SPECIFIC CHANGES IN RAT FEMURS INDUCED BY TWO EXERCISE REGIMENS AND VITAMIN C SUPPLEMENTATION

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

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1976

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ABSTRACT

SPECIFIC CHANGES IN RAT FEMURS INDUCED BY TWO EXERCISE REGIMENS AND VITAMIN C SUPPLEMENTATION

By

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This investigation was undertaken to determine the effects of eight weeks of sprint (SPT) or endurance (END) training and/or vitamin C supplementation on the wet weight, dry weight, length and ash content of the femur in the male albino rat (Sprague-Dawley Strain). Wet weights were recorded using a Mettler Balance. Dry weights were obtained following 24 hours in a 110 degree (C) drying oven by the same method. The dried samples were ashed for 24 hours at 600 degrees centigrade to determine the mineral content. All statistical analyses were run using a two-way fixed effects AOV with Neuman-Keuls tests run on significant F values.

The SPT and END exercise regimens both produced a number of alterations in the femurs of the animals. The CON animals had the greater absolute wet weights and dry weights when compared to the SPT and END animals. The END and CON groups had longer bones (absolute) than the SPT group and the CON group had a greater ash content than the SPT group (absolute).

In all relative measurements, the SPT and END animals had greater values than the CON group.

Margaret Anne Kolka

The vitamin C supplementation did not appear to have any beneficial or other effect on training performance or long bone growth with the high intensities of training in this investigation.

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Margaret Anne Kolka

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

1976

DEDICATION

To my family and friends.

ACKNOWLEDGEMENTS

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

A very special thank you is extended to Dr. W. D. VanHuss 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. D. VanHuss, Dr. R. R. Roy and Dr. K. W. Ho for making the writing of this thesis a worthwhile experience.

A special thank you is extended to Dr. R. R. Roy for his assistance in statistical procedures and editing of early drafts, to David Anderson for his assistance in laboratory techniques, to Dr. G. Mikles for his continued support and to Ann Snyder, Dee Ulmer and Charlene Troyano for their assistance during the processing of the tissues. A very special thank you is offered to Nancy McNames, Jennifer Parks, Bonnie Smoak and Crystal Fountain for their friendship and concern.

Gratitude is due to Dr. O. Mickelsen for the extensive use of the facilities of his laboratory, Department of Food Science and Human Nutrition.

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CHAPTER ONE

THE PROBLEM

The stress and strain of muscular activity and weight bearing is thought to provide a stimulation for the growth and development of bone. However, research results on the effects of exercise on bone growth in young animals are not clear at this point. It is believed that there is a resultant increase in the rate of calcium deposition to the bone with exercise and conversely, a decrease in the rate of calcium resorption from the bone (4,6). Current literature indicates that exercise induced changes in bone may be related to the intensity of the training program. High intensity and relatively short duration repetitive running, 60-80 m/min for 30 sec, apparently stimulates the growth of new bone around the diaphyses of rat tibias (51). Training at slow speeds and low intensities has not produced any noticable alterations in the bones of rats (40,44). However, swimming of moderate intensity has been shown to retard the long bone growth in young animals (30,51,52).

The response of bone to vitamin C supplementation during high intensity training should be positive since vitamin C is involved in the formation of the fibrous structures of the body. The effects of vitamin C on performance has been studied in both man and animals (7,22,25,53). Animal data presented by Demarais, tend to confirm the need for larger than normal quantities of vitamin C with training (8). However, no studies have been found which evaluate the effects of vitamin C supplementation as a means of preventing the retardation of long bone growth which has been observed in endurance training.

Current literature has indicated that exercise consists of a continuum of specific levels, each of which elicits a somewhat different response within the organism. However, little is known about the specific effects of the different exercise on long bone growth. The two training programs used in this study were designed to provide functional overloads at distinctly different points in the metabolic continuum. The intensities of the two programs were designed to provide very strenuous physical stress which stimulates overtraining in short-duration high-intensity work and in long-duration low-intensity work.

The femur of the rat is a long bone which provides a workable tool for bone analysis, is easily accessible and directly involved in the flexion and extension of the hip. Use of a long bone from the hindlimb of the rat provides comparable data with previous and current investigations.

Previous findings in the rat indicate a depletion of the intrinsic supply of vitamin C in the adrenals during heavy stress (1,54). These data indicate the supply of the vitamin is not readily replaced even though the rat is known to synthesize this vitamin. Normal growth of the fibrous tissues, including bone, is dependent on an adequate supply of vitamin C within the animal (7,19,47). It was reasoned that supplementation might prevent the vitamin C depletion, thus have a favorable effect upon bone growth.

Statement of the Problem

To determine the effects of high intensity training of short and long duration and/or vitamin C supplementation on the wet weight, dry weight, length and ash content in the right femur of the rat. It was hypothesized that the high intensity training regimens in this investigation and the vitamin C supplementation should result in a greater relative weight, relative length and relative mineral content of the femur in the trained animals when compared with control animals.

Significance of the Problem

Exercise related alterations in the long bones, although controversial, are well documented (4,6,9,11,21,26,28,30,40,41,44,46,51). In particular, confusion exists in attempting to determine the work intensity in the various programs. The need for further assessment of bone response to various controlled, reproducible levels of training is needed, preferable with high levels or intensity. Changes in the femur of the highly trained animals in this investigation should provide further insight into the relationship between the intensity of training and long bone growth.

The effect of vitamin C on bone during high intensity exercise is not clear in the literature. The inclusion of vitamin C in this investigation should lead to a better understanding of the relationship between vitamin C and high levels of training on bone growth.

Limitations of the Study

1. The results are limited to the right femur of adult male rats.
2. The rat synthesizes its own supply of vitamin C, therefore, the significance of the supplemental vitamin C effect may not be fully revealed in this study.
3. The training programs were not of equal intensity, thus may not have been truly comparable regarding the type of exercise used.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

This review focuses on the composition of bone, stress and bone growth, the mechanism of vitamin C, vitamin C and its relationship to athletic performance and specificity of training.

Composition of Bone

Bone consists of an organic and an inorganic component (5,6,11, 12,14,19). The organic element consists of collagenous fibers embedded in a ground substance. This organic component determines the density of the tissue. An appatite crystal of calcium and phosphate constitutes the inorganic component which provides rigidity to the bone. The two constituents are consolidated in a matrix, resulting in great strength and adaptability to the tissue.

The inorganic matrix, usually comprising one third to one half of whole bone, is primarily calcium phosphate (60-80%), calcium carbonate (10%), magnesium phosphate (1-2%) and magnesium carbonate (1-2%) (11,13,19). The inorganic matrix, or mineral content of the bone, can be represented by the ash content of the bone. Ash content is a desirable gravimetric reference state for analysis of bone samples (31).

Mechanical Stress and Bone Growth

The first suggestion of an intensity of training-bone growth relationship was cited by Steinhaus (46), who hypothesized that the pressure produced by exercise upon bone epiphyses stimulated bone growth until an optimal length had been achieved. If the pressure continued beyond this point bone growth would be retarded. Rarick, in support of this contention, concluded that an undefined minimum of physical activity is essential for normal bone growth (41). Donaldson (9) has presented evidence in support of the stress involvement in the growth of long bones in rats and dogs; and increases in bone growth have been reported with low-intensity exercise programs (41). However, impaired growth following training is also supported in well-controlled investigations (40,46,52).

A number of investigators have trained growing animals and recorded lengths and weights of bones. Training has been reported to both increase bone length and decrease bone length; to increase bone weight and decrease bone weight. Current literature has indicated that the exercise induced changes in bone may be related to the intensity of the training program (6,46). Experiments with animals have demonstrated that the bones of animals receiving stressful programs are short and dense (44) and can tolerate large lateral forces before breaking (43).

Growing animals (rats) trained by running up to six hours a day at a low intensity showed no effect on bone growth as indicated by length and weight when compared to body weight (40,44). Alteration in bone growth in mice occured in a training program of 18 m/min for 80 min a day for 12 weeks with longer and heavier femurs appearing (28). However, when the training regimen was altered to 80 min/day for 21 weeks or 120 min/day for 12 weeks, shorter and lighter bones appeared. This is in support of the premise that higher duration will lead to inhibition. In a training regimen involving speeds of 26-30 m/min for 60 minutes a day, Tipton found shorter and lighter bones in rats when compared to body weight (51).

High intensity training has been shown to inhibit the growth in length of the long bones in laboratory animals, and furthermore to increase the mineral content of the specific bone (6). King found that with high intensity repetitive exercise, 30 sec at 60-80 m/min, density of the bone in rats increased (29). However, there was no apparent change in the density of the bone following 26-30 m/min one hour a day for ten weeks (29). Work by Tipton (51) and Saville (44) showed no changes in the mineral content of the long bones in rats. An increase in the relative mineral content (calcium) of mice trained in a high-intensity program was shown in work by Kiiskinen and Heikkinen (21,28).

Removal of the local physiological environmental factors (mechanical stress, weight-bearing and muscle action) results in metabolic alterations in the bone tissue (4,6,11,19). Cortical thinning, loss of trabeculation (supporting structures), increased fragility and loss of mineral are apparent. The loss of mineral appears to occur without significant alteration in the chemical composition or histology of the remaining bone.

On the basis of the literature review it was expected that the two high intensity training programs would result in heavier, denser bones relative to body weight. However, it was expected that the overall growth of the trained animals would be less, therefore the absolute bone lengths and weights would be less.

Mechanism of Vitamin C

All animals with exception of the guinea pig, one species of bat, monkey and man synthesize vitamin C (ascorbic acid) (7,19,47). Vitamin C is involved in the formation of intracellular substances to include the collagen of fibrous structures, and the matrices of bone, cartilage and dentin. In the absence of this vitamin, the condition known as scurvy develops. The pathology of scurvy induces the cessation of bone formation and a breakdown in collagen (47). Resorption of mineral depositions occurs and a resultant atrophy and bone fragility is apparent. Supplementation of vitamin C is shown histologically to reverse this trend within a few hours through tissue regeneration.

Normal bone formation has been found to be dependent on an adequate supply of vitamin C (39,50). The need for this vitamin appears to be increased during exercise in children involved in strenuous and/or prolonged training programs (55). A search of the literature has revealed no evidence available which evaluates the effect of supplementary vitamin C intake as a measure of preventing the bone growth retardation which accompanies heavy exercise.

Vitamin C and Athletic Performance

Previous investigations (25,53) have shown that a slight increase in ascorbic acid with training is desirable. Doses of up to one gram daily have shown a slight, but not significant, alteration in performance (25). Beneficial effects in both cardiovascular, as measured by PWC_{170}, and metabolic responses have been reported in exercise studies using vitamin C supplementation. However, very high doses of vitamin C may increase O_2 utilization by metabolically active systems and thus have a negative effect on performance by disturbing the equilibrium between O_2 transport and O_2 utilization (25).

Ascorbic acid appears to improve the biosynthesis of epinephrine and norepinephrine (25). The increased secretion of norepinephrine subsequently enhances stroke volume by increasing cardiac contractility. The heart can reach the same cardiac output by working at a lower heart rate and therefore working more efficiently. The addition of vitamin C during training also enhances the utilization of the plasma free-fatty-acids as an energy source in the working muscle (25). This shift in metabolism is a result of the glycogen-sparing effect on muscle tissue and the liver. Beneficial effects would be most prominent during endurance exercise.

Specificity of Training

Gross measurements of total-body oxygen uptake and debt have been used to reflect human metabolic responses to physical activity. Repeated bouts of exhaustive sprint running lead to an increased tolerance of oxygen debt which presumably reflects a greater compacity 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 oxidative muscle metabolism and tends to increase total-body oxygen uptake compacity. Moderate or light workloads and relatively long bouts of continuous exercise are typical of endurance training programs (23,42,49). The two training programs used in this study were developed to simulate in animals, the sprint and endurance programs of man.

CHAPTER THREE

RESEARCH METHODS

This investigation was designed to determine possible exercise and/or vitamin C induced changes in the wet weight, dry weight, length and ash content in the right femur of the rat.

Experimental Animals

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. A standard period of 12 days was allowed for adjustment to the laboratory conditions. Activity was initiated when the animals were 84 days old.

Research Design

This study was organized as a two-way design with three activity groups and two dietary supplement groups. The supplement (vitamin C) was crossed throughout the activity groups.

	CON	SPT	END
C	10	10	10
No C	10	10	10

The duration of the experiment was eight weeks, a period which has been sufficient to produce marked changes in body weight, body ash and muscle responses (52). The three activity groups are as follows:

Sedentary Control Group

The 24 animals in the second shipment constituted the sedentary control (CON) group. These animals received no exercise and were forced to remain relatively inactive throughout the experiment. The animals were housed in individual sedentary cages (24 cm by 18 cm by 18 cm) during both the adjustment and activity periods.

Sprint Group

The sprint running (SPT) group was comprised of the 30 animals in the first shipment. Each of these animals was housed in individual voluntary activity cages (sedentary cages with access to a freely revolving wheel) during the adjustment period and in individual sedentary cages during the experimental period. The SPT animals were subjected to an interval training program of high-intensity sprint running. The workload of the SPT group was gradually increased until on the 27th day of training and thereafter, the animals were expected to complete six bouts of exercise with 2.5 minutes of inactivity between bouts. Each bout included five 15 second work periods with four 30 second rest periods. During the work periods, the animals were expected to run at the extremely fast speed of 108 m/min.

Endurance Group

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

The two dietary supplement groups are as follows:

Vitamin C Group

One half of the animals in each activity group received vitamin C supplementation (C group). Vitamin C supplementation was administered orally by syringe with a dosage of 2.4 mg vitamin C (Merck) in a .1 ml 5% sugar solution per 100 g body weight between 7 and 9 pm daily. The administration of the supplement was begun the day prior to the initiation of activity and terminated the day prior to sacrifice. The dosage used was equivalent to 1680 mg daily for a 70 kg man.

Placebo Group

The remaining animals in each of the activity groups (no C group) received an identical quantity of the sugar solution per unit of body weight.

Training Procedures

The SPT and END groups were trained in a battery of individual controlled running wheels (CRW). The 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. (58)

Animals learn to run in the CRW by avoidance-response operant conditioning. A low intensity controlled shock current, applied through alternating grids comprising the running surface, provides the motivation for the animal 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 period, the light remains on and shock is administered. As soon as the animal reaches the desired speed, the light is immediately extinguished 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 the work periods.

Performance data are prescribed 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 criteria used to evaluate and compare training performance. A secondary criteria is provided by the percentage of shock-free time (PSF) which is calculated from the CDS and the total work time (TWT):

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

In this study all exercise treatments were administered once a day, Monday through Friday, between 12:30 and 5:30.

Animal Care

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

The animals received food (Wayne Lab Blox) and water *ad libitim*. 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 daily sequence of 12 hours of light followed by 12 hours without light. Since the rat is normally a nocturnal animal, the light sequence was established so that the lights were off between 1:00 pm and 1:00 am and on between 1:00 am and 1:00 pm. 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

Twelve animals were sacrificed from each of the six subgroups. Since one of the inherent purposes of the study was to compare various parameters in two groups of highly trained animals, three extra rats originally were included in the SPT and END groups. Twelve animals were selected for sacrifice from each of the six groups on the basis of their health and 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 minimal performance criteria were established. However, individual daily records of PEM and PSF values were examined and those animals making the best adaptations to the training regimens were selected for sacrifice. All 24 CON animals were judged to be healthy and were sacrificed.

Three sacrifice periods of two days duration (Monday and Tuesday) were established. All animals within a treatment group were killed during a single sacrifice period (i.e., 12 animals each day). The trained animals were killed either 72 or 96 hours after their last bouts of exercise were completed. This procedure was followed to eliminate any transient effects of acute 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 ml/100 g body weight) of a 6.48% sodium pentobarbital (Halatal) solution. After selected tissues were removed for analysis by other investigators, the animals were eviscerated, placed in plastic bags and frozen at 20 degrees Fahrenheit.

Tissue Removal and Analysis

The frozen carcasses were removed after 9 months of storage and thawed overnight. The right femur was excised and all extraneous tissue removed. A wet weight was determined to the nearest .001 g using a Mettler Balance. Bone length as measured from the greater trochanter to the lateral condyle was recorded to the nearest .1 mm using a vernier caliper (37). The tissues were dried at 110 degrees centigrade for 24 hours, placed in a dessicator, and the dry weight determined using the same procedure as followed for the wet weight. The dried samples were crushed with a mortar and pestle, to allow for complete ashing. The samples were then placed in vycor crucibles in a 600 degree centigrade oven for ashing, a method for removing or destroying organic matter associated with bone (2,15,16,17,18,27,31,33,34,44,56). After 24 hours the samples were removed and returned to room temperature in a dessicator and ash measured to the nearest .001 g (2,16,17,27). The ash samples were then placed in capped one dram bottles to await further analysis.

Statistical Analysis

Body weight, absolute and relative wet weight, dry weight, length and ash content were analyzed using a two-way fixed effects (activity and vitamin C) analysis of variance routine on the Michigan State University Control Data 6500 Computer (CDC 6500). Neuman-Keuls tests were used whenever a significant overall F ratio (<.05) was obtained.

CHAPTER FOUR

RESULTS AND DISCUSSION

The material in this chapter is organized into four main sections. The first part deals with the training results from the Controlled-Running Wheel (CRW) programs. It includes the percentage of body weight lost during the daily exercise periods, the environmental conditions during the training period and the training response as reflected by the two performance criteria. Secondly, body weight results are given. Thirdly, the femur data and analyses are presented. Finally, a discussion is offered that attempts to relate the present findings to those of other investigations reported in the literature.

Training Results

The sprint (SPT) and endurance (END) Controlled-Running Wheel (CRW) training programs are presented in 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 to the end of the program, the animals were expected to run at velocities ranging from 90 to 108 m/min. No comparable exercise program for small animals has been found in the literature. The results indicate the animals could not maintain the program requirements. PEM values fell to approximately 45 during the last three weeks of training.

Several possible explanations could account for these relatively poor performance data. The required running velocities may have been too fast, however observations during the training sessions revealed that the animals were capable of sprinting at the desired speeds. Low PEM 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 and percent humidity, but again the END data make this explanation improbable (See Appendix B). The most likely cause of the low PEM values is that the SPT regimen may have produced a state of overtraining. The data presented in Figure 1 supports the view that the SPT regimen produced a state of overtraining. Increases in the required velocity were expected repeatedly from the SPT animals before they were fully adapted to the previous velocity. The animals clearly could not handle the training program. The constant additional stress could have resulted in overtraining.

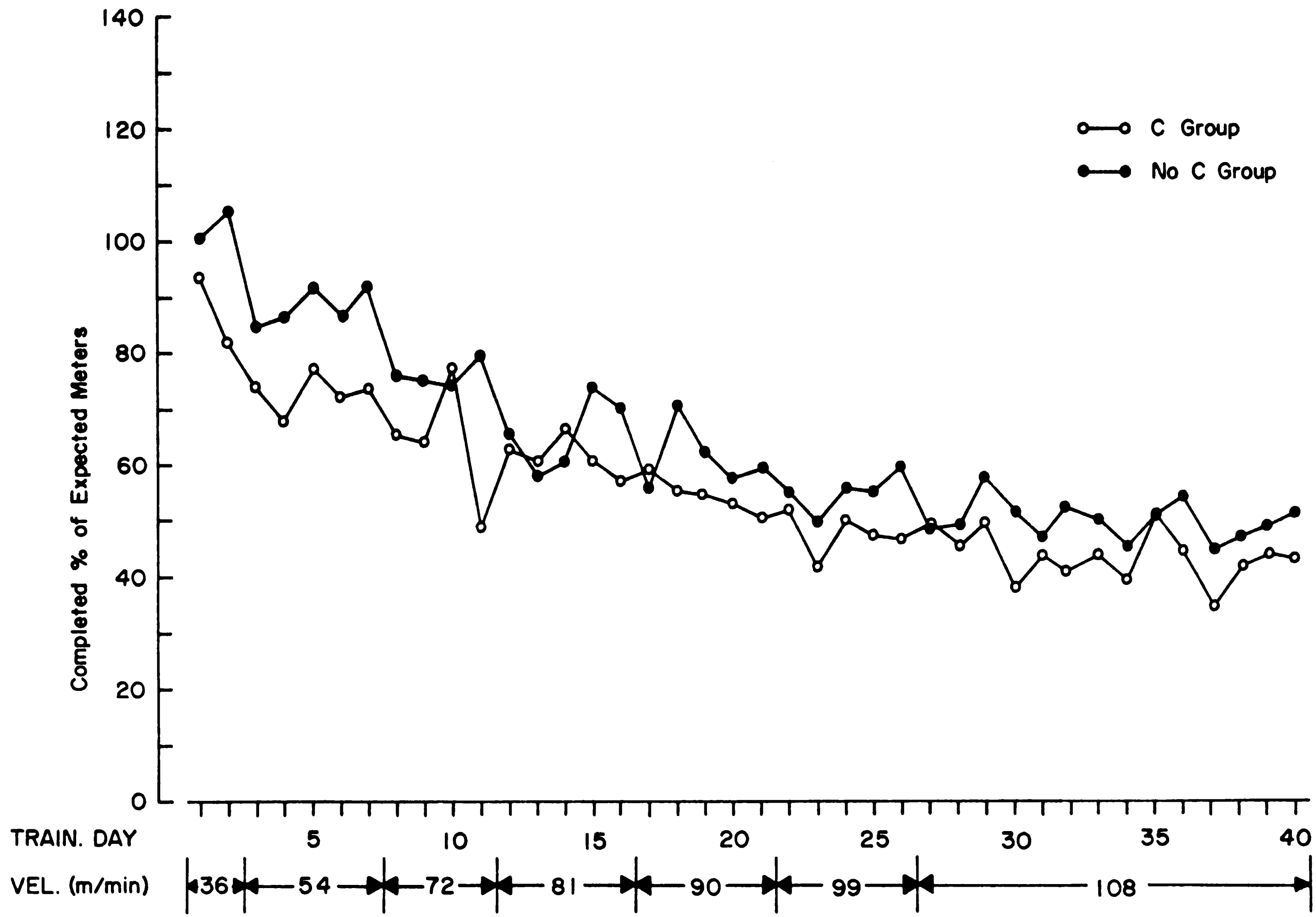

Fig. 1. Mean Daily Percent Expected Meters Run for SPRINT Animals

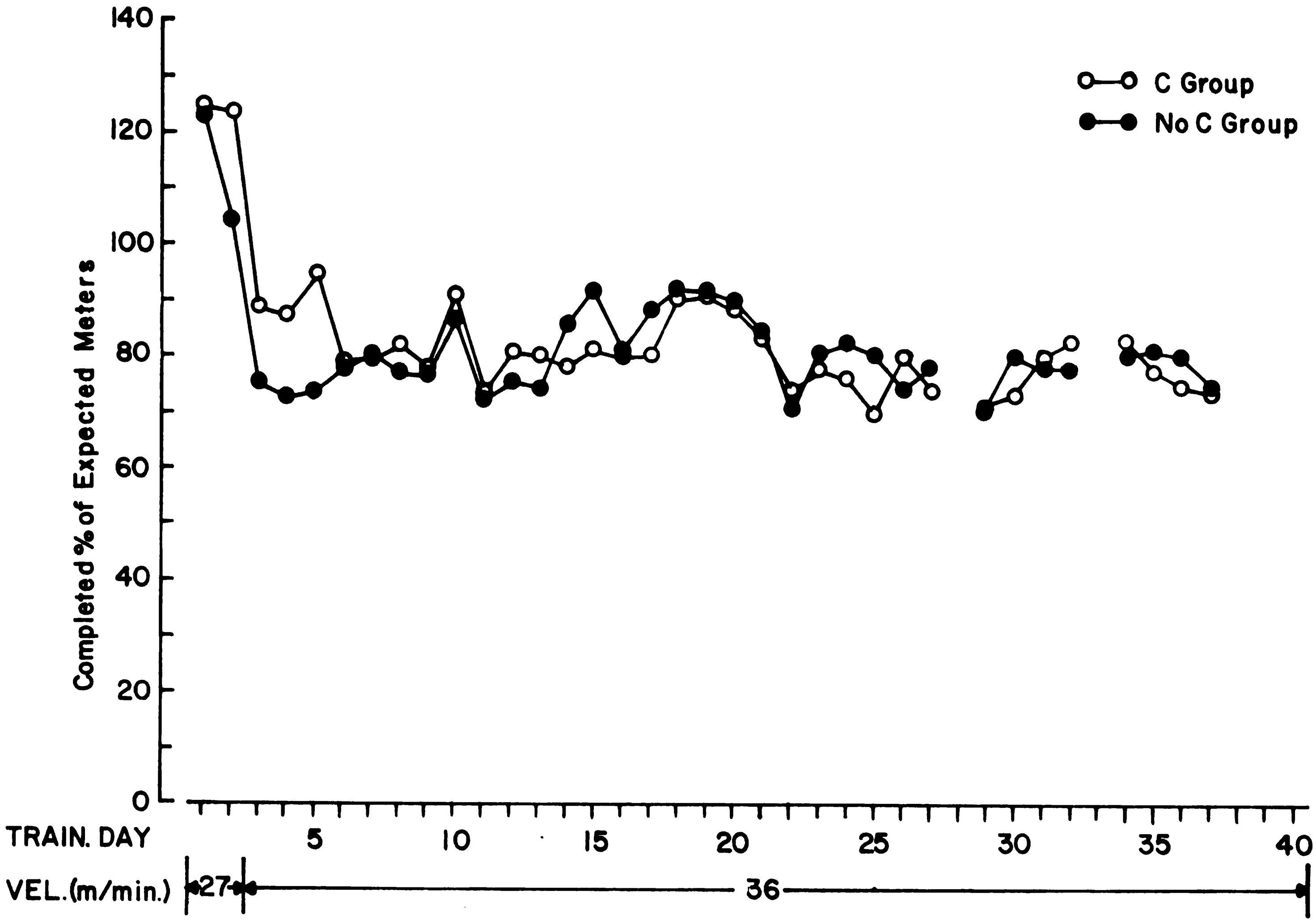

Fig. 2. Mean Daily Percent Expected Meters Run for ENDURANCE Animals

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 group and the No C group. These results indicate that the animals were able to maintain the daily requirements of the END program relatively well.

The END animals ran at the relatively slow speed of 36 m/min. Periods of continuous running were progressively increased to 60 minutes at the end of five weeks of training and were maintained at this level for the remainder of the eight week program (Appendix A). The single bout of exercise was determined subjectively to result in daily physical exhaustion of the animals. Repeated exposure to this level of stress could have resulted in a mild state of overtraining. On the average, the END animals lost 2.55% of their body weight during each training session. 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 days 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 training groups of animals was not statistically significant. Both trained groups were approximately 20% lighter than the CON group. These results are in agreement with those of previous studies using the CRW (23,49) and support the general observation that strenuous exercise slows the usual gain in

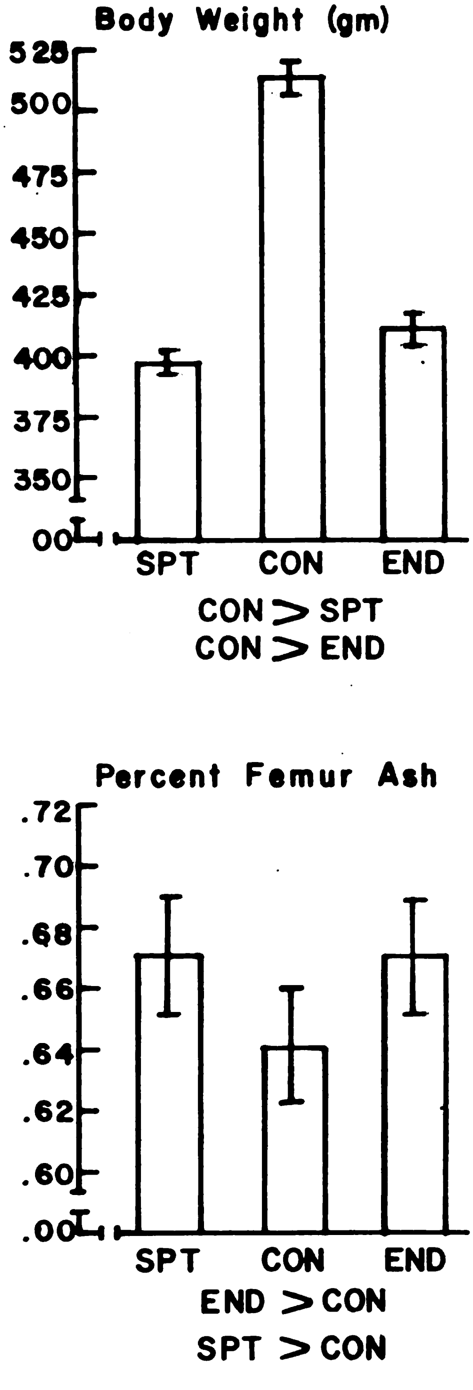

Fig. 3 Means and S.E. (n=20) with significant ($p<0.05$) Newman— Keuls Contrasts

body weight seen in the male rat over time (42). The slower rate of weight gain is usually attributed to an increase in caloric expenditure associated with exercise. In some instances the growth impairment has been attributed to a significant reduction in food intake (38).

Vitamin C Effects

The training responses of the animals supplemented with vitamin C as compared to those performances of the No C groups are shown in Figures 1 and 2. The vitamin C supplementation did not appear to affect the training performance of the animals.

In the ten analyses run on vitamin C effects (Tables 1 and 2), only two showed significant P values. Absolute femur length indicated that the No C group had longer bones. The relative ash measurement showed greater values for the C groups. However, post hoc tests (Neuman Keuls) failed to show significance in any comparisons.

Femur Results

The femur absolute wet weight and absolute dry weight results followed the same pattern as was found for the body weight (Figure 4). The CON group had significantly larger mean absolute wet and dry weights than did either of the trained groups. The absolute wet and dry weights of the SPT and END groups were not significantly different.

Absolute femur lengths were significantly shorter in the SPT group when compared to the END and CON groups (Figure 5). There was no difference however, between the END and CON groups. The CON group was

Table 1. Analysis of Variance for overall vitamin C effects for body weights at sacrifice and absolute femur results.

Dependent Variable	Treatment Means C	Treatment Means No C	F Value	P Value
Body weight at sacrifice (g)	451.533	445.500	3.162	.077
Femur weight (g)	1.183	1.215	1.637	.204
Dry femur weight (g)	.790	.811	1.725	.192
Femur Length (cm)	3.698	3.857	7.946	.007*
Ash weight (g)	.489	.499	.987	.999

*Significant overall vitamin C effect at the 0.05 level. Post Hoc test revealed no significant differences between the means.

Table 2. Analysis of Variance for overall vitamin C effects for relative femur results.

Dependent Variable	Treatment Means C	Treatment Means No C	F Value	P Value
Femur weight (g)	2.773	2.748	.213	.999
Dry femur weight (g)	1.854	1.837	.255	.999
Femur length (g)	8.703	8.756	.091	.999
Relative Ash	1.575	1.492	5.133	.026*
Percent Ash	.664	.654	1.952	.165

*Significant overall vitamin C effect at the 0.05 level. Post Hoc test revealed no significant differences between the means.

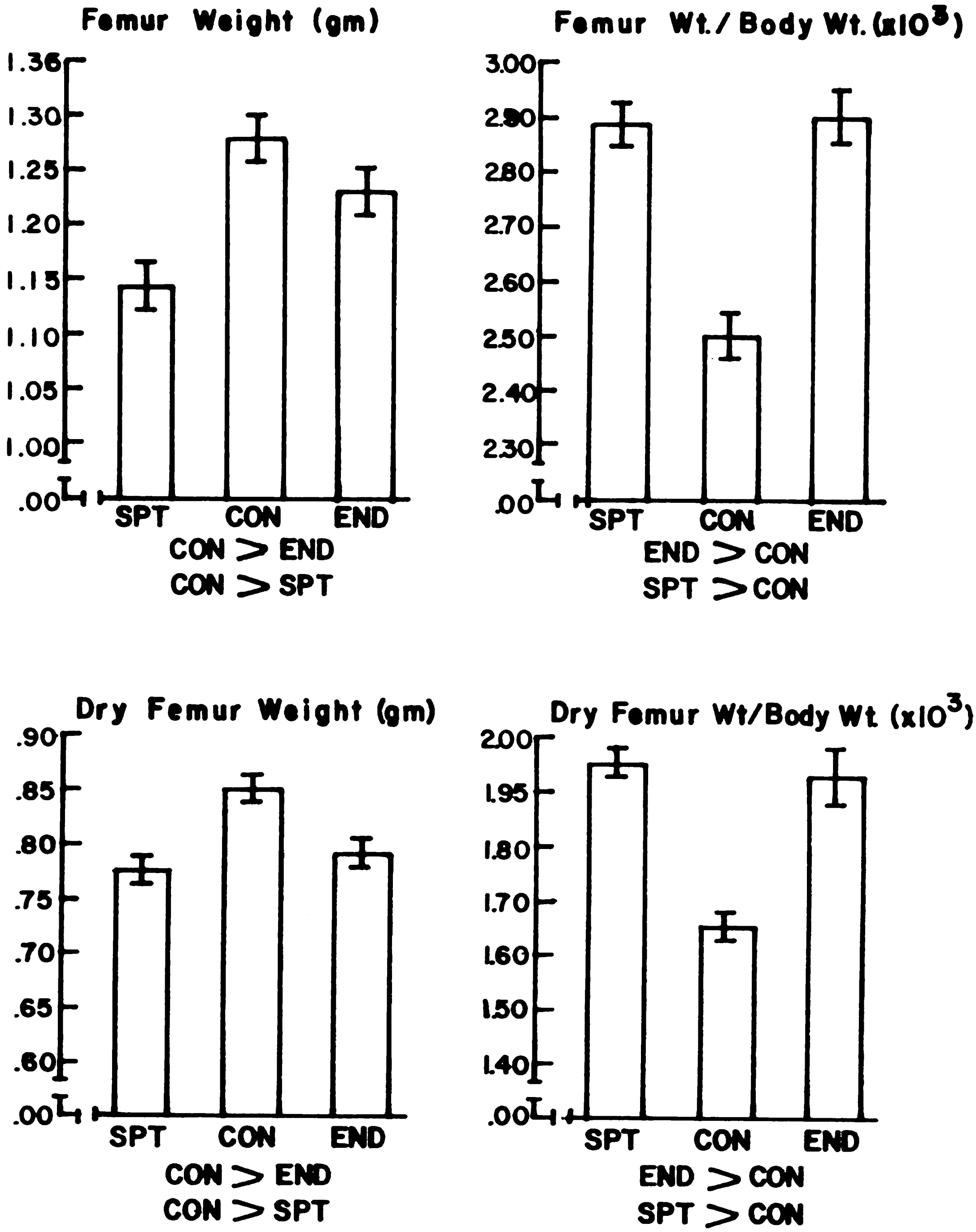

Fig. 4. Means and S.E. ($n=20$) with significant ($p<0.05$) Newman—Keuls Contrasts

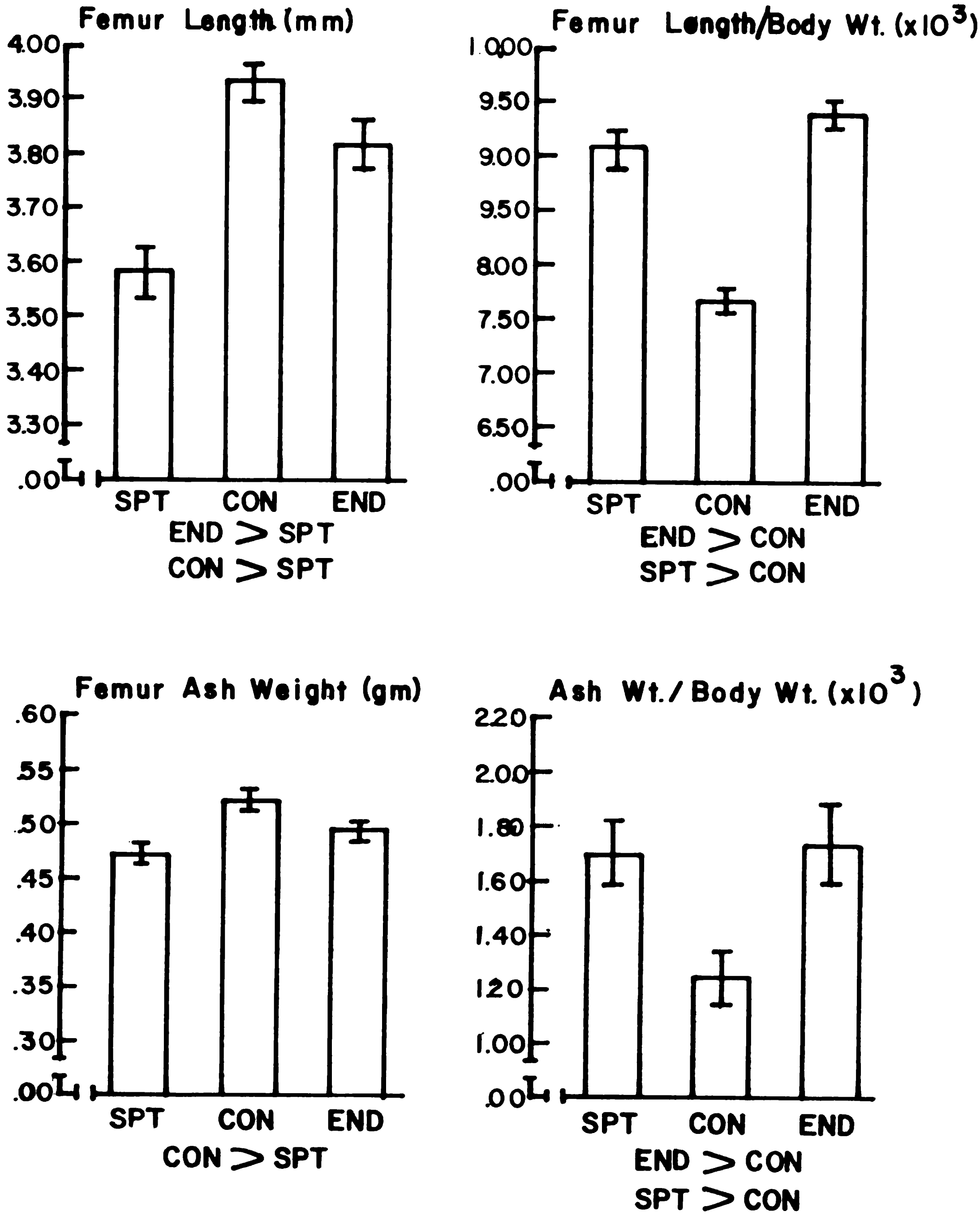

Fig. 5 Means and S.E. (n=20) with significant ($p < 0.05$) Newman–Keuls Contrasts

significantly greater in absolute ash content than the SPT group. No significant differences appeared between the CON and END or the SPT and END groups (Figure 5).

The relative wet and dry femur weights for both of the trained groups were significantly larger than those of the CON group (Figure 4). Relative femur lengths, percent ash and relative ash content all followed the same pattern as the relative weights (Figures 3,4,5). In all cases the SPT and END animals had significantly larger relative values than the CON animals. There was no significant difference in any group between the SPT and END animals.

Discussion

Exercise related alterations in long bone growth are a result of the intensity of the training program (6,46). The increase in relative density as a result of an increase in the mineral content as measured by relative ash weight, agrees with previous investigations (6,29,44). Kiiskinen and Heikkinen reported that increased mineral content with stressful exercise is probably a result of an increase in the calcium and hydroxyproline concentrations (6,21,28).

The greater relative weight of the SPT and END bones is in agreement with Wolff's Law that bone will develop the structure most suited to resist the forces acting upon it (10,11). Longer bones have been observed in mice following intense training (21,28). The vast amount of the data detailing the effects of strenuous exercise on long bone growth is consistent with the findings of this investigation (40,44,46,51,52). The consensus among these experiments is that there is a retardation of long bone growth with high intensity training.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study was undertaken to determine the effects of two high-intensity exercise programs and/or vitamin C supplementation on various femur 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 (58). The modified programs, an endurance running routine (END) and a sprint (SPT) running routine, 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 activity group received vitamin C supplementation (C group) and the remaining animals received a placebo (No C group). Vitamin C supplementation was administered orally by syringe with a dosage of 2.4 mg vitamin C (Merck) in a .1 ml 5% sugar solution per 100 g body weight. The No C group received an identical quantity of the sugar solution per unit of body weight. An eight week treatment period began when the animals were 84 days of age. Selected animals 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, dry weight, length and ash content were determined for the right femur of each animal. Relative weights were determined for each of the measurements to account for the size of the animal.

Absolute weights indicated significantly ($P<.05$) heavier bones in the CON group. The END and CON groups had significantly greater absolute lengths than did the SPT animals. Absolute ash weight in the CON group was also significantly larger than the SPT group.

In all relative measurements, the SPT and END groups had significantly larger values than did the CON animals ($P<.05$).

Analysis of variance of the C group and the No C group indicated significant differences in absolute length and relative ash, however post hoc tests revealed no significant differences between the C and the No C groups on these measurements.

Conclusions

1. Both the SPT and END training regimens resulted in greater relative weight, relative length and relative mineral content in the femur of the trained animals when compared to the control animals.
2. The inclusion of vitamin C in this investigation did not appear to serve as a preventive to long growth retardation in the trained animals.
3. On the basis of this investigation, it appears that judgment must be withheld on the effect of vitamin C on long bone growth.

Recommendations

1. The present study should be repeated with further analysis of the bone ash as to specific mineral content.

2. 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.
3. Further investigation into the mechanism of vitamin C in the bone during high intensities of training should be investigated.

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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.	Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (m/min)	Total Time of Prog. (min: sec)	Total Exp. Meters TEM	Total Work Time (sec) TWT
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
1	1=M	1	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

				Simple Correlations				
Variable	N^a	Mean	Standard Deviation	Air Temp	Per Humid	Bar Press	Per Body Wt Loss	PEM
<u>SPT</u> <u>C</u>								
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
<u>SPT</u> <u>No</u> <u>C</u>								
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
<u>END</u> <u>C</u>								
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 Body 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
<u>END</u> <u>No</u> <u>C</u>								
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