award an unplanned recovery day on Wednesday of each of the last three weeks of training. The animals were run on the 39th and 40th day of the program, but the results were not recorded due to a technician error.

Body Weights at Sacrifice

At the end of eight weeks of exercise, the trained animals were significantly lighter than the sedentary control animals (Figure 3). The differences in body weight between the SPT and END groups of animals were not statistically significant (Table 1). Both trained groups were approximately 20% lighter than the CON group. These results are in agreement with those of previous studies using the CRW (26, 76) and support the general observation that strenuous exercise slows the usual gain in body weight seen in the male rat over time (13). The slower rate of weight gain usually is attributed to an increase in caloric expenditure associated with exercise. In some instances the growth impairment has been ascribed to a

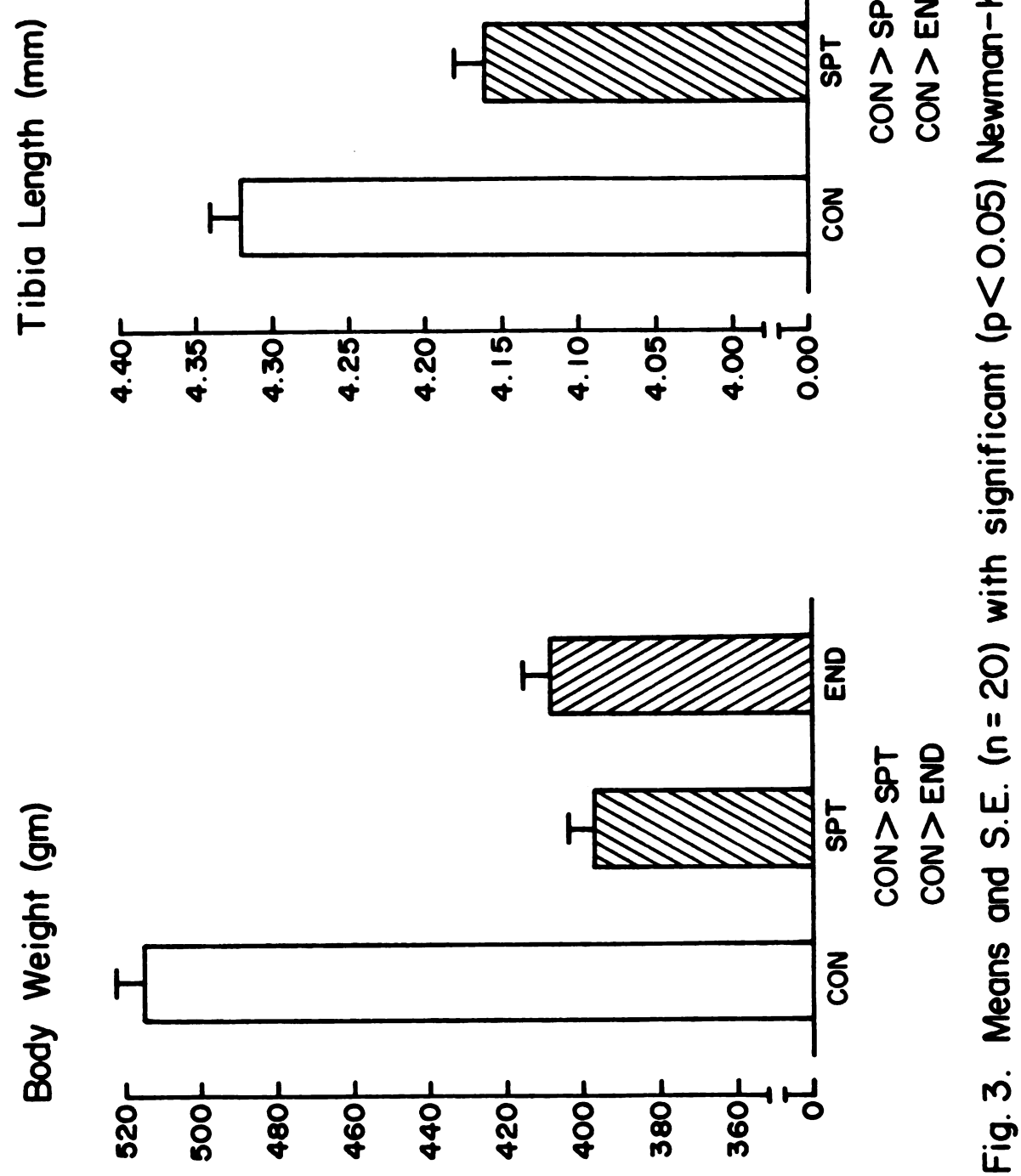

Fig. 3. Means and S.E. (n = 20) with significant ($p < 0.05$) Newman-Keuls contrasts

Table 1. Analysis of variance for overall training effects and Newman-Keuls tests of paired comparisons for absolute and relative bone data.

Dependent Variable	Treatment Means CON	SPT	END	F Value	P Value	Newman-Keuls Test**
Body weight at sacrifice (g)	515.1 ±6.9	397.3 ±6.1	408.3 ±7.0	96.731	< 0.0005*	SPT<CON END<CON
Tibia weight (g)	.991 ±.016	.633 ±.018	.947 ±.011	190.329	< 0.0005*	SPT<END <CON
Rel. tibia weight ($x10^{-3}$)	1.926 ±.026	1.592 ±.034	2.330 ±.041	118.850	< 0.0005*	SPT<CON <END
Tibia length (mm)	4.319 ±.022	4.158 ±.023	4.205 ±.017	15.577	< 0.0005*	SPT<CON END<CON

*Significant overall treatment effect at the 0.05 level.
** Newman-Keuls Tests were run at the 0.05 level of significance

Table 2. Analysis of variance for overall vitamin C effects for body weights at sacrifice and absolute and relative bone data.

Dependent Variable	Treatments Means C	No C	F Value	P Value
Body weight at sacrifice (g)	433.367 ±12.02	447.033 ±10.25	3.202	.079
Tibia weight (g)	.834 ±.034	.880 ±.030	7.742	.007*
Relative tibia weight ($x10^{-3}$)	1.927 ±.067	1.971 ±.057	1.236	.271
Tibia length (mm)	4.210 ±.023	4.245 ±.018	2.042	.159

*Significant overall treatment effect at the 0.05 level.

significant reduction in food intake (13, 62). However, these parameters were not monitored in the present study.

Effects of Vitamin C Supplementation

The training response of the animals receiving supplements of vitamin C and those animals receiving the placebo are shown in Figures 1 and 2. The vitamin C supplementation did not appear to affect the training performance of the rats.

An analysis of the body weight and bone data of the C and No C groups (Table 2) indicated only one significant F-value. The overall mean tibia weight for the three No C groups ($\bar{X} = 0.0880$) was significantly greater ($p < .05$) than that for the three C groups ($\bar{X} = 0.834$). However, this was not the case for relative tibia weight. These results do not support the hypothesis that vitamin C should serve as a preventive for the impairment of bone growth during strenuous training. In fact, the generally lower bone weight and bone length values for the C group indicate that the dosage of vitamin C used may have produced greater growth impairment.

Activity Level Results

All overall bone comparisons between activity levels were statistically significant. Post-hoc test results (SNK) identify the groups that differed significantly from each other ($p \leq .05$) (Table 1).

Absolute tibia lengths of the END and SPT animals were significantly shorter than those of the CON group (Figure 3).

However, there was no difference between the two training groups. These data support the hypothesis that training may induce bone growth impairment.

Both the absolute and relative tibia weights of the SPT group were significantly less than those of the other groups (Figure 4). The END animals had the largest relative tibia weights while the SPT group had the smallest. Relative and absolute bone weights were 46% and 49% higher respectively for the END animals than for the SPT animals. These results were not anticipated. Expectations were that the greater stress involved in the SPT program would result in heavier bones.

Discussion

Several possible explanations could account for the relatively poor performance data of the SPT group. The required running velocities may have been too fast, but observations during the training sessions indicated that the animals were capable of sprinting at the desired speeds. Low PEM and PSF values might suggest that the animals responded to the unconditioned shock stimulus rather than to the conditioned light stimulus. Improper initial training and defects in the CRW equipment could lead to such a learning problem, but the END animals learned to run under the same conditions and had no such difficulties (see Figure 2). A lack of control of environmental factors affecting training performance might have accounted for these results. This is particularly true for air temperature

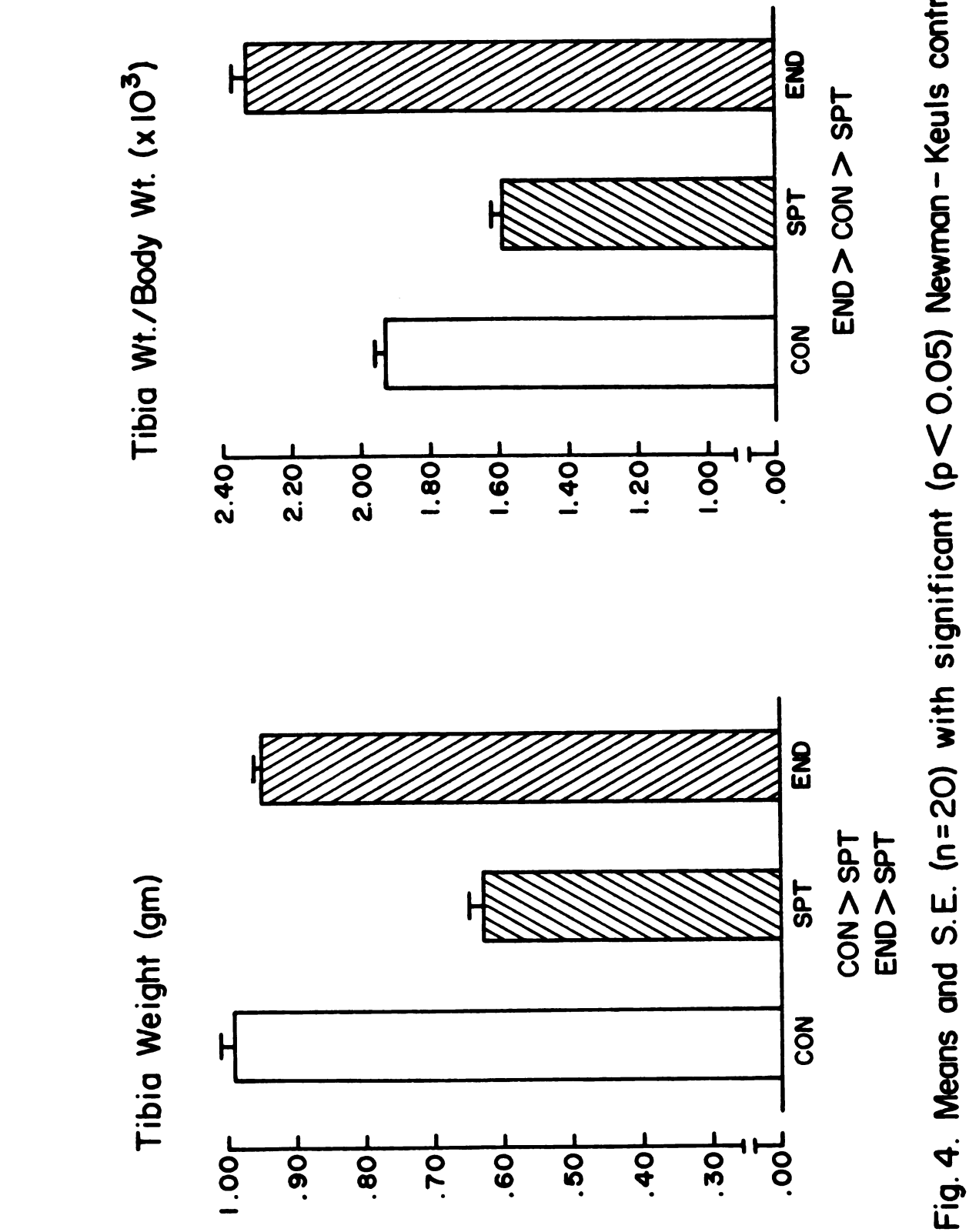

Fig. 4. Means and S.E. (n=20) with significant ($p < 0.05$) Newman-Keuls contrasts

and percent humidity, but again the END data make this explanation improbable (see Appendix B). The most likely cause of the low PEM and PSF values is that the SPT regimen may have produced a state of overtraining. The data in Figure 1 support this hypothesis. Repeated increases in the required velocity may have been expected from the SPT animals before they were fully adapted to the previous velocity. The constant additional stress could have resulted in overtraining.

The bone data strongly reinforce the hypothesis that overtraining was produced by an overly demanding SPT regimen. The intensity of exercise has been hypothesized to play an important role in determining the adaptation of long bones to different training programs (7, 75). The significantly lighter weight of the tibias of the SPT group as compared to the bones of the CON group appears to be a result of the strenuousness of the SPT program.

The heavier relative weights of the tibias of the END animals when compared to those of the CON group are in agreement with the findings of previous investigators (14, 70, 71, 80). These results were anticipated in light of Wolff's Law which states that bone will develop the structure most suited to resisting the forces acting upon it (84).

The tibia length data obtained in this study are consistent with the findings of a number of investigations (40, 65, 78, 80). The consensus among these experiments is

that there is a retardation of long bone growth with high-intensity training.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study was undertaken to determine the effects of two high-intensity exercise programs and vitamin C supplementation on various tibia measurements. Normal male adult rats (Sprague-Dawley strain) were used as subjects. The two training regimens were modifications of Controlled-Running Wheel routines previously reported from this laboratory (82). The modified programs, an endurance running routine (END) and a sprint running routine (SPT), represented attempts to stimulate selectively either aerobic or anaerobic metabolic processes in the experimental animals.

Eighty-four animals were brought into the laboratory and randomly assigned to CON, SPT and END activity groups. One half of the animals in each training group received vitamin C supplementation (C group) and the remaining animals received a placebo (No C group). Both supplements were administered daily by oral syringe. Vitamin C dosage was 2 mg in a .1 cc 5% sugar solution per 100 gm body weight. The animals were 84 days of age at the start of the eight-week treatment period. Selected rats were sacrificed 72 to 96 hours after their last training session.

Selection criteria developed for training performance resulted in a final cell frequency of ten animals per treatment group. Wet weight and length were determined for the left tibia. Relative weight also was determined to account for the size of the animal.

Absolute tibia lengths of the END and SPT animals were significantly shorter ($p < .05$) than those of the CON group. The END animals had the greatest relative tibia weights. Both the absolute and relative bone weights of the SPT group were significantly less than those of the other groups.

Analysis of variance of the C group and the No C group data indicated a significant difference only in absolute tibia weight.

Conclusions

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

1. Both the SPT and END training regimens resulted in shorter absolute tibia lengths in the animals than did the CON treatment.
2. The SPT animals had significantly lower absolute and relative tibia weights when compared to the CON and END animals.
3. The inclusion of vitamin C in this investigation did not appear to serve as a preventive to long bone growth retardation in the trained animals.

Recommendations

1. The present study should be repeated to further investigate the effects of the SPT training regimen on long bone growth.
2. In any follow-up study using the END program, treatment should continue for at least sixteen weeks to determine whether a state of overtraining may be produced.
3. Power-type events for animals must be included to add to the present knowledge of bone adaptations to intense training. High jumping and weight lifting programs should be developed for this purpose.
4. Parameters which measure bone strength and deformation characteristics should be included in future studies.
5. Further investigations are needed to determine whether the sugar administered to the animals may have acted as an intervening variable in the present study.
6. The mechanism of vitamin C in the adaptation of bone to strenuous training should be explored further.

REFERENCES

REFERENCES

1. Bachinsky, P. P. Vlijanie fizičeskih upražneij na obespečnnost organisma vitaminami C i B_1 v vesennij period. Vop. Pitan. 18:53, 1959. From Nutr. Abstr. 30:221, 1960.

2. Bailey, D. A., A. V. Carron, R. G. Teece and H. Wehner. Effect of vitamin C supplementation upon the physiological response to exercise in trained and untrained subjects. Int. Z. Vitaminforsch. 40:435, 1970.

3. Baker, E. M. Vitamin C requirements in stress. Amer. J. Clin. Nutr. 20:583, 1967.

4. Baker, E. M., R. E. Hodges, J. Hood, H. E. Sauberlich and S. C. March. Metabolism of ascorbic - 1-^{14}C acid in experimental human scurvy. Amer. J. Clin. Nutr. 22:549, 1969.

5. Barnes, M. J. Function of ascorbic acid in collagen metabolism. Ann. N. Y. Acad. Sci. 258:264, 1975.

6. Bates, C. J., C. J. Prynne and C. I. Levene. Ascorbate-dependent differences in the hydroxylation of proline and lysine in collagen synthesized by 3T6 fibroblasts in culture. Biochim. Biophys. Acta. 278:610, 1972.

7. Booth, F. W., and E. W. Gould. Effect of training and disuse on connective tissue. In: Exercise and Sport Review. Edited by J. Wilmore. Vol 3, 1975, pp. 83.

8. Brunner, H. Vitamin C experiences with Redoxon in the Swiss army in Frauenfeldt. Schweiz. Med. Wochschr. 71:715, 1941.

9. Buskirk, E. R., K. L. Anderson and J. Brozek. Unilateral activity and bone and muscle development in the forearm. Res. Quart. 27:127, 1956.

10. Carey, E. J. Studies in the dynamics of histogenesis. Experimental, surgical and roentgenographic studies in the architecture of human cancellous bone, the resultant of back pressure vectors of muscle action. Radiology. 13:127, 1929.

11. Clayton, B. E., J. E. Hammant and P. Armitage. Increased adrenocorticortrophic hormone in the sera of acutely scorbutic guinea-pigs. J. Endocr. 15:284, 1957.

12. Comstock, J. P., T. J. Gribble and S. Udenfriend. Further study on the activation of collagen proline hydroxylase in the cultures of L-929 fibroblasts. Archives of Biochem. Biophys. 137:115, 1970.

13. Crews, E. L., III, K. W. Fuge, L. B. Oscai, J. O. Holloszy and R. E. Shank. Weight, food intake, and body composition: effects of exercise and of protein deficiency. Am. J. Physiol. 216:359, 1969.

14. Donaldson, H. H. Summary of data for the effects of exercise on the organ weights of the albino rat; comparison with similar data from the dog. Amer. J. Anat. 56:57, 1935.

15. Donaldson H. H., and S. B. Conrow. Quantitative studies on the growth of the skeleton of the albino rat. Amer. J. Anat. 26:237, 1919.

16. Evans. J. R., and R. E. Hughes. The growth maintaining activity of ascorbic acid. Br. J. Nutr. 17:251, 1963.

17. Faulkner, J. A. New perspectives in training for maximum performance. J. Am. Med. Assoc. 205: 741, 1968.

18. Fox, F. W., L. F. Dangerfield, S. F. Gottlich and E. Jokl. Vitamin C requirements of native mine laborers. Br. Med. J. 2:143, 1940.

19. Gelbke, H. Tierexperimentelle untersuchungen zur frage des enchondralen knochenwachstums unter zug. Arch. Deutsche Ztschr. Chir. 266:271, 1950. Cited by: Evans, F. G. Stress and Strain in Bones. Springfield, Illinois: Charles C. Thomas, 1957.

20. Gey, G. O., K. H. Cooper and R. A. Bottenberg. Effect of ascorbic acid on endurance performance and athletic injury. J. Am. Med. Assoc. 211:105, 1970.

21. Giroud, A., M. Martinet and M. T. Bellon. Dépendance de la fonction cortico-surrénalee chez l'homme, vis-a-vis de l'acide ascorbique: Variations de l'élimination urinaire de l'homme corticale. C. r. Séanc. Soc. Biol. 135:514, 1941.

22. Griffith, W. H., and F. W. Morthland. A study of the military applicability of research on ascorbic acid. Washington, D. C.: Life Sciences Res. Office, FASEB, 1963, pp. 47.

23. Harper, A. A., I. F. S. Mackay, H. S. Raper and G. L. Camm. Vitamins and physical fitness. Br. Med. J. 1:243, 1943.

24. Hayano, M., N. Saba, R. I. Dorfman and O. Hechter. Some aspects of the biogenesis of adrenal steroid hormones. Recent Prog. Horm. Res. 12:79, 1956.

25. Henschel, A., H. L. Taylor, J. Brozek, O. Mickelson and A. Keys. Vitamin C and ability to work in hot environments. Am. J. Trop. Med. 24:259, 1944.

26. Hickson, R. Exercise-induced biochemical alterations in different types of skeletal muscle. Unpublished Ph. D. Thesis, Department of Health, Physical Education and Recreation, Michigan State University, East Lansing, Michigan, 1974.

27. Hodges, J. R., E. M. Baker, J. Hood, H. E. Sauberlich and S. C. March. Experimental scurvy in man. Am. J. Clin. Nutr. 22:535, 1969.

28. Hodges, J. R., and R. T. Hotston. Ascorbic acid deficiency and pituitary adrenocortical activity in the guinea pig. Br. J. Pharmac. 40:740, 1970.

29. Hodges, J. R., and R. T. Hotston. Suppression of adrenocorticotrophic activity in the ascorbic acid deficient guinea pig. Br. J. Pharmac. 42:595, 1971.

30. Hodges, R. E., J. Hood, J. E. Canham and E. M. Baker. Clinical manifestations of ascorbic acid deficiency in man. Am. J. Clin. Nutr. 24:432, 1971.

31. Hutton, J. J., A. L. Tappel and S. Udenfriend. Requirements for α-keto-glutarate, ferrous iron and ascorbate by collagen proline hydroxylase. Biochem. Biophys. Res. Commun. 24:179, 1966.

32. Irwin, M. I., and B. K. Hutchins. Research on vitamin C requirements of man. J. Nutr. 106:823, 1976.

33. Jeffrey, J. J., and G. R. Martin. The role of ascorbic acid in the biosynthesis of collagen. II. Site and nature of ascorbic acid participation. Biochim. Biophys. Acta. 121:281, 1966.

34. Johnson, R. E., R. C. Darling, F. Sargent, P. Robinson, M. Bartlett and A. Kibler. Effects of variations in dietary vitamin C on the physical well-being of manual workers. J. Nutr. 29:155, 1945.

35. Jones, R. S., L. Peric-Golia and K. Eik-Nes. Ascorbic acid deficiency and adrenocortical function in the guinea pig. Endocrinology. 63:659, 1958.

36. Kato, S., and T. Ishiko. Obstructed growth of children's bones due to excessive labor in remote corners. In: Proceedings of International Congress of Sport Sciences. 1964. Edited by K. Kato. Tokyo: Japanese Union of Sport Sciences, 1966.

37. Kersten, H., W. Kersten and H. Staudinger. Zum wirkungsmechanismus der ascorbinsäure. I. Isolierung einer ascorbinsäureabhangigen DPNH-oxydase aus nebennierenmikrosomen. Biochim. Biophys. Acta. 27:598, 1958.

38. Kersten, H., S. Leonhäuser and H. Staudinger. Zum wirkungsmechanismus der ascorbinsäure. II. Ascorbinsäureabhangige DPNH-oxydase und hydroxylierungsreaktionen bei steroiden. Biochim. Biophys. Acta. 29:350, 1958.

39. Keys, A., and A. F. Henschel. Vitamin supplementation of U. S. army rations in relation to fatigue and the ability to do muscular work. J. Nutr. 23:259, 1942.

40. Kiiskinen, A., and E. Heikkinen. Effect of prolonged physical training on the development of connective tissues in growing mice. Proc. Int. Symp. Exercise Biochem., 2nd., 1973, pp. 253.

41. Kirchhoff, H. W. Über den einfluss von vitamin C auf energieverbrauch, kreislauf-und ventilationsgrössen im belastungsversuch. Nutritio Dieta. 11:184, 1969.

42. Kitabchi, A. E. Ascorbic acid in steroidogenesis. Nature (London). 215:1385, 1967.

43. Kitabchi, A. E., A. H. Nathans and C. L. Kitchell. Adrenal gland in vitamin E deficiency. III. Inhibition of ACTH-induced steroidogenesis in isolated adrenal cells by ascorbic acid. J. Biol. Chem. 248:835, 1973.

44. Koch, J. C. The laws of bone architecture. Amer. J. Anat. 21:177, 1917.

45. League of Nations Technical Commission on Nutrition. Quart. Bull. Hlth. Org., L. of Nat. 7:460, 1938.

46. Lehninger, A. L. Biochemistry. New York, N. Y.: Worth Publishers, 1975, pp. 696.

47. Liakakos, D., D. G. Ikkos, P. Vlachos, K. Ntalles and Ch. Coulouris. Effect of ascorbic acid on urinary hydroxyproline of children receiving corticosteroids. Archives of Dis. Childhood. 49:400, 1974.

48. Liakakos, D., N. L. Doulas, D. Ikkos, C. Amoussakis, P. Vlachos and G. Jouramani. Inhibitory effect of ascorbic acid (Vitamin C) on cortisol secretion following adrenal stimulation in children. Clin. Chim. Acta. 65:251, 1975.

49. Lipscomb, H. S., and D. H. Nelson. Dynamic changes in ascorbic acid and corticosteroids in adrenal vein blood after ACTH. Endocrinology. 66:144, 1960.

50. Long, C. N. H. The conditions associated with the secretion of the adrenal cortex. Fed. Proc. 6:461, 1947.

51. Maksjutinskaya, O. V., T. K. Pavlenko and O. I. Snigur. Ėkskrecija vitaminov C močoj u škol' nikov pri različnyh nagruzhah po plavaniju. Gig. Sanit. 12:82, 1967. From Nutr. Abstr. 38:931, 1968.

52. Matthes, S. Athletic performance and vitamin C. Untersuch. Lebensm. 81:81, 1941.

53. Medical Research Council (Accessory Food Factors Committee). Vitamin C requirements of human adults. Medical Research Council, Special Report No. 280. London: Her Majesty's Stationary Office, 1953, pp. 179.

54. Moffroid, M. T., and R. H. Whipple. Specificity of speed of exercise. Physical Therapy. 50:1692, 1970.

55. Namyslowski, L. Vitamin C requirements in sportsmen depending on physical exertion. Roczniki Panstwowego Zakladu Hig. 7:97, 1956. From Chem. Abstr. 51:4518.

56. Namyslowski, L. Estimation of ascorbic acid requirements of athletes in relation to physical exertion. Roczniki Panstwowego Zakladu Hig. 7:97, 1956. From Chem. Abstr. 54:13303.

57. Namyslowski, L. Influence of stress on ascorbic acid content of rat adrenals. Roczniki Panstwowego Zakladu Hig. 7:425, 1956. From Chem. Abstr. 51:3789.

58. Namyslowski, L. Ascorbic acid content of the suprarenals of rats during physical effort. Roczniki Panstwowego Zakladu Hig. 8:79, 1957. From Chem. Abstr. 51:10695.

59. Namyslowski, L. Course of return to normal of ascorbic acid content in rat adrenals after exhaustive exercise. Roczniki Panstwowego Zakladu Hig. 8:265, 1957. From Chem. Abstr. 52;1418.

60. Namyslowski, L. Effect of training on the adaptation of rat adrenals to efforts. Endokrynologia Polska. 9:223, 1958. From Chem. Abstr. 53:8350.

61. National Research Council, Food and Nutrition Board. Recommended Dietary Allowances, revised 1974. Washington, D. C.: National Academy of Sciences, 1974.

62. Oscai, L. The role of exercise in weight control. In: Exercise and Sport Sciences Reviews, Vol. 1. Edited by J. Wilmore. New York: Academic press, 1973, pp. 103.

63. Pincus., G. Regulation of adrenal cortical secretion. In: Adrenal cortex, transactions of Third Conference, Josiah Macy, Jr., Foundation. Edited by E. P. Ralli, 1951.

64. Poal-Manresa, J., K. Little and J. Trueta. Some observations of the effects of vitamin C deficiency on bone. Br. J. Exp. Path. 51:372, 1970.

65. Price-Jones, C. The effect of exercise on the growth of white rats. Quart. J. Exp. Physiol. 16:61, 1926.

66. Prokop, L. Vitamins and athletic performance. Med. u. Ernährung. 2:174 and 199, 1961. From Nutr. Abstr. 33:1064.

67. Ram, M. M. Growth rate and protein utilization in vitamin C deficiency. Indian J. Med. Res. 54: 964, 1966.

68. Rasmussen, S. Exercise physiology at the cellular level. J. Sports Med. Phys. Fitness. 12:97, 1972.

69. Ratsimamanga, R. Variations in the ascorbic acid content of the suprarenal glands during work. C. R. Soc. Biol. 131:863. 1939. From Nutr. Abstr. 9:1659.

70. Roy, R. R. Specific changes in a histochemical profile of rat hindlimb muscle induced by two exercise regimens. Unpublished Ph. D. Thesis, Department of Health, Physical Education and Recreation, Michigan State University, East Lansing, Michigan, 1976.

71. Saville, P. D., and M. P. Whyte. Muscle and bone hypertrophy. Clin. Orthop. 65:81, 1969.

72. Saville, P. D., and R. Smith. Bone density, breaking force and leg muscle mass as functions of weight in bipedal rats. Am. J. Phys. Anthrop. 25:35, 1966.

73. Sayers, G., M. A. Sayers, H. L. Lewis and C. N. H. Long. Effect of adrenotrophic hormone on ascorbic acid and cholesterol content of the adrenal. Soc. Exp. Biol. Med. 55:238, 1944.

74. Selye, H. Textbook of Endocrinology, Second edition. Montreal, Canada: Acta Endocrinologica, 1949.

75. Steinhaus, A. H. Chronic effects of exercise. Phys. Rev. 13:103, 1933.

76. Taylor, J. Histochemical profiles of rat triceps surae and plantaris after seven exercise regimens. Unpublished Ph. D. Thesis, Department of Anatomy, Michigan State University, East Lansing, Michigan, 1971.

77. Thornton, P. A. Effect of ascorbic acid deprivation in guinea pigs on skeletal metabolism. J. Nutr. 95:388, 1968.

78. Tipton, C. M., R. D. Matthes and J. A. Maynard. Influence of chronic exercise on rat bones. Med. Sci. Sports. 4:55, 1972.

79. Udenfriend, S. Formation of hydroxyproline in collagen. Proline is incorporated into peptides before it is hydroxylated. Science. 152:1335, 1966.

80. Van Huss, W. D., W. W. Heusner and O. Mickelson. Effects of prepubertal exercise on body composition. In: Exercise and Fitness, 1969. Edited by D. Franks. Chicago: Athletic Institute, 1970, pp.201.

81. Weinmann, J. P., and H. Sicher. Bone and Bones, Second edition. St. Louis: C. V. Mosby, 1955.

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

83. Wiebel, H. Vitamin C dosage in female athletic students. Deut. Med. Wochschr. 65:60, 1939.

84. Wolff, J. Cited in Rasch, P. J., and R. K. Burke. Kinesiology and Applied Anatomy. Philadelphia: Lea and Febiger, 1974, pp. 12.

85. Yakovlev, N.N. Vitamins B_1 and C requirements of sportsmen. Vop. Pitan. 17:3, 1958.

APPENDICES

APPENDIX A

TRAINING PROGRAMS

Table A-1. Modified Eight Week Sprint Training Program for Postpubertal and Adult Male Rats in Controlled-Running Wheels

Wk.	Day of Wk.	Day of Tr.	Acceler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repeti-tions per Bout	No. of Bouts	Time Be-tween Bouts (min)	Shock (ma)	Run Speed (m/min)	Total Time of Prog. (min: sec)	Total Exp. Meters TEM	Total Work Time (sec) TWT
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
1	1=M	1	2.0	00:10	10	10	8	2.5	1.2	36	42:50	480	800
	2=T	2	2.0	00:10	10	10	8	2.5	1.2	36	42:50	480	800
	3=W	3	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
	4=T	4	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
	5=F	5	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
2	1=M	6	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
	2=T	7	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
	3=W	8	1.5	00:15	30	6	7	2.5	1.2	72	43:00	756	630
	4=T	9	1.5	00:15	30	6	7	2.5	1.2	72	43:00	756	630
	5=F	10	1.5	00:15	30	6	7	2.5	1.2	72	43:00	756	630
3	1=M	11	1.5	00:15	30	6	7	2.5	1.2	72	43:00	756	630
	2=T	12	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
	3=W	13	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
	4=T	14	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
	5=F	15	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
4	1=M	16	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
	2=T	17	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
	3=W	18	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
	4=T	19	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
	5=F	20	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
5	1=M	21	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
	2=T	22	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
	3=W	23	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
	4=T	24	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
	5=F	25	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
6	1=M	26	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
	2=T	27	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	3=W	28	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	4=T	29	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	5=F	30	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
7	1=M	31	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	2=T	32	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	3=W	33	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	4=T	34	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	5=F	35	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
8	1=M	36	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	2=T	37	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	3=W	38	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	4=T	39	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	5=F	40	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450

This training program is a modified version of a standard program designed using male rats of the Sprague-Dawley strain (23,49).

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

APPENDIX A--continued

Table A-2. Modified Eight Week Endurance Training Program for Postpubertal and Adult Male Rats in Controlled-Running Wheels

Wk.	Day of Wk.	Day of Tr.	Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Complete Bouts	Partial Bouts (min: sec)	Time Between Bouts (min)	Shock (ma)	Run Speed (m/min)	Total Time of Prog. (min: sec)	Total Exp. Meters TEM	Total Work Time (sec) TWT
0	4=T	-2	3.0	40:00	10	1	1		5.0	0.0	27	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1		5.0	0.0	27	40:00	---	---
1	1=M	1	2.0	02:30	0	1	6		2.5	1.2	27	27:30	405	900
	2=T	2	2.0	02:30	0	1	6		2.5	1.2	27	27:30	405	900
	3=W	3	1.5	05:00	0	1	3		5.0	1.2	36	25:00	540	900
	4=T	4	1.5	05:00	0	1	3		5.0	1.2	36	25:00	540	900
	5=F	5	1.5	05:00	0	1	3		5.0	1.2	36	25:00	540	900
2	1=M	6	1.5	05:00	0	1	3		5.0	1.2	36	25:00	540	900
	2=T	7	1.0	07:30	0	1	2		5.0	1.2	36	20:00	540	900
	3=W	8	1.0	07:30	0	1	2		2.5	1.2	36	17:30	540	900
	4=T	9	1.0	07:30	0	1	2		1.0	1.2	36	16:00	540	900
	5=F	10	1.0	15:00	0	1	1		0.0	1.2	36	15:00	540	900
3	1=M	11	1.0	15:00	0	1	1	05:00	1.0	1.2	36	21:00	720	1200
	2=T	12	1.0	15:00	0	1	1	07:30	1.0	1.0	36	23:30	810	1350
	3=W	13	1.0	15:00	0	1	1	10:00	1.0	1.0	36	26:00	900	1500
	4=T	14	1.0	15:00	0	1	1	12:30	1.0	1.0	36	28:30	990	1650
	5=F	15	1.0	15:00	0	1	2		1.0	1.0	36	31:00	1080	1800
4	1=M	16	1.0	15:00	0	1	2	05:00	1.0	1.0	36	37:00	1260	2100
	2=T	17	1.0	15:00	0	1	2	07:30	1.0	1.0	36	39:30	1350	2250
	3=W	18	1.0	15:00	0	1	2	10:00	1.0	1.0	36	42:00	1440	2400
	4=T	19	1.0	15:00	0	1	2	12:30	1.0	1.0	36	44:30	1530	2550
	5=F	20	1.0	15:00	0	1	3		1.0	1.0	36	47:00	1620	2700
5	1=M	21	1.0	15:00	0	1	3	05:00	1.0	1.0	36	52:00	1800	3000
	2=T	22	1.0	15:00	0	1	3	07:30	1.0	1.0	36	54:30	1890	3150
	3=W	23	1.0	15:00	0	1	3	10:00	1.0	1.0	36	57:00	1980	3300
	4=T	24	1.0	15:00	0	1	3	12:30	1.0	1.0	36	59:30	2070	3450
	5=F	25	1.0	15:00	0	1	4		1.0	1.0	36	63:00	2160	3600
6	1=M	26	1.0	15:00	0	1	4		1.0	1.0	36	64:00	2160	3600
	2=T	27	1.0	30:00	0	1	2		5.0	1.0	36	65:00	2160	3600
	3=W	28	1.0	30:00	0	1	2		2.5	1.0	36	62:30	2160	3600
	4=T	29	1.0	30:00	0	1	2		1.0	1.0	36	61:00	2160	3600
	5=F	30	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
7	1=M	31	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	2=T	32	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	3=W	33	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	4=T	34	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	5=F	35	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
8	1=M	36	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	2=T	37	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	3=W	38	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	4=T	39	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	5=F	40	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600

This training program is a modified version of a standard program designed using male rats of the Sprague-Dawley strain (23,49).

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

APPENDIX B

BASIC STATISTICS FOR TRAINING DATA

Basic statistics for Percentage of Body Weight Loss, Environmental Factors and Performance Criteria

Variable	N[a]	Mean	Standard Deviation	Simple Correlations: Air Temp	Per Humid	Bar Press	Per Body Wt Loss	PEM
SPT C								
Air Temp (F)	367	72.9	4.8					
Per Humid	367	39.0	12.1	.110				
Bar Press (mmHg)	367	740.7	4.3	-.276	-.713			
Per Body wt loss	367	1.7	.5	-.066	-.206	.044		
PEM	367	55.4	20.2	-.199	-.359	.121	.258	
PSF	367	56.8	19.5	-.398	-.312	.164	.196	.872
SPT No C								
Air Temp (F)	376	73.1	4.6					
Per Humid	376	38.5	12.3	.125				
Bar Press (mmHg)	376	740.8	4.2	-.263	-.715			
Per Body wt loss	376	1.7	.6	.000	-.200	.046		
PEM	376	61.6	25.9	-.165	-.383	.210	.115	
PSF	376	62.6	23.4	-.260	-.271	.129	.032	.868
END C								
Air temp (F)	340	73.9	4.0					
Per Humid	340	47.1	10.7	.134				
Bar Press (mmHg)	340	739.5	3.8	-.288	-.675			
Per Bocy wt loss	340	2.5	1.0	.374	.139	-.240		
PEM	340	82.8	24.7	-.279	-.173	.232	-.069	
PSF	340	70.7	18.6	-.403	-.083	.159	-.118	.685
END No C								
Air Temp (F)	348	73.9	4.0					
Per Humid	348	47.0	10.6	.151				
Bar Press (mmHg)	348	739.5	3.8	-.294	-.677			
Per Body wt loss	348	2.6	1.0	.439	.114	-.159		
PEM	348	82.2	18.7	-.231	-.253	.286	-.003	
PSF	348	69.6	19.2	-.254	-.102	.153	.021	.748

N[a] = total days training, all animals