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THE EFFECTS OF EXERCISE AND VITAMIN C SUPPLEMENTATION ON VARIOUS MORPHOLOGICAL PARAMETERS IN THE FEMUR OF MALE ALBINO RATS

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THE EFFECTS OF EXERCISE AND VITAMIN C SUPPLEMENTATION ON VARIOUS MORPHOLOGICAL PARAMETERS IN THE FEMUR OF MALE ALBINO RATS

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ABSTRACT

THE EFFECTS OF EXERCISE
AND VITAMIN C SUPPLEMENTATION
ON VARIOUS MORPHOLOGICAL PARAMETERS
IN THE FEMUR
OF MALE ALBINO RATS

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This study was undertaken to determine the effects of eight weeks of strenuous sprint (SPT) or endurance (END) exercise and Vitamin C supplementation on various morphological growth parameters of the femoral shaft of normal male albino rats.

At the end of this period ten animals per treatment group were sacrificed. The left femurs were removed, wet weights and lengths determined, and then sectioned transversely at three levels along the shaft and processed for morphological studies.

In absolute terms, both SPT and END animals had decreased body weights and femurs of decreased weight, length, and cross-sectional size when compared to sedentary (SED) control animals. However, relative to body weight, the femurs of trained animals were actually greater in size when compared to those of untrained animals.

Neither training performance nor long bone growth was effected by the Vitamin C supplementation.

To my husband and parents

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CHAPTER 1

THE PROBLEM

Physical activity is known to produce a variety of changes in the normal anatomical and physiological function of animals and man (2, 3, 4, 7, 8, 23, 24, 25, 27, 36, 39, 63, 65, 84, 102, 108, 109, 120). More specific to the interest of this study however, are the types and degree of changes found in growing long bones due to exercise. Review of literature has found limited and sometimes contradictory reports concerning the response of growing bone when subjected to varied types of strenuous physical activity. Some data indicate that physical activity tends to increase bone weight, length, diameter, bone wall thickness and density (2, 12, 13, 14, 15, 24, 25, 56, 57, 58, 71, 88, 91, 96, 97, 108, 109, 114). In contrast, other reports indicate a decrease in overall stature with shorter long bone length, no change or slight increase in bone diameter and weight and accelerated maturation of long bones resulting in early closure of their epiphyseal plates (2, 3, 4, 13, 54, 56, 57, 58, 59, 60, 66, 68, 87, 108, 109, 112). With reference to such conflicting data it can therefore be stated that adaptation of bone to physical training is a function of intensity of exercise

(13, 64, 97). Regardless of the potentially negative effects of strenuous exercise on bone growth, implementation of rigorous conditioning programs during the rapid growth period of late childhood and early adolescence have been promoted. Not only is there a need for concrete identification of specific training effects on growing bone at various levels of work intensity but also determination of the factors which might prevent the occurrence of negative training effects.

The concept that exercise consists of a continuum of specific activities each of which elicits a specific response within the organism has evolved from current research on the topic (30, 75, 92, 115). The spectrum ranges from activities of very high intensity work of short duration utilizing uniquely anaerobic metabolic pathways to those activities characterized by very low intensity work of long duration utilizing uniquely aerobic metabolic pathways (115). Unfortunately, little information is available regarding the specific response of bone when subjected to this spectrum of activity levels.

An adequate supply of Vitamin C has been found to be a necessity for normal overall growth and bone formation (22, 29, 49, 50, 69, 70, 73, 86, 90). Although the specific function of Vitamin C in the body has not been adequately identified it is known to be a key factor in collagen formation, bone matrix deposition, prevention of scurvy and adrenal steroid release (31, 46, 47, 50, 51, 53, 61, 69,

70, 73, 86, 93, 111, 113, 121). In addition, Vitamin C also appears to play a role in adaptation to stress (9, 78, 79, 80, 81, 82, 83, 93). However, its specific function in bone adaptation to stress is unknown.

Research Hypotheses

The hypotheses to be tested in this study are as follows:

1. The body weights of sedentary animals should be significantly greater than those of exercised animals.
2. There should be a significant difference between the body weights of sprint and endurance trained animals.
3. The femoral bones of exercised animals should be significantly shorter in length but heavier in weight than the femoral bones of sedentary animals.
4. There should be a difference in femur length and weight between the sprint exercised and endurance exercised animals.
5. The total greatest cross-sectional diameter of femurs from exercised animals should be greater than in femoral bones of sedentary animals.
6. There should be a difference in the total greatest cross-sectional diameter of femurs between sprint exercised animals and endurance exercised animals.
7. Femoral bones of exercised animals should have a greater cortical wall area than femoral bones of sedentary

animals.

8. There should be a difference in the femoral cortical wall areas between sprint exercised animals and endurance exercised animals.

9. The femurs of exercised animals should have greater marrow cavity areas than femurs of sedentary animals.

10. There should be a difference in the marrow cavity area of femurs between the sprint exercised animals and endurance exercised animals.

11. There should be changes in the cross-sectional shape of femurs from exercised animals but not in femurs from sedentary animals.

12. There should be a difference in the cross-sectional shape of femurs between the sprint exercised animals and endurance exercised animals.

13. Exercised animals receiving Vitamin C supplementation should have significantly less long bone growth impairment than exercised animals not receiving 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 (SED), (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 .1 cc of 5% sugar water solution per 100 gm. of body weight daily. The remaining animals only received similar amounts of sugar water solutions according to their own body weight as a placebo.

The SPT and END training regimens were implemented using electronically controlled running wheels (119). The two programs were more intensive than any exercise routines previously used in this laboratory (see Appendix). The animals in the SPT group were subjected to an eight week 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 11 days these animals were expected to complete a 60-min. continuous run at a speed of 36 m/min. The END program has been designed to overload the aerobic capacity of the experimental animals.

Ten animals in each of the six activity-diet subgroups were sacrificed at the conclusion of eight continuous weeks of treatments. Health and training performances throughout the treatment period were used as selection criteria for these animals. After removal of various tissues and organs for use in a larger activity-diet study the animals were

frozen and stored in a deep freezer at a temperature of 0° C. The animals were then removed from the cold and allowed to thaw at room temperature for a 24 hour period. The left femur was removed from each animal and refrigerated in an air-tight container. Each bone was then sectioned transversely at three different levels along its shaft, hand ground to approximately 50 microns in thickness, stained and mounted on glass slides. Various morphological parameters were determined with use of a Praedo micro-projector. The weight and length of the femurs were determined from the right femur of each animal which was used in a bone mineral content study. Analysis of variance procedures were used to analyze the data.

Rationale

The SPT and END training regimens have been designed to simulate high intensity exercise programs for humans. It was expected that the work intensity of the two training regimens would induce growth and morphological changes in the femurs of the exercised animals. In addition, it was expected that there would be a differential response in the bone parameters under study between the SPT and END training programs.

Diet supplementation with Vitamin C (ascorbic acid) was included in the study as a possible preventative of expected decrement in bone growth of the exercised animals.

Vitamin C is known to be a key factor in the normal formation of bone matrix (39, 40, 49, 86, 105, 111), collagen production (10, 39, 40, 49, 51, 70, 105, 111, 113), bone growth (29, 39, 40, 49, 50, 73, 86, 90, 121), adrenal gland function (10, 31, 46, 47, 53, 61, 69, 73, 78, 79, 80, 81, 82, 83, 93, 121), and adaptation to stress (46, 47, 49, 52, 53, 55, 61, 73, 78, 79, 80, 81, 82, 83, 89, 93, 121).

Although the rat synthesizes its own supply of Vitamin C, there are several reasons why it was used in this study rather than the guinea pig which, like man, does not synthesize the vitamin. Unlike the guinea pig, the rat is easily trained to run in a wheel. Since exercise is a major component of this study it is mandatory that trainable animal subjects be used. Also, unique to the rat is that although the animal is postpubertal at 84 days of age, which is when all experimental treatments were initiated, their skeletal growth continues until the animals are over 400 days of age (24). Thus, data concerning exercise effects on skeletal growth is obtainable. In addition, many of the earlier studies concerned with the subject of exercise effects on long bone growth have used the rat femur as their model (13, 16, 25, 59, 71, 87, 96, 97, 112).

The techniques used for data collection of all morphological parameters have been described by Frost (33, 34), Schock (99, 100), Villanueava (116, 117), and Weibel (118).

Limitations

1. Direct inferences to human beings cannot be made with results from animal studies.
2. The power of the statistical analysis may be limited due to the small size of the diet-activity subgroups.
3. Optimal durations of treatments have not been clearly established for the achievement of significant results between groups.
4. Optimal types of training regimens may not have been selected to show differential growth effects on bone.
5. Since the rat synthesizes its own supply of Vitamin C, the full effect of Vitamin C supplementation on bone growth may not be revealed in this study.
6. The results of this study are specific to the femoral bones of male albino rats. Therefore, the data apply only to the SPT and END training regimens used in this study.
7. The animals for this investigation were received in three separate shipments due to limitations of personnel and equipment. The activity treatments were not randomized across shipments. This lack of randomization could introduce a bias in the exercise related data. Since the animal supplier (Hormone Assay, Inc., Chicago, Illinois) had a well-controlled substrain of Sprague-Dawley rats the probability of a genetic bias is small. Every possible effort was made to control unique external factors in the laboratory. The diet treatments were randomized across all

shipments.

8. Due to unavailability of personnel and feasible technical procedures for preparation of bone for morphological analysis, the rat femurs were left intact with the remaining animal carcasses and frozen at a temperature of 0° C. This procedure may have produced some variation in the morphological data of the rat femurs.

9. The procedure followed for preparation of bone sections for microscopic analysis resulted in some incomplete sections thus limiting the final sample size of the study.

CHAPTER II

LITERATURE REVIEW

The literature review will cover six primary topics. Literature describing normal bone morphology and growth patterns will be covered in the first section. The following two sections will summarize literature concerning immobilization effects and exercise effects on bone, respectively. In the fourth section, the general function of Vitamin C in the body will be discussed. The relationship of Vitamin C and adaptation to stress will be covered in the fifth section while the last section will be devoted to reviewing literature on the recommended dietary allowances of Vitamin C needed to meet these various conditions of stress.

Normal Compact Bone Morphology and Growth

Long bone, such as the femur, is characterized by having a hollow shaft composed of compact lamellar bone with an epiphysis at each end composed of trabecular or cancellous bone. Osteoblasts produce the matrix of compact bone in the form of concentric lamellar rings surrounding Haversian canals. Once the osteoblast ceases to produce bone matrix it resides in a lacuna in the midst of its matrix and is

now termed an osteocyte. The osteocytes of lamellar bone remain alive and in contact with each other via small nutrient transport channels called canaliculi. However, the major transportation of nerve and blood supplies is accomplished vertically via Haversian canals and horizontally via Volkman's canals (11, 19, 35, 40).

According to Wolff's law, as described by Koch (64), Saville and Smith (96), and Moss (77), there is a direct relationship between skeletal shape and function. In observations by Currey (20), Epker (28), Frost (35), Hooper (48), Koch (64), and Moss (77), not only will the application of mechanical stresses such as weight bearing or pull by muscle attachments initiate a variety of adaptive changes in the structural aspects of the skeleton but also these changes will be specifically related to the type, intensity, and duration of the applied activity. When compressive forces are applied, such as that created by weight bearing due to physical activity, a concave surface develops on one side of the effected bone shaft. The physiological response is increased osteoblastic activity resulting in increased bone deposition in an attempt to fill in the hollowed out area of the bone shaft. In contrast, when tensile forces are applied to bone, such as that created by the pull of muscle attachments, a convex surface develops on the side of the effected bone shaft opposite to the concave surface. In this case, the physiological response is increased osteoclastic activity resulting in

increased bone resorption in an attempt to smooth out the bulging area of the shaft. The overall effect is to cause change in shape and structure in the compactum of long bones specific to the type of stress applied.

The process of maintaining bone continuity and adaptation to stress is called bone remodelling. In a study by Takahashi et al. (110), it was found that osteoclastic activity resulting in removal of old necrotic osteocytes and bone tissue preceded invasion by active bone producing osteoblasts. Collins (19), Frost (34, 35) and Larson (68) confirmed this remodelling sequence of resorption before deposition. In addition, Frost (35) stated that the remodelling process serves two primary functions. First, it provides a mechanism for self repair of fatigue-like damage resulting from excessive exposure to mechanical stresses. And secondly, it functions to ensure maintenance of an effective electrolyte storage depot and blood-bone buffering system.

The growth process generally involves increasing long bone size in conjunction with maturation of its cellular and inorganic components. As described by Belanger (11), Collins (19), Frost (35), Ham (40) and Larson (68), long bone growth is accomplished by two key processes. The process of endochondral ossification increases long bone length via interstitial growth of fetal cartilage rudiments and maturation of epiphyseal plates while appositional growth increases long bone width. During interstitial

growth, chondroblasts congregate at the midpoint of the cartilage rudiment shaft to form a primary ossification center. The chondroblasts then produce large quantities of avascular cartilage matrix thus expanding the primary ossification center towards each end of the shaft. As the avascular cartilage matrix increases, nutrient and waste products are no longer able to diffuse the added distance to and from the chondrocytes located at the midpoint of the shaft. The chondrocytes in the area become necrotic and die. Blood vessels then invade the area along with bone producing osteoblasts. The osteoblasts immediately begin manufacturing osteoid tissue which soon becomes calcified thus completing the replacement of cartilage with bone and increasing the length of the shaft.

In addition to the interstitial growth process, long bone length is also increased with the formation of secondary ossification centers at the epiphyseal plates. Belanger et al. (11), as well as Moss (77), Schenk (98), Siegling (103), and Sissons (104), described this process in great detail. The cartilage cells of the epiphyseal plate are characteristically arranged in columns like stacks of coins. Within each column of cells are four zones of chondrocytes having various shapes and functions. The uppermost zone of chondrocytes is composed of a few layers of immature cartilage cells evenly distributed throughout the intercellular matrix. The proliferative zone, which actually forms the top of the stacks, contains

chondrocytes which are flat and thin in appearance and function as producers of new cartilage cells. Below this zone is a region of hypertrophied chondrocytes. These cells appear rounded and swollen and function as a producer of intercellular matrix. The bottom of the epiphyseal plate houses the zone of calcifying cartilage. It extends into the bone shaft and is the area where carilage matrix is resorbed and replaced by bone. As this bone deposition front advances toward the epiphysis, the length of the bone is gradually increased. Once all of the chondrocytes composing the various zones of an epiphyseal plate have matured and been replaced with bone, growth stops. Therefore, both interstitial growth and epiphyseal plate maturation as subprocesses of endochondral ossification result in maturation and increased length of long bone.

In order to attain normal long bone growth however, there must be an increase in width commensurate with any increase in length. The process of increasing long bone width is referred to as appositional growth and has been adequately described in works by Belanger (11), Collins (19), Frost (35), and Ham (40). Surrounding the periosteal surface or exterior of the shaft of long bone is a layer of connective tissue called the periosteum. Lying underneath the periosteum are undifferentiated stem cells and osteoblasts. Stimulation of the osteoblasts as part of the growth process results in addition of bone tissue to the periosteal surface of the shaft. However, the addition of

bone to the periosteal surface of the shaft by osteoblastic activity must be balanced by resorption of the interior or endosteal surface of the shaft by osteoclastic activity. The net result is an increase in the total width and marrow cavity area in the shaft of long bones.

Immobilization and Disuse Effects on Long Bones

Abundant information regarding immobilization and disuse effects on the skeleton is available. However, there appears to be some variation in the observed skeletal changes reported concerning this topic.

In a review article by Booth (13), data by several authors concerning immobilization effects on bone were cited. Allison and Brooks (5), in a denervation study using dogs, found that length as well as matrix and mineral contents were decreased in long bones. They also found that the marrow cavity diameter was increased while the total diameter was decreased indicating that the cortical wall thickness of the bone shaft was thinner. Along these same lines, they noted increased porosity in the bone shafts. In addition, immobilization increased the width of the epiphyseal plates while inhibiting the growth in length of the long bones.

Booth (13) also referred to several other immobilization studies reporting results similar to those of Allison and Brooks (5). Klein (62) in a study also using dogs,

reported that the long bones showed decreased weight, matrix, and mineral content, and decreased cortical wall thickness. The cortical walls of the shafts of these bones were also very porous. Kittens with a denervated hindlimb were used to study immobilization effects on bone in an investigation conducted by Gillespie (38). His observations confirmed those of Allison and Brooks (5) and Klein (62) as previously stated. In a study using rats, Armstrong (6) also showed decreases in the length, diameter, matrix deposition and mineral content of long bones after immobilization. In works by Geiser and Trueta (1958) as cited by Booth and confirmed by Heaney (42), it has been observed there is increased bone resorption with immobilization due not only to greater osteoclastic activity but also to increased blood flow to the effected area. Reductions in bone mineral content and density due to immobilization have been reported in studies by Donaldson (23), Manegold (72), and Abramson (1). Hattner (41), summarizing the effects of weightlessness on the skeleton, substantiates these findings and adds that enhanced vascularization may act as an adjunct to bone rarefaction.

In contrast to some of the findings published in Booth's (13) article, Steinhaus (108, 109) and Malina (71) have referred to studies reporting some contradictory data concerning immobilization effects on bone. Experimental rat data of Müller (1923), as referred to by Steinhaus (108, 109), showed that bones from immobilized limbs are longer

in length than bones from active limbs. Malina (71) and Steinhaus (108, 109) went on to report additional data of Friedlander and Thierse (1928) showing immobilized bones to have decreased diameter, increased porosity, decreased mineral content but increased length. They also observed a reduction in the blood supply of inactive bones. Further, they found that the epiphyseal plates of immobilized bones had increased growth activity.

Collins (19), in his book on bone pathology, has also reported that immobilization produced increased bone porosity due to enhanced osteoclastic resorption as well as decreased blood flow to the effected area.

Exercise Effects on Compact Bone

Exercise exists as a continuum of specific activity levels having variation in type, point of application, intensity, and duration of activity. As a result of such variation, exercise elicits an assortment of changes in compact bone specific to each applied activity level. Authors such as Saville and Smith (96), Koch (64), Moss (77), and Kiiskinen (56) have referred to Wolff's law in an attempt to explain the diversity of exercise-related bone changes. In general, Wolff's law states that every change in the function of a bone is followed by definite changes in its internal architecture and external configuration. Booth (13), in a review article, makes application of

Wolff's law with reference to exercise effects on bone by stating that "...adaptation of bone to training is inextricably a function of the intensity of training". Thus, as reflected in the literature, inconclusive results are available regarding the specific response of bone to specific levels of activity.

In an article by Beyer (12), cadets entering the U.S. Naval Academy were used as subjects to study the effects of physical training on various growth and physiological parameters. He found that over a four year period, the cadets had favorable increases in height and weight as well as in the physiological parameters under investigation.

In a strenuous low-intensity running exercise study using rats, Price-Jones (87) found that the growth rate of the exercised animals was impaired during the treatment period as indicated by decreased body weights when compared with sedentary control animals. There were also decreases in the humerus weights and lengths and femur weights and lengths of exercised animals when compared to those of control animals. Price-Jones's overall conclusion was that strenuous low-intensity exercise of long duration retards growth in length and weight of long bones as well as total body length and weight.

In two classic review articles, Steinhaus (108, 109) cited the work of several researchers studying the effects of physical activity on skeletal growth. Some of the research presented had results indicating that exercise

stimulates growth thus increasing arm and leg length in students participating in physical education programs. On the other hand, research involving strenuously exercised rats claimed that physical activity had a detrimental effect on growth by decreasing bone length and weight as well as body weight. At the same time it was noted that exercise of moderate intensity seemed to stimulate growth reflected by increased body weight although total body length was unaffected. In x-rays of bones and joints of highly specialized athletes such as baseball pitchers, various shape and size changes in the bones of exercised limbs were observed. With reference to all of this conflicting data, Steinhaus concluded that "...pressure on the epiphyses of long bones from participation in exercise stimulates growth up to an optimal level and beyond this point retards growth".

Bipedal rats were used in two studies by Saville (96) and Smith (107) to evaluate the effects of weight bearing on various characteristics of the femur. The femurs of bipedal rats showed increased bone density, volume and weight, increased breaking strength and increased mineral content. There was also an increase in the cross-sectional diameter and cortical wall thickness of the femurs from the bipedal rats although their body weights were lower than controls. It was also noted that the external shape of the femurs varied somewhat between the bipedal and control rats.

Rats exposed to a strenuous endurance running program described by Saville and Whyte (97), had hindlimb bones of increased weight, mineral content, density, volume and breaking strength. However, unlike bones from bipedal rats used in previous studies by Saville (96, 107), the bones from exercised rats had no change in their cortical wall thickness.

King and Pengelly (60) in an investigation also using rats, subjected their animals to a high intensity sprint running program and a moderate intensity endurance running program. The tibias from the sprint runners were the only bones having a significant increase in density. However, the adrenal glands were also excised from all animals and were found to be heavier in both groups of exercised animals when compared to control animals.

Rats exposed to strenuous exercise programs of various intensities and durations designed by Tipton et al. (112), had femurs and tibia-fibula complexes with decreased length, width, and water content. Evaluations of the mineral content and epiphyseal plate maturation of these bones however, showed no change between the various treatment groups. Likewise, Lamb et al. (66), using moderately exercised rats also found that the animals had lower body weights and shorter tibial lengths when compared to sedentary animals.

On the other hand, Donaldson and Meeser (25) and Borer and Kuhns (14), exposed rats and hamsters respectively, to

voluntary running exercise of low intensity. Results from both investigations showed decreased total body lengths and weights as well as decreased long bone lengths and weights in exercised animals when compared to their respective controls.

In several investigations conducted by Kiiskinen et al. (56, 57, 58, 59), concerned with exercise effects on bone, mice were assigned to various endurance treatment groups and forced to run on a treadmill. Those animals subjected to the endurance program of light intensity had no change in body weight and femur length. However, the femurs of these animals did have increased weight, mineral content, density, bone deposition and an increased number of bone cells when compared to sedentary animals. On the other hand, those mice participating in the moderate and heavy endurance programs when compared to sedentary animals, not only had lower body weights indicating impaired growth, but also had decreased long bone length as well as decreased vascularity. But, the bones did have increased weight, mineral content, bone deposition, density, and breaking strength. In addition, Kiiskinen et al. (56, 57) made note of the fact that the heavy endurance running program, when continued over a long duration, produced some detrimental effects in the bones of his mice. The most obvious change was a decrease in bone weight. Consequently, he concluded that exercise of excessive intensity and duration produces an overload

thus creating an over-training situation resulting in detrimental bone changes.

Mice were also used in two studies by Heikkinen et al. (43, 44) in which the animals were forced to participate in moderate endurance programs of treadmill running. The bone data from these animals paralleled the previously described data from the moderately trained animals of Kiiskinen et al. (56, 57).

Several studies concerned with investigating the effects of hard physical labor and exercise on skeletal growth of humans are also prevalent in the literature. Adams (2) found that young black women engaged in hard strenuous labor were taller, heavier, and generally larger in overall size when compared to women not exposed to physical labor. In a roentgenographic examination of bones of Russian laborers, Prive (88) found that physical activity increases bone length, delays ageing and increases bone hypertrophy. He also commented that the observed skeletal changes were specific to the types of physical load placed upon the bones. In an anthropometric study of children on hand preference for work, VanDusen (114) found that the arm bone lengths and widths of preference limbs were significantly greater than those of non-preferred limbs. Buskirk et al. (15) found similar increases in the length of arm bones of tennis players although he did not detect any changes in the width of these bones. Adolescent boys actively involved in various physical activities of low to moderate intensity, were

found to have increased height and body weight when compared to inactive adolesdent boys as described in a study by Ekblom (27). With reference to ageing, research by Castillo (18), Dalen (21) and Smith et al. (106) shows that physical activity of low to moderate intensity increases the mineral content of bones and delays the onset of osteoporosis in the aged. Additional age and physical activity studies have been conducted by Reeve et al. (94) and Lane et al. (67). They found blood flow was increased in the bones of physically active mature adults over that in bones of physically inactive mature adults. Thus, the application of mechanical stressors, such as that created by participation in physical activity, results in increased bone vascularization.

Kato and Ishiko (54), reporting on the results of hard physical labor on the growth of Japanese children, have found an overall detrimental effect. Consequential to carrying heavy loads on their shoulders, the children were found to be very small in stature, especially in leg length, the femurs and tibias showed early epiphyseal plate closure, and the bones of their feet were deformed. Correspondingly, in an investigation involving growth of adolescent boys participating in a weight training program, Kusinitz and Keeney (65) found that weight and height gains in these boys were impaired when comparisons were made to similar boys not participating in a weight training program. Likewise, in a x-ray study of elbows of pre-adolescent baseball pitchers, Adams (3) found the bones in pitching arms to have shorter

lengths due to early epiphyseal plate closure, increased density, fractures of various types, and damaged articular cartilage. In a review article, Malina (71) cites another x-ray report by Adams (4) in which shoulders of throwing arms of young pitchers were studied. He found the humerus of throwing arms to have a low mineral content, bone fragmentation, and a widened proximal epiphyseal plate indicating accelerated bone growth. Other supportive data indicating possible negative effects of strenuous physical activity on bone growth have been reported in a publication by Larson (68). Bone x-rays of physically active growing children have portrayed various types of trauma such as: the development of osteochondrosis designating derangement of the normal growth process, osteochondritis, traumatic epiphysitis, traumatic epiphyseal separation, and various bone and epiphyseal fractures. Thus, it would appear that an optimal level of physical activity stimulates and enhances overall bone growth while excessive physical activity impairs bone growth.

Vitamin C and Bone

Vitamin C (ascorbic acid) is known to play a major role in the bone growth process and in the maintenance of normal bodily functions. However, its specific involvement in these processes has not been clearly identified in the literature. In fact, most of the information available on the

function of Vitamin C in the body has been derived from studies where subjects are placed on diets deficient in the vitamin. The resultant effects of such deficiency have provided the foundation for development of what is currently known about the functions of Vitamin C.

According to Guyton (39), ascorbic acid, a water soluble vitamin, plays a fairly major role in regulating food intake and growth. More specifically, Vitamin C is involved in the reversible oxidation-reduction of food stuffs while also possibly acting as a cofactor in the anabolic metabolism of proteins. With reference to growth and maintenance of cellular constituents, Vitamin C is necessary for the hydroxylation of proline to form hydroxyproline which is a requisite for the formation of collagen. Collagen is the primary component of almost all supportive connective tissues in the body having functions ranging from maintenance of capillary wall integrity to management of normal skeletal growth. Vitamin C also affects alkaline phosphatase production by osteoblasts, thus influencing bone matrix formation. Therefore, with collagen and bone matrix production so dependent upon an adequate supply of ascorbic acid, Guyton states it is not surprising that Vitamin C deficiency results in impaired skeletal growth as well as generalized capillary and cell membrane fragility, all of which are symptomatic signs of scurvy. He concludes that an adequate intake of Vitamin C above that required to prevent the onset

of scurvy is necessary for normal maintenance and growth of the skeleton.

Similar statements regarding the known functions of Vitamin C have been made by Ham (40). In addition, Ham notes that the vitamin is largely stored in the adrenal gland plus having some regulatory control over its function.

In a biochemical study, Udenfriend (113) also indicates that ascorbic acid is involved in the overall maintenance of connective tissue and implicates that it has specific involvement in the creation of hydroxyproline which is a necessary precedent to collage formation. Jeffrey (51), in another biochemical study, reported findings corroborating those of Udenfriend.

Children receiving large pharmacological dosages of corticoids were used as subjects in a study by Liakakos et al. (70) where large dosages of Vitamin C were also administered. The Vitamin C was given in an attempt to neutralize the inhibitory effects that corticoids have on collagen formation. His findings indicate that not only does ascorbic acid inhibit the negative effects of corticoids on growth but also acts as a co-factor in hydroxyproline production which is preliminary to collagen formation.

In a vitamin deficiency study, Thornton (111) found scorbutic guinea pigs to have reduced osteoblastic activity resulting in decreased collagen and bone matrix formation. In addition, he found that the quality of the bone matrix was also impaired due to decreased alkaline phosphatase

activity. As a consequence, the scorbutic guinea pigs had bones that were incompletely calcified and very labile in nature. He concluded that an adequate intake of Vitamin C is necessary to maintain normal skeletal growth.

Similar decrements in the quantity and quality of bone matrix in tibias of scorbutic guinea pigs were also reflected in the data of Poal-Manresa et al. (86). More generalized findings were reported by Ram (90) in that his guinea pigs had reductions in adrenal Vitamin C content, food intake, and body weight when fed on an ascorbic acid deficient diet. Evans and Hughes (29) also found that growth rate was impaired in their scorbutic guinea pigs but noted that it occurred as a result of insufficient food metabolism rather than low food intake.

Literature on the topic of Vitamin C deficiency effects on skeletal growth has also been reviewed and reported on by Irving (49). The histological changes noted in such studies included increased shaft resorption with thinning of bone walls, disruption of normal metaphyseal cartilage maturation with hemorrhaging in the area, and dense calcification of pre-existing metaphyseal osteoid tissue. Chemical changes resulting from inadequate ascorbic acid intake included reduced cartilage and bone matrix production, decreased alkaline phosphatase activity consequently hindering the normal calcification of matrix, and insufficient collagen production.

More detailed information regarding the functions of Vitamin C in the body has been presented in another review article by Meiklejohn (73). Review of biochemical studies found ascorbic acid capable of being reversibly oxidized (accepts hydrogen) and reduced (donates hydrogen). It was also found that the majority of the vitamin in the body is present in the reduced form such that it primarily functions as an anti-oxidant in various metabolic processes. One process that Vitamin C is known to participate in is regulation of epiphyseal plate (metaphysis) maturation as described by Sledge (105). Its anti-oxidant function specifically protects chondrocyte lysosomes from breaking down when exposed to oxygen. It allows the cartilage matrix of the metaphysis to mature before being resorbed by these chondrocyte lysosomes such that the proper environment for calcification can be maintained. Furthermore, according to Meiklehohn (73), Vitamin C has additional control over metaphysis maturation by regulating alkaline phosphatase activity and matrix calcification as well.

In addition, Meiklejohn (73) goes on to say, that Vitamin C also seems to play a key role in the function of the adrenal gland which is its major storage depot. He states that ascorbic acid does not appear to be actively involved in the actual synthesis of the adrenocortical hormones but rather with inhibiting their formation and release.

More detailed information on the relationship between Vitamin C and adrenal gland function has been reported in

the literature by several other researchers. Guinea pigs placed on a Vitamin C deficient diet were used in a study by Jones et al. (53) where it was found that adrenal ascorbic acid content decreased, body weights decreased, and plasma adrenocorticoids increased. Damage to the epiphyseal plates in the femoral and tibial bones of these animals was also observed. In another study where Vitamin C intake was restricted, Fordyce and Kassouny (31) found hypertrophied adrenal glands with low ascorbic acid content, enhanced adrenal steroid production, and elevated plasma corticoid levels as well as decreased food intake accompanied by weight losses. In two investigations by Hodges and Hotston (46, 47), where scorbutic guinea pigs were used, similar findings of increased adrenal activity with enhanced corticoid synthesis and release were also reported. They concluded that because Vitamin C seemingly has such a great impact on regulating adrenal gland activity, it may also be an important regulating factor in various metabolic adaptations to stress.

On the other hand, many investigations have been conducted where large dosages of Vitamin C have been administered in an attempt to study its effect on adrenal gland function. In a study by Liakakos et al. (69), children were given large dosages of Vitamin C to see if plasma adrenal corticoid levels would be altered. Their findings suggest that high intakes of ascorbic acid exert a braking effect on adrenal corticoid synthesis and release. Consequently,

large amounts of ascorbic acid may prove to have no particular beneficial effect in adaptation to conditions of stress. Wilbur (121) and Kitabchi (61) reported similar findings and added that adrenal ascorbic acid stores have to be depleted before steroid synthesis and release can proceed. Furthermore, Kitabchi (61) stated that adrenal cholesterol levels were exceptionally low after high intakes of Vitamin C.

Vitamin C and Exercise as a Stressor

As the literature indicates, Vitamin C exerts a rather strong regulatory influence over adrenal gland function and skeletal growth. It further indicates that active participation in strenuous exercise or physical labor results in various functional and morphological adaptations in both of these structures. Therefore, under conditions of stress, such as that imposed by physical activity, it could be presumed that additional Vitamin C may be required by the adrenal glands and skeleton in order to meet their functional needs. Several investigations on this topic have been reported in the literature.

Numerous studies on the relationship between ascorbic acid and stress induced by participation in physical activity have been cited in a review article by Irwin (50). Some data indicate a need for higher requirements of Vitamin C when doing strenuous work or exercise in either hot or cold environments. On the other hand, other reports

indicated that megadosages of Vitamin C had no effect in improving the health status of physically active subjects exposed to similar environmental conditions. Irwin specifically cited a study by Henschel et al. (45) where U. S. Army soldiers walked at a pace of 3.25 miles per hour on a motor driven treadmill at a 7.5 per cent graded incline for six ten-minute alternating work and rest periods. The subjects were also given varied dosages of Vitamin C. It was found that soldiers on a high intake of ascorbic acid showed no improvement in their physiological response or their ability to do work while in a hot environment when compared to control subjects. However, Irwin also cited an in vitro frog muscle study where it was shown that Vitamin C enhanced contraction and delayed the onset of fatigue.

Urinary ascorbic acid excretion and blood ascorbic acid levels were used as indicators of Vitamin C requirements by adult skiers as reported in a study by Namyslowski (80). Both parameter values were very high indicating depletion of adrenal Vitamin C stores. As a result of these findings, Namyslowski recommended that megadoses of Vitamin C be taken by athletes during training and prior to competition in their sport. Thus, the result is enhanced post-exercise recovery via adrenal ascorbic acid restoration.

Other biochemical studies using athletes as subjects were also reviewed by Irwin. In general, most researchers indicated a need for increased Vitamin C intake by athletes partaking in various sport events. However, it should be

noted that this recommendation was based solely on biochemical data since there was no improvement in the physiological responses or athletic performances of these subjects.

In contrast to the reports supporting Vitamin C supplementation by athletes, Irwin also presented data showing no beneficial effects from Vitamin C supplementation. Soldiers receiving high dosages of Vitamin C were subjected to rigorous programs of marching on an inclined motor driven treadmill as described in a study by Keys and Henschel (55). The response of various physiological parameters, such as heart rate, oxygen uptake and blood lactate levels, to such programs indicated that Vitamin C supplementation had no beneficial effects.

Johnson et al. (52), conducted a study where volunteers from a Civilian Public Service Camp were placed on diets either devoid of Vitamin C or supplemented with the vitamin. The data from physical fitness tests on these subjects showed that there was no significant difference in the physical efficiency of these two groups.

In a study by Fox et al. (32), natives working in the gold mines of South Africa received dietary supplements of Vitamin C in an attempt to improve their physical work performance and overall health status. The net result was that Vitamin C supplementation had no significant beneficial effect on improving the health status or working efficiency of the natives. Two separate studies by Gey (37) and Bailey et al. (9) also reported findings of no beneficial effects

from Vitamin C supplementation on the various physiological responses of their subjects to physical training.

However, other studies have reported findings indicating that Vitamin C supplementation does have a positive effect on improving physical performance. In a study by Prokop (89), athletes participating in strenuous, fast running events were given dietary supplements of several vitamins, including Vitamin C. Examination of the data showed that Vitamin C supplementation improved the physiological response of the athletes by reducing their post-exercise oxygen debts, pulse rates and blood pressures.

Adrenal ascorbic acid levels of rats and guinea pigs exposed to a regimen of excessive exercise were surveyed in a study by Ratsimamanga (93). His findings indicated that excessive exercise depleted adrenal ascorbic acid stores in both animal subjects. As a result of such findings, he suggested that ascorbic acid may be connected in some way with the function of adrenal cortical hormones. However, no specific recommendations for Vitamin C supplementation were given.

Rats were also used as subjects in several investigations conducted by Namyslowski (78, 79, 81, 82). All of his results denote depletion of adrenal ascorbic acid contents following participation in various exercise programs. However, Namyslowski also pointed out that the restoration of adrenal ascorbic acid back to normal levels occurred much sooner and more completely in trained animals than in

control animals. Thus, he concluded that the adrenal glands of trained animals are better adapted in their ability to restore ascorbic acid levels back to normal following exercise. Further, he recommends that dietary Vitamin C supplementation be mandatory for athletes participating in strenuous physical activity.

Recommended Dosages of Vitamin C

As reflected in the literature, Vitamin C is a necessary dietary constituent required for normal adrenal gland function and skeletal growth. However, the exact dosage level of Vitamin C needed to meet its functional roles has not been adequately identified in the literature. In fact, recommended Vitamin C supplemental dosages seem to vary according to individual growth rate and physical activity levels.

In a review article by Baker (10), the League of Nations Technical Commission on Nutrition was cited as recommending that 30 mg. of Vitamin C is the daily requirement necessary to meet the functional needs of a normal human adult. However, Baker as well as Meiklejohn (73), also reported that 70 mg./day of Vitamin C is required for a normal human adult as recommended by the United States National Research Council. The League of Nations Commission interpreted this discrepancy by stating that: "... so long as there is no evidence to support the view that an intake of more than

30 mg. daily has beneficial effects, there is no basis for recommending an intake greater than the amount."

However, under conditions of stress such as that imposed by participation in various exercise programs, some researchers have indicated the need for increased daily intakes of Vitamin C. Namyslowski (83), in a study on physical performances of adult snow skiers, has recommended supplemental Vitamin C dosages of 200-250 mg./day for athletes actively partaking in rigorous training or athletic programs. In a separate study on the physiological responses of athletes participating in heavy, fast running events, Prokop (89) suggests that for a 70 kg. athlete 100-140 mg. of ascorbic acid should be ingested daily during training periods and increased to 140-200 mg. daily during competition periods.

In a review article completely devoted to reporting Vitamin C requirements of man, Irwin and Hutchins (50) have presented a wide variety of recommendations. Summary of the data presented on the ascorbic acid requirements necessary for a human adult under normal living conditions designated dosages of between 50 mg. to 100 mg. per day as being adequate. While under conditions of physical stress, the recommended daily Vitamin C requirement was increased to a range of 100 mg. to 600 mg. for an active athlete. For active, growing school-age children, a minimal dosage of 25 mg./day of Vitamin C has been recommended although the optimal dosage level for the vitamin was closer to 50 mg./day.

CHAPTER III

METHODS AND MATERIALS

Human metabolic responses to physical activity can be reflected by gross measurements of total-body oxygen debt and oxygen uptake. Stressing the muscular anaerobic metabolic pathway results in an increased oxygen debt tolerance as induced by a program of exhaustive sprint running. Maximum workloads and short bouts of repeated exercise are characteristic of this type of training regimen. On the other hand, endurance running, characterized by light to moderate workloads and relatively long bouts of continuous exercise stimulates the muscular aerobic metabolic pathway. Stimulation of this pathway results in an increased capacity to do cellular work in the presence of oxygen and is directly related to oxygen uptake. This study was designed to investigate the morphological changes produced in the left femur of the male albino rat after exposure to eight weeks of sprint and endurance training with dietary supplementation of megadoses of Vitamin C.

Experimental Animals

Eighty-four normal male albino rats (Sprague-Dawley strain) were obtained from Hormore 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 of age.

Exercise Groups

The exercise treatment consisted of three levels of activity which were administered daily between 12:30 p.m. and 5:30 p.m., Monday through Friday for a duration of eight continuous weeks. The exercise treatment groups were as follows.

Control Group

The 24 animals in the second shipment constituted the sedentary (SED) control group. During the adjustment period and treatment period these animals were housed in individual sedentary cages (24 cm. X 18 cm. X 18 cm.) and were not forced to exercise.

Sprint Group

The sprint (SPT) running 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 adjustment period and in an individual sedentary cage during the treatment period. The SPT animals were subjected to an interval training program of high-intensity sprint running (Appendix A-1). The workload of the SPT

program was increased gradually until 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 the relatively fast speed of 108 m./min.

Endurance Group

The endurance (END) running group was comprised 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 (Appendix A-2). The workload was progressively 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.

Diet Subgroups

In addition to the three exercise treatments half of the animals in each activity group received one of two dietary supplements. The animals were given their dietary supplement by oral syringe, seven days a week, between 7:00 p.m. and 9:00 p.m. The diet treatments were administered beginning on the day before initiation of the exercise treatments and were terminated the day before sacrifice.

Vitamin C

Approximately .1 cc of a 5% sugar solution with 2 mg. ascorbic acid/100 gm. of body weight was given to one-half the animals (Vit-C) in each exercise group.[1]

Placebo Group

Approximately .1 cc of a 5% sugar solution/100 gm. of body weight was given as a placebo to the remaining animals (No-C) in each activity group.

Training Procedures

The SPT and END groups were trained in a battery of individually 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 (119).

Animals learn to run in the CRW by avoidance-response operant conditioning. Motivation for the animals to run is provided by a controlled low-intensity electrical current, applied through the running surface. A light located 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

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

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 shock is discontinued. The light-shock sequence is repeated if the animal fails to maintain the desired speed throughout the work period. Most animals learn to react to the light-shock stimulus after only a few days of training.

A typical training session consists of alternating work and rest periods. During all rest periods the wheel is braked automotically to prevent spontaneous activity. The brake is released and the wheel is free to turn during the 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 was employed to alter the normal day-night schedule for the animals so that they could be 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 SED 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 principle 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 treatment period, ten animals were sacrificed from each of these four subgroups. They were selected for sacrifice on the basis of their health and their training performances throughout the treatment period. Those animals subjectively determined to be in good health were chosen for sacrifice. Because the training regimen was extremely vigorous, no absolute minimal performance standard was established. However, individual daily records of PEM and PSF were examined, and healthy animals that made the best adaptations to the training regimens were selected for sacrifice.

In each of the sedentary subgroups, two extra animals were included originally to allow for the unlikely possibility that some of the unexercised animals may become ill during the course of the study. At the termination of the treatment period all of the animals in the sedentary subgroups were subjectively determined to be in good health and were sacrificed.

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 cessation of their last exercise bout. This procedure was followed to eliminate any transient acute exercise affect. At sacrifice, the animals were either 140 or 141 days of age.

Immediately prior to sacrifice, the final body weight of each animal was recorded. Each animal was anesthetized by an interperitoneal injection (4 mg./100 gm. body weight) of a 6.48% sodium pentabarbital (Halatal) solution. Several organs, muscles, and the tibia-fibula complex were removed and processed according to varied protocal for use in a larger diet-exercise investigation. After completion of each of the sacrifice sessions all of the dead animals were slow-frozen and stored as such for approximately nine months.

The animals were then allowed to thaw for a 24 hour period. The left hindlimb was skinned and the overlying musculature removed thus exposing the femur. The femur was then detached from its articulation with the acetabulum of the pelvis and the head of the tibia at the knee. All remaining connective tissue and articular cartilage was removed and the bone weighed to the nearest milligram on a Mettler Balance. A Vernier caliper was used to measure the femur length to the nearest .1 mm. The length measurement was taken from the greater trochanter at the proximal end of the femur to the lateral femoral condyle at the distal end of the bone. Bone length is expressed in absolute terms (millimeters) and bone weight is expressed in both absolute terms (milligrams) and relative terms (percentage

of body weight). The bones were then placed in individual airtight vials and refrigerated until sectioned.

Bone Sectioning Procedures

In morphological bone studies one of the most acceptable methods of bone preparation incorporates the use of undecalcified bone sections (33, 34, 74, 76, 99, 110, 116, 117). Morphological measurements taken from undecalcified bone sections are more representative of the actual bone size since the procedure is devoid of any chemical reagents which could distort the size and shape of the bones under study (33, 34, 115, 116). Cross-sectional samples were taken from three levels of the left femur of the rats following a method for preparation of thin undecalcified bone sections as described by Frost (33, 34). Sections were taken at approximately one-fourth the total length of the femur from both its proximal and distal ends as well as at the midpoint of the bone shaft. The distal end of the femur was cushioned between two pieces of foam rubber and clamped in a small table vice. Using a fine-toothed jeweler's saw, the bone was cut into three cross-sectional slabs approximately one millimeter thick.

Each slab was then hand-ground between two pieces of 400 grade abrasive waterproof carborundum sandpaper while cooled with running tap water to reduce possible heat effects due to friction produced by the grinding process.

The sections were ground using a circular motion to a thickness of approximately 50 microns. Residue from the sandpaper was cleansed from the bone sections by soaking them in a 0.01% mild soap water solution for two minutes followed by a tap water wash and distilled water rinse respectively.

Each section was placed in an individual vial containing Villanueva's Bone Stain for fresh mineralized bone for a 48 hour period (116). The individual bone sections were then differentiated in a 0.01% acetic acid in 95% methanol solution, dehydrated in ascending alcohols and cleared in xylene. The three sections of each femur were mounted on a glass microscope slide and permanently mounted with Permount mounting media.

Morphological Measurements

In addition to the femur length and absolute and relative femur weights, several other morphological parameters were studied. The additional parameters were employed as indicators of change in bone size and shape and were taken from sections of all three levels of each femur. The parameters under study are as follows.

Cortical Wall Area

This parameter is commonly used among researchers as an indicator of bone wall size and structure (34, 85, 99, 100, 116). The cortical wall area (CWA) studied was that portion of the bone lying between the periosteal and endosteal

surfaces of each section of each bone. The CWA was determined using a slightly modified point-count technique as described by Frost (33, 34), Villanueva (116), Wiebel (118), and Sedlin (101). A magnification of 21 times was used for all measurements of this type since it allowed each section to be viewed in its entirety.

Using a Praedo microslide projector (Leitz Wetzlar, Germany) each bone section was individually projected on a square-ruled graph paper grid having a total of 391 equidistantly spaced intersections with a known grid area (GA) of 1 cm^2 per square. The point of intersection of any two lines on the graph paper grid which fell within the cortical wall of the projected section was counted as a "hit" (H). Where a point of intersection lied tangent to either the endosteal surface or periosteal surface of a section, the count was recorded as a half hit. The sum of all total cortical hits and tangents (TCH) was recorded for each separate measurement (termed a "throw") of each bone section. Six to eight throws (T) per section were taken to accumulate approximately 150 total hits so that the calculated area for each section was equal to the true area of each section (34, 116). The cortical wall area of each bone section was calculated using the following equation:

$$\text{Cortical Wall Area} = \frac{(\text{Total no. of cortical hits}) \times (\text{Known grid area})}{(\text{Total no. of throws}) \times (\text{Total no. of intersections in the whole grid})}$$

and is rewritten as:

$$CWA = \frac{TCH \times KGA}{TT \times TGI}$$

All area measures were recorded in square centimeters (cm^2).

Marrow Cavity Area

The marrow cavity area (MCA) studied was that portion of the bone entirely surrounded by the endosteal surface of each section of each bone. This parameter also served as an indicator of bone size and structure especially as it related to the total bone area. The MCA was determined using the same method described for deriving the cortical wall area of each bone section. In the CWA equation, appropriate terms were substituted so that MCA replaced the CWA and the total marrow hits (TMH) replaced the TCH.

Cortical Wall Area/Total Area Ratio

The ratio of cortical wall area to total section area (TSA) indicates that portion of each bone section that is actually consumed by bone tissue. This relationship was also used as an indicator of the amount of bone deposition or conversely, the amount of bone resorption that occurred on the bone surfaces as part of the remodelling process (34, 99, 100). The total section area was determined by addition of the cortical wall area to the marrow cavity area for each section (99, 100).

Greatest Wall Thickness and Least Wall Thickness

Mechanical stressors ranging from immobilization to high levels of physical activity are known to have an effect on bone morphology. The determination of the greatest cortical wall thickness (GWT) and least cortical wall thickness (LWT) of each section of each bone reflects any change in the shape and symmetry of the bone that may have occurred as a result of the experimental treatments (34, 99, 100). Those portions of each section subjectively identified as having the greatest wall thickness and the least wall thickness were measured from the appropriate endosteal surface to the appropriate periosteal surface respectively. The measurements were taken directly from the projected image of each section of each bone with a clear plastic ruler divided into millimeter units. All measurements of this type were recorded in millimeter units.

Greatest Total Diameter and Greatest of the Least Total Diameter

Another indicator of change in shape and symmetry of bone is the greatest total diameter (GTD) and the greatest of the least total diameter (LTD) (see Figure 1). The LTD was measured from that portion of each bone section having the greatest of the least total diameter between opposing periosteal surfaces and lying perpendicular to the GTD. The same procedure and measurement tool was used for determining these parameters as was used for the GWT and LWT.

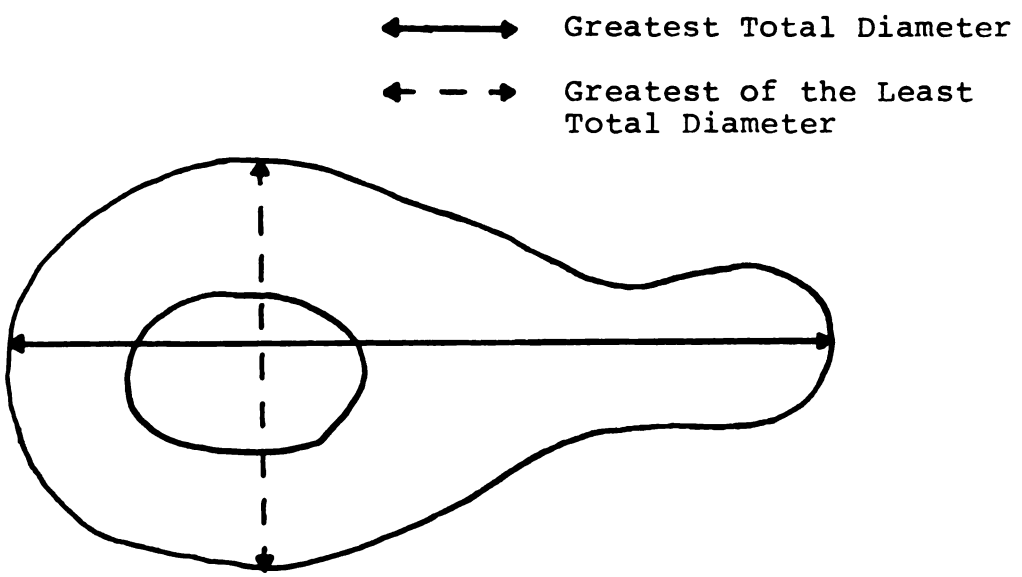

Figure 1. Diagram representing the proximal section of the femoral shaft demonstrating the Greatest Total Diameter and the Greatest of the Least Total Diameter.

The morphological parameter values obtained from the sedentary-diet subgroups served as a reference standard for the various morphological parameters under study. All morphological measurements for each section of each bone were performed at the same time without knowing the identity of the treatment group.

Analysis of Data

This study was conducted as a two-way (3 X 2) factorial design with three levels of activity and with two levels of diet. All of the morphological parameters under study were analyzed using a two-way fixed-effects analysis of variance

routine on the Michigan State University Control Data 6500 Computer (CDC 6500). Newman-Keuls tests were used to evaluate the differences between pairs of means whenever a significant (P = .05) F-ratio was obtained.

CHAPTER IV

RESULTS AND DISCUSSION

Four major sections of material will be covered in this chapter. The results of the Controlled-Running Wheel (CRW) training programs, which include the environmental factors that operated during training, the percentage of body weight lost during daily exercise sessions, and the performance criteria used to reflect training responses are covered in the first section. The second section deals with results of specific activity levels as indicated by the various morphological parameters under study. In the following section, the results of Vitamin C supplementation on performance are reported and discussed. Finally, a general interpretation and discussion of the results are set forth in the last portion of this chapter.

Training Results[1]

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

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[1]Some of the material in this section has been adapted, in part, from the unpublished Ph.D. dissertation of Roland R. Roy (95).

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 and aerobic metabolic processes in the animals. The performance of the animals was 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 SPT-No C groups are presented in Figure 2. 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 2 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 3. PEM values were 70% or higher each day of training in both the C and No C animals. These results indicate that the animals were able to maintain the daily requirements of the END program relatively well.

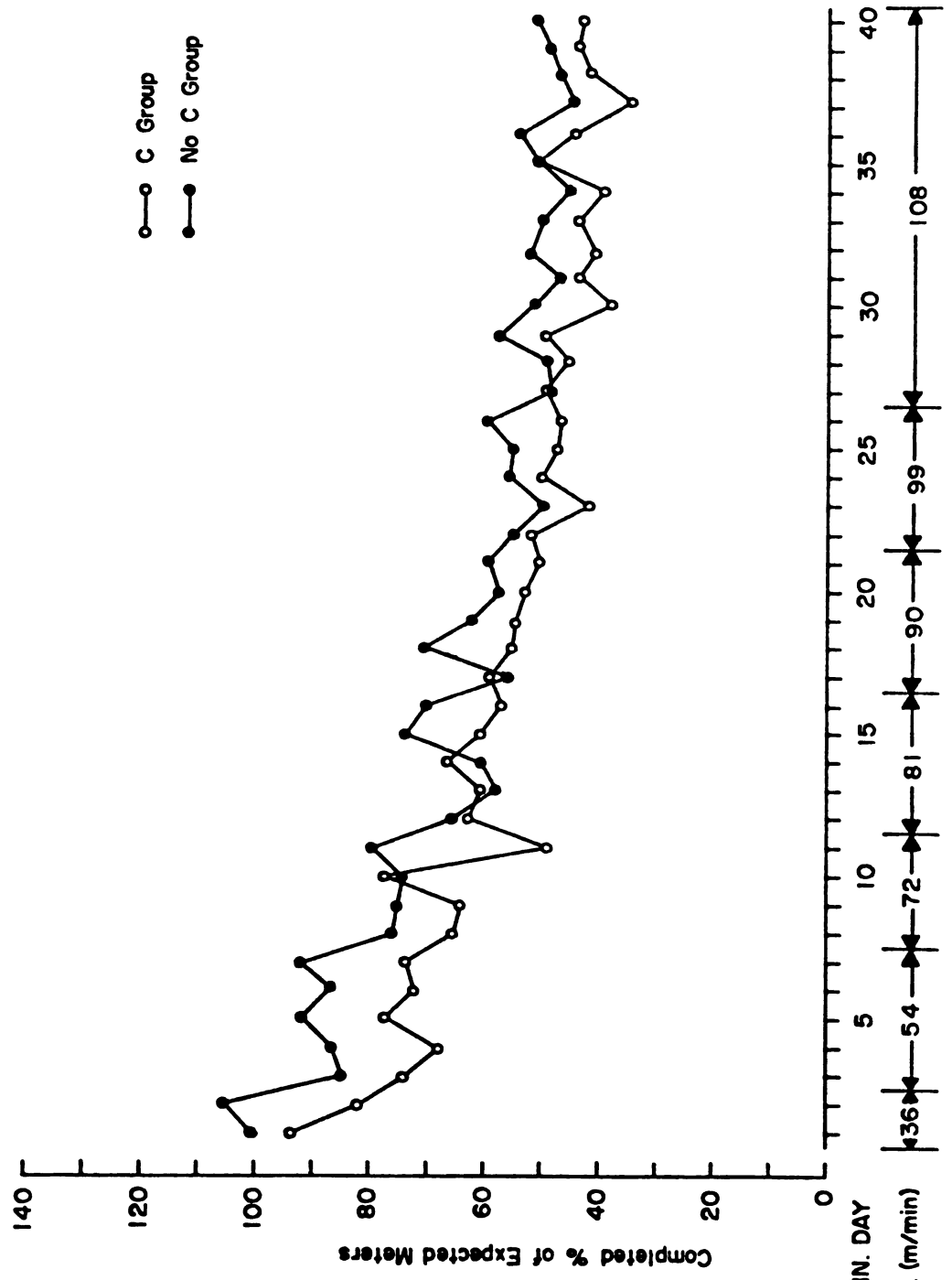

FIGURE 2. Mean Daily Percent Expected Meters Run for SPRINT Animals

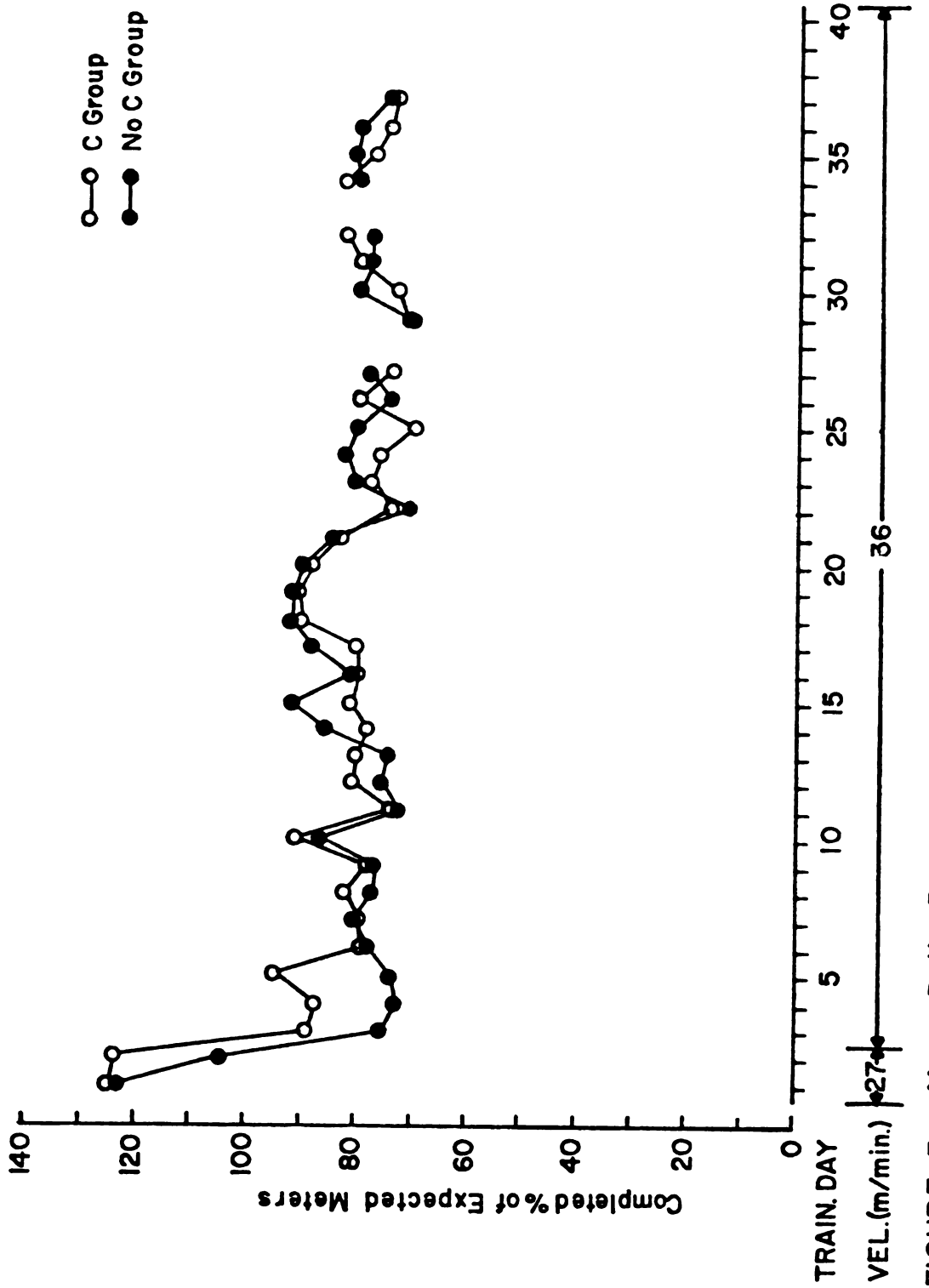

FIGURE 3. Mean Daily Percent Expected Meters Run for ENDURANCE Animals

The END animals ran at the relatively slow speed of 36 m/min. Periods of continuous running were progressively increased to 60 minutes at the end of five weeks of training and were maintained at this level for the remainder of the eight week program (see Figure 3 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.

Activity Level Results

Body Weights at Sacrifice

At the conclusion of the eight week training period, all exercise treatment groups had body weights that were significantly different from each other ($P = .001$) (see Table 1). Both SPT and END groups were considerably lighter in weight than the SED group. The SPT group was determined to be approximately 23% smaller in size while the END group had a size reduction of only 18% when compared to the SED group. In addition, the SPT animals also had a 6% greater weight loss than the END animals. In general terms, the data indicates that growth is impaired, as reflected by body weight,

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

DEPENDENT VARIABLES	ABSOLUTE TREATMENT MEANS SED	SPT	END	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Body Wt. (g)	507.06 ± 26.95	395.06 ± 22.60	417.63 ± 32.57	73.47	.001	SPT < END SPT < SED END < SED
Femur Wt. (g)	1.26 ± .09	1.15 ± .12	1.19 ± .08	4.98	.019	SPT < SED
Femur Lg. (cm)	3.80 ± .66	3.75 ± .28	3.71 ± .70	.10	.903	
Great Tot Diam (mm) Prox. Sect.	131.00 ± 7.05	123.25 ± 10.34	128.00 ± 8.17	3.43	.041	SPT < END SPT < SED END < SED
Mid. Sect.	99.31 ± 4.75	96.00 ± 6.26	96.56 ± 5.16	1.70	.193	
Dist. Sect.	109.56 ± 5.62	98.37 ± 8.48	105.31 ± 6.51	10.48	.001	SPT < END SPT < SED
Least Tot Diam (mm) Prox. Sect.	80.25 ± 4.55	76.69 ± 5.24	78.13 ± 4.45	2.24	.118	
Mid. Sect.	77.44 ± 4.94	73.00 ± 7.71	76.44 ± 4.24	2.55	.089	
Dist. Sect.	68.06 ± 3.17	62.81 ± 5.41	67.13 ± 2.19	8.54	.001	SPT < END SPT < SED END < SED

DEPENDENT VARIABLES	RELATIVE TREATMENT MEANS SED	SPT	END	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Body Wt. (g)	------	------	------	-----	----	----------
Femur Wt. (g) ($\times 10^{-3}$)	2.50 ± .20	2.90 ± .20	2.90 ± .20	22.41	.001	SED < SPT SED < END
Femur Lg. (cm) ($\times 10^{-3}$)	7.53 ± 1.40	9.56 ± 1.00	8.90 ± 1.70	8.65	.002	SED < END SED < SPT
Great Tot Diam (mm) Prox. Sect. ($\times 10^{-3}$)	258.60 ± 17.60	312.30 ± 24.50	309.00 ± 13.20	40.12	.001	SED < END SED < SPT
Mid. Sect.	196.10 ± 14.00	241.80 ± 16.90	232.00 ± 13.60	34.49	.001	SED < END SED < SPT
Dist. Sect.	217.00 ± 17.60	250.90 ± 24.50	253.10 ± 13.20	21.09	.001	SED < SPT SED < END
Least Tot Diam (mm) Prox. Sect. ($\times 10^{-3}$)	158.50 ± 11.80	194.20 ± 11.00	187.70 ± 13.20	40.20	.001	SED < END SED < SPT
Mid. Sect.	153.00 ± 13.00	183.50 ± 15.60	183.60 ± 11.10	26.39	.001	SED < SPT SED < END
Dist. Sect.	134.90 ± 10.30	160.20 ± 11.30	161.40 ± 10.70	30.88	.001	SED < SPT SED < END

Table 1. Continued.

DEPENDENT VARIABLES	ABSOLUTE TREATMENT MEANS SED	SPT	END	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Ave Tot Diam (mm) Prox. Sect.	82.35 ± 4.20	81.27 ± 5.19	82.37 ± 3.94	.31	.732	
Mid. Sect.	82.37 ± 3.12	80.29 ± 6.41	81.26 ± 2.66	.90	.412	
Dist. Sect.	82.31 ± 4.13	77.38 ± 5.67	81.41 ± 3.15	5.60	.007	SPT < END SPT < SED
Great Wall Thick (mm) Prox. Sect.	68.25 ± 8.33	61.41 ± 7.89	65.97 ± 5.86	3.51	.038	SPT < SED
Mid. Sect.	26.22 ± 4.55	26.91 ± 12.04	24.56 ± 4.46	.38	.689	
Dist. Sect.	18.81 ± 2.46	18.53 ± 1.49	17.22 ± 1.60	3.20	.050	
Least Wall Thick (mm) Prox. Sect.	13.12 ± 3.34	12.68 ± .70	12.28 ± .94	.68	.512	
Mid. Sect.	10.84 ± 1.07	11.68 ± 1.07	11.71 ± .75	4.10	.023	SED < SPT SED < END
Dist. Sect.	10.75 ± 1.25	11.00 ± .98	10.28 ± 1.03	1.78	.181	

DEPENDENT VARIABLES	RELATIVE TREATMENT MEANS SED	SPT	END	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Ave Tot Diam (mm) Prox. Sect. ($\times 10^{-3}$)	162.60 ± 10.90	206.00 ± 12.70	198.20 ± 15.90	48.36	.001	SED < END SED < SPT
Mid. Sect.	162.70 ± 10.30	202.00 ± 13.90	195.30 ± 10.60	51.65	.001	SED < END SED < SPT
Dist. Sect.	163.00 ± 10.70	197.40 ± 11.60	195.80 ± 13.60	41.69	.001	SED < END SED < SPT
Great Wall Thick (mm) Prox. Sect. ($\times 10^{-3}$)	134.50 ± 15.50	155.60 ± 19.70	158.20 ± 11.50	10.69	.001	SED < SPT SED < END
Mid. Sect.	51.70 ± 9.00	68.00 ± 30.90	58.90 ± 10.20	2.76	.076	
Dist. Sect.	37.20 ± 4.90	47.40 ± 4.50	41.50 ± 5.50	16.74	.001	SED < END SED < SPT END < SPT
Least Wall Thick (mm) Prox. Sect.	25.80 ± 5.90	32.20 ± 2.20	29.50 ± 2.40	10.83	.001	SED < END SED < SPT
Mid. Sect.	21.40 ± 1.90	29.50 ± 3.30	28.20 ± 2.20	47.65	.001	SED < END SED < SPT
Dist. Sect.	21.30 ± 4.00	28.10 ± 2.80	24.70 ± 3.10	22.54	.001	SED < END SED < SPT END < SPT

Table 1. Continued

DEPENDENT VARIABLES	ABSOLUTE TREATMENT MEANS SED	SPT	END	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Cort Wall Area (cm^2) Prox. Sect.	10.30 ± 1.00	9.39 ± 1.02	10.01 ± 1.00	3.35	.044	SPT < SED
Mid. Sect.	7.75 ± .62	7.48 ± 1.26	7.62 ± .70	.37	.691	
Dist. Sect.	7.12 ± .49	6.55 ± .60	6.74 ± .43	5.04	.011	SPT < SED END < SED
Mar. Cav Area (cm^2) Prox. Sect.	3.36 ± .44	2.92 ± .57	3.01 ± .32	3.93	.027	SPT < SED END < SED
Mid. Sect.	4.94 ± .52	4.05 ± .92	4.38 ± .42	7.50	.002	SPT < SED END < SED
Dist. Sect.	5.88 ± .91	4.31 ± .99	5.45 ± .58	14.59	.001	SPT < END SPT < SED
Tot. Bone Area (cm^2) Prox. Sect.	13.66 ± .99	12.32 ± 1.49	13.03 ± 1.16	4.71	.014	SPT < SED
Mid. Sect.	12.70 ± .80	11.53 ± 1.82	12.01 ± .91	3.46	.040	SPT < SED
Dist. Sect.	13.00 ± 1.07	10.86 ± 1.58	12.20 ± .95	12.22	.001	SPT < END SPT < SED

DEPENDENT VARIABLES	RELATIVE TREATMENT MEANS SED	SPT	END	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Cort Wall Area (cm^2) Prox. Sect. ($\times 10^{-2}$)	20.30 ± 2.20	23.80 ± 2.00	24.00 ± 1.70	17.19	.001	SED < SPT SED < END
Mid. Sect.	15.30 ± 1.30	18.80 ± 2.90	18.30 ± 1.50	13.88	.001	SED < END SED < SPT
Dist. Sect.	14.10 ± 1.10	16.70 ± 1.30	16.20 ± 1.10	22.39	.001	SED < END SED < SPT
Mar. Cav Area (cm^2) Prox. Sect. ($\times 10^{-2}$)	6.60 ± 1.00	7.40 ± 1.30	7.20 ± .70	2.62	.084	
Mid. Sect.	9.80 ± 1.10	10.10 ± 2.00	10.50 ± .90	1.10	.342	
Dist. Sect.	11.70 ± 1.80	11.00 ± 2.30	13.10 ± 1.20	5.61	.007	SPT < END SED < END
Tot. Bone Area (cm^2) Prox. Sect. ($\times 10^{-2}$)	27.00 ± 2.30	31.20 ± 2.90	31.20 ± 1.70	16.96	.001	SED < SPT SED < END
Mid. Sect.	25.10 ± 1.80	28.90 ± 3.90	28.80 ± 1.60	11.03	.001	SED < END SED < SPT
Dist. Sect.	25.80 ± 2.30	27.70 ± 3.50	29.30 ± 2.00	6.99	.002	SED < END

Table 1. Continued

DEPENDENT VARIABLES	ABSOLUTE TREATMENT MEANS SED	SPT	END	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)	DEPENDENT VARIABLES	RELATIVE TREATMENT MFANS SED	SPT	END	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Cortical Area/ Total Area							Cortical Area/ Total Area						
(cm^2) Prox. Sect.	.75 ± .03	.76 ± .02	.76 ± .02	1.15	.327			------	------	------	-----	----	---------
Mid. Sect.	.61 ± .03	.65 ± .04	.63 ± .02	4.72	.014	SED < END SED < SPT		------	------	------	-----	----	---------
Dist. Sect.	.54 ± .03	.60 ± .03	.55 ± .01	17.70	.001	SED < SPT END < SPT		------	------	------	-----	----	---------
Mar Cav Area/ Total Area							Mar Cav Area/ Total Area						
(cm^2) Prox. Sect.	.24 ± .03	.23 ± .02	.23 ± .02	1.15	.327			------	------	------	-----	----	---------
Mid. Sect.	.38 ± .03	.35 ± .04	.36 ± .02	4.72	.014	SPT < SED END < SED		------	------	------	-----	----	---------
Dist. Sect.	.45 ± .03	.39 ± .03	.44 ± .01	17.70	.001	SPT < END SPT < SED		------	------	------	-----	----	---------

when pubescent animals are subjected to strenuous running exercise regimens. This finding coincides with data of similar investigations (14, 25, 56, 57, 58, 59, 66, 87) presented in the literature review. Furthermore, the greatly diminished size of the SPT group in particular, would seem to indicate that these animals were subjected to an overtraining situation under the sprint exercise regimen employed in this study.

Femur Weights at Sacrifice

Due to the great body size variability between the activity groups, both absolute and relative measurements will be reported on the femur weight data, as well as all subsequent morphological data to follow in this section. It is the feeling of this investigator that consideration of both absolute and relative data provides a more accurate picture of the training regimen effects in the parameters under study.

The absolute femur weights of both activity groups are less than that of SED group (see Table 1). However, only the SPT group has an average femur weight that is significantly lighter than that of the SED animals. When average body weight is taken into consideration for each respective exercise group, the opposite trend holds true. That is, the relative femur weight of the SED group is significantly less than the relative femur weights of both SPT and END groups. Therefore, it appears superficially that exercise adversely effects femur growth as reflected by absolute

femur weights. On the other hand, the relative femur weight data suggests that exercised animals actually have heavier bones proportionate to their overall body size when compared to unexercised animals.

Femur Lengths at Sacrifice

Examination of the absolute femur length data reveals no significant differences between any of the activity groups (see Table 1). On the other hand, relative femur length data shows the SED animals to have significantly shorter bones than their exercised counterparts. These findings indicate that long bone growth is enhanced by participation in strenuous physical activity when the overall size of the subjects is taken into consideration.

Femoral Diameter Measurements

The data from three related diameter measures are presented under this heading. They reflect the size and shape variations seen throughout the three cross-sectional levels of bone, inclusive of the marrow cavity, studied in this investigation. It should be noted that effects of the different exercise regimens varies considerably among the three levels of bone examined.

The absolute average total diameters show the SPT and END animals to have femurs that are smaller in cross-sectional size than those of SED animals (see Table 1). However, only the distal section of the SPT group demonstrated an average total diameter significantly smaller than either of the other two groups. In relative terms, the converse is true.

The average total diameters of all three bone levels show the SED animals to have significantly (P = .001) smaller femurs in cross-sectional size than the trained animals.

Because of the great diversity in cross-sectional shape along the femoral shaft, a single measurement of the greatest total diameter and the greatest of the least total diameter was taken at each section of bone examined. The absolute values for each of these parameters show the bones of both trained groups to be smaller in size when compared to the untrained group (see Table 1).

More specifically, not only were the SPT and END groups significantly (P = .041) smaller in greatest total diameters at the proximal section than the SED group but the SPT group was also significantly smaller than the END group. At the distal section, only the SPT animals had a smaller maximum cross-sectional diameter of any statistical repute when compared to the other two groups.

Similarly, the distal section absolute values for the greatest of the least diameter measures, also show both groups of trained animals to have femurs that are significantly smaller in size than those of untrained animals (see Table 1). In addition, there was also a notable statistical difference between the two exercised groups. Again, the converse was true when body size was taken into consideration with reference to these particular diameter measures. Consistently, the relative values show the SED animals to have statistically significant smaller femoral

cross-sectional diameters than their trained counterparts. Taking into consideration relative body size and the data previously set forth, one might speculate that strenuous running exercise enhances the strength of the femoral shaft.

Femoral Area Measurements

The most accurate and valid indicators of cross-sectional bone size are the various area assessments included under this heading. Two indicators of cross-sectional shape are also incorporated in this material.

The absolute cortical wall area data is characteristic of previously described material (see Table 1). The trained exercise groups generally have smaller bone areas than the untrained group. However, only those animals subjected to the SPT program proved to have significantly decreased cortical wall areas at the proximal level of their femoral shafts while both sets of exercised animals had such indications at the distal level. Conversely, the relative cortical wall areas of all three bone levels showed the SED animals to have statistically notable smaller bones than either sets of exercised animals. It would appear that exercise has a positive effect on bone growth by increasing the proportional size of the femoral shaft actually composed of bony tissue.

Further indications of size and shape changes along the femoral shaft, supportive of the cortical wall area data, are provided by the greatest and least wall thickness measurements. Significant focal bone increases along the

femoral shaft have been determined on both an absolute and relative basis as indicated by a single measurement of the greatest wall thickness (see Table 1). The evidence indicates that focal bony enlargements occurred at both proximal and distal ends of the femur as a result of strenuous sprint and endurance running. However, the most notable bony expansions occurred in the distal portion of the femur of SPT animals. This is probably due to the additional stress imposed upon these animals as an inherent part of the sprint exercise program.

Concurrently, a single measurement of the least wall thickness was taken on each bone section in an attempt to identify any focal areas of bone deprivation. Interestingly, unlike previously reported trends, both absolute and relative data indicated that the SED animals had focal regions of significant bone wall thinness with respect to their exercised counterparts (see Table 1). Furthermore, the most pronounced effect was noted at the middle section of the femur with respect to both absolute and relative data. It is evident from the greatest and least wall thickness data, in conjunction with the cortical wall area information, that participation in a strenuous program of either sprint or endurance running enhances the appositional growth of the femur.

The marrow cavity area was also determined for each of the three bone levels examined. The absolute data demonstrates that both SPT and END treatment groups have

significantly smaller marrow cavity areas than the SED treatment group (see Table 1). On the other hand, when body size was taken into consideration, the SED animals generally had smaller marrow cavity areas compared to the trained animals. However, the only significant between group difference resulted at the distal section with both SPT and SED animals having notably smaller marrow cavity areas than the END animals. Therefore, based on both cortical wall and marrow cavity areas, it would appear that strenuous sprint and endurance running increases the total cross-sectional girth of the femoral shaft via the proportional expansion of both the marrow cavity as well as the cortical wall shaft.

Overall bone size changes are reflected by the absolute and relative total bone area determinations. These parameters are composed of both cortical wall and marrow cavity areas. Both absolute and relative values confirm the findings of previously reported size indicators (see Table 1). However, of particular interest is the fact that only the SPT exercised animals proved to have significantly smaller absolute total femoral areas at all three levels of bone when compared to the other two groups. Conversely, in relative terms, the SED animals were determined to have femurs of significantly smaller girth than their exercised counterparts. It can be inferred from the data presented, that exercise enhances overall bone growth proportional to body size. However, with respect to the absolute data, it should be noted that not only were the femurs of SPT animals

notably smaller in size than those of SED animals but also obviously smaller than those of END animals. Based on this information, there is an indication that the sprint exercised animals may have been exposed to an exercise regimen of much greater stress than the endurance trained animals.

Further indication of femoral size changes due to strenuous running exercise is reflected by the ratio of cortical wall area to total bone area. Again, the exercised animals are characterized by having femurs composed of considerably more bony tissue proportional to their total cross-sectional area than the untrained animals (see Table 1). In addition, the reciprocal data, reflected by the ratio of marrow cavity area to total bone area also confirms these findings.

Vitamin C Supplementation Results

As with the exercise data, both absolute and relative values were obtained on the Vitamin C data. However, all of the diet supplementation results, except for five random parameters at various bone levels, showed no significant differences (see Table 2) between the Vitamin C (Vit C) and no Vitamin C (No C) groups.

A tally of the directional size and shape changes for each of the parameters at all three bone levels was tabulated. The findings show that 60% of the parameters had values demonstrating that the animals receiving the dietary Vitamin C supplement had femurs smaller in size than those

Table 2. Analysis of variance for overall Vitamin C effects and Newman-Keuls tests of paired comparisons for absolute and relative bone data.

DEPENDENT VARIABLES	ABSOLUTE TREATMENT MEANS VIT-C	ABSOLUTE TREATMENT MEANS NO-C	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Body Wt. (g)	447.90 ± 54.29	431.93 ± 57.53	4.09	.049	VIT-C > NO-C
Femur Wt. (g)	1.22 ± .10	1.18 ± .11	1.24	.297	
Femur Lg. (cm)	3.85 ± .26	3.67 ± .77	1.16	.325	
Great Tot Diam (mm) Prox. Sect.	130.04 ± 6.86	125.33 ± 10.46	3.73	.060	
Mid. Sect.	99.21 ± 5.31	95.38 ± 5.12	6.55	.014	VIT-C > NO-C
Dist. Sect.	105.88 ± 8.45	102.96 ± 7.99	2.09	.156	
Least Tot Diam (mm) Prox. Sect.	79.00 ± 4.93	77.71 ± 4.92	.84	.365	
Mid. Sect.	76.75 ± 5.22	74.50 ± 6.64	2.19	.146	
Dist. Sect.	66.71 ± 4.25	65.29 ± 4.53	1.66	.204	

DEPENDENT VARIABLES	RELATIVE TREATMENT MEANS VIT-C	RELATIVE TREATMENT MEANS NO-C	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Body Wt. (g)	------	------	-----	----	----------
Femur Wt. (g) ($X10^{-3}$)	2.73 ± .30	2.80 ± .20	.31	.650	
Femur Lg. (cm) ($X 10^{-3}$)	8.70 ± 1.10	8.60 ± 2.00	.15	.735	
Great Tot Diam (mm) Prox. Sect. ($X 10^{-3}$)	293.70 ± 32.30	292.80 ± 30.40	.03	.873	
Mid. Sect.	223.20 ± 24.80	223.40 ± 25.00	.01	.930	
Dist. Sect.	239.20 ± 23.30	241.50 ± 25.20	.19	.660	
Least Tot Diam (mm) Prox. Sect. ($X 10^{-3}$)	175.50 ± 20.30	181.80 ± 19.20	.91	.345	
Mid. Sect.	173.10 ± 23.40	173.70 ± 15.40	.54	.467	
Dist. Sect.	151.10 ± 16.80	153.20 ± 15.90	.43	.515	

Table 2. Continued

DEPENDENT VARIABLES	ABSOLUTE TREATMENT MEANS VIT-C	ABSOLUTE TREATMENT MEANS NO-C	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Ave Tot Diam (mm) Prox. Sect.	82.44 ± 4.94	81.55 ± 3.86	.45	.504	
Mid. Sect.	82.37 ± 4.14	80.25 ± 4.45	3.09	.086	
Dist. Sect.	81.11 ± 5.15	79.62 ± 4.53	1.31	.258	
Great Wall Thick (mm) Prox. Sect.	66.77 ± 5.20	63.65 ± 9.64	2.10	.154	
Mid. Sect.	27.25 ± 9.38	24.54 ± 5.59	1.38	.248	
Dist. Sect.	18.10 ± 1.81	18.27 ± 2.19	.11	.747	
Least Wall Thick (mm) Prox. Sect.	13.02 ± 2.68	12.38 ± 1.01	1.24	.272	
Mid. Sect.	11.37 ± .98	11.46 ± 1.12	.08	.776	
Dist. Sect.	10.39 ± 1.12	10.96 ± 1.05	3.37	.074	

DEPENDENT VARIABLES	RELATIVE TREATMENT MEANS VIT-C	RELATIVE TREATMENT MEANS NO-C	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Ave Tot Diam (mm) Prox. Sect. ($X 10^{-3}$)	186.50 ± 23.60	191.30 ± 22.90	1.52	.225	
Mid. Sect.	185.60 ± 23.30	187.70 ± 18.50	.38	.544	
Dist. Sect.	183.80 ± 20.30	187.00 ± 19.70	.85	.361	
Great Wall Thick (mm) Prox. Sect. ($X 10^{-3}$)	150.80 ± 18.00	148.10 ± 20.10	.32	.576	
Mid. Sect.	61.40 ± 23.60	57.60 ± 16.40	.11	.741	
Dist. Sect.	41.30 ± 7.30	42.80 ± 5.40	1.16	.288	
Least Wall Thick (mm) Prox. Sect. ($X 10^{-3}$)	29.20 ± 5.00	29.10 ± 4.40	.02	.897	
Mid. Sect.	25.70 ± 3.80	27.00 ± 4.80	3.57	.066	
Dist. Sect.	23.60 ± 3.70	25.80 ± 4.00	8.22	.006	NO-C > VIT-C

Table 2. Continued

DEPENDENT VARIABLES	ABSOLUTE TREATMENT MEANS VIT-C	ABSOLUTE TREATMENT MEANS NO-C	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Cort Wall Area (cm^2) Prox. Sect.	10.07 ± .91	9.73 ± 1.19	1.33	.256	
Mid. Sect.	7.81 ± .98	7.44 ± .79	1.98	.167	
Dist. Sect.	6.88 ± .49	6.73 ± .62	.94	.337	
Mar Cav Area (cm^2) Prox. Sect.	3.13 ± .40	3.08 ± .57	.15	.697	
Mid. Sect.	4.65 ± .61	4.27 ± .83	4.27	.045	VIT-C > NO-C
Dist. Sect.	5.42 ± 1.18	5.02 ± .92	2.71	.107	
Tot Bone Area (cm^2) Prox. Sect.	13.20 ± 1.18	12.81 ± 1.46	1.22	.276	
Mid. Sect.	12.45 ± 1.19	11.71 ± 1.39	4.58	.038	VIT-C > NO-C
Dist. Sect.	12.29 ± 1.56	11.75 ± 1.42	2.33	.134	

DEPENDENT VARIABLES	RELATIVE TREATMENT MEANS VIT-C	RELATIVE TREATMENT MEANS NO-C	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Cort Wall Area (cm^2)-Prox. Sect. ($X 10^{-3}$)	22.70 ± 2.70	22.70 ± 2.50	.01	.919	
Mid. Sect.	17.60 ± 3.00	17.30 ± 1.90	.16	.690	
Dist. Sect.	15.60 ± 1.70	15.80 ± 1.60	.34	.562	
Mar Cav Area (cm^2)-Prox. Sect. ($X 10^{-3}$)	7.10 ± 1.10	7.10 ± .90	.05	.823	
Mid. Sect.	10.40 ± 1.50	9.90 ± 1.30	1.99	.165	
Dist. Sect.	12.20 ± 2.30	11.70 ± 1.70	.86	.359	
Tot Bone Area (cm^2)-Prox. Sect. ($X 10^{-3}$)	29.80 ± 3.60	29.80 ± 2.50	.00	.997	
Mid. Sect.	28.00 ± 3.90	27.20 ± 2.20	1.15	.289	
Dist. Sect.	27.70 ± 3.40	27.40 ± 2.70	.14	.709	

Table 2. Continued

DEPENDENT VARIABLES	ABSOLUTE TREATMENT MEANS VIT-C	ABSOLUTE TREATMENT MEANS NO-C	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Cortical Area/ Total Area (cm^2) Prox. Sect.	.76 ± .02	.76 ± .04	.18	.672	
Mid. Sect.	.63 ± .04	.64 ± .04	1.08	.304	
Dist. Sect.	.56 ± .04	.58 ± .04	1.87	.179	
Mar Cav Area/ Total Area (cm^2) Prox. Sect.	.24 ± .02	.24 ± .04	.18	.670	
Mid. Sect.	.37 ± .04	.36 ± .04	1.08	.304	
Dist. Sect.	.44 ± .04	.42 ± .04	1.87	.179	

DEPENDENT VARIABLES	RELATIVE TREATMENT MEANS VIT-C	RELATIVE TREATMENT MEANS NO-C	F VALUE	P VALUE (a=.05)	SNK TEST (a=.05)
Cortical Area/ Total Area (cm^2) Prox. Sect.	------	------	-----	----	----------
Mid. Sect.	------	------	-----	----	----------
Dist. Sect.	------	------	-----	----	----------
Mar Cav Area/ Total Area (cm^2) Prox. Sect.	------	------	-----	----	----------
Mid. Sect.	------	------	-----	----	----------
Dist. Sect.	------	------	-----	----	----------

animals not receiving Vitamin C. On the other hand, 31% of the parameters indicated the Vit-C group had larger bones than the No-C group while only 9% of the parameters had equivalent values.

When the tabulated data was analyzed further, some interesting trends between absolute and relative data were also noted. Forty-three percent of the parameters, consisting of just absolute values, demonstrated the Vit-C animals actually had femurs of smaller size than those animals not receiving a Vitamin C supplement while only 17% of the relative parameters exhibited such a trend. In contrast, the relative data shows that 22% of the parameters had values indicating the Vit-C animals had femurs of increased size although only 9% of these animals demonstrated this trend when absolute values were examined. However, it must be emphasized that none of these results represent a statistically significant difference between the Vit-C and No-C groups. Thus, it would appear that diet supplementation with Vitamin C had little or no effect on enhancing bone growth in rats placed on strenuous running exercise programs.

Because of the great diversity of information on the functional roles of Vitamin C in bone growth and adaptation to stress, it is very difficult to interpret the diet supplementation findings reported in this study. It was hypothesized that high dosages of Vitamin C would enhance long bone growth, especially in the trained animals, by increasing their ability to adapt to the physical stress

imposed by the exercise programs. However, it is evident from the data that the findings do not support this hypothesis. In fact, it would appear that high intakes of Vitamin C may actually be detrimental to long bone growth. A possible explanation of such results may lie in the regulatory role that Vitamin C plays in controlling functions of the adrenal gland. Various hormones synthesized in the adrenal gland are known to be released into the blood stream upon subject exposure to stress. However, adrenal Vitamin C stores must be depleted prior to synthesis and release of adrenal hormones. Under ordinary circumstances, physical exertion is known to deplete adrenal Vitamin C stores thus allowing the normal sequence of adrenal hormone synthesis and release to occur. However, under the conditions imposed by this study, large dosages of Vitamin C were administered daily throughout the eight week training period. Although adrenal Vitamin C stores may have been depleted following each exercise session, it may be possible that the daily Vitamin C intake was excessive enough to continuously replenish the adrenal gland storage depot, prior to body excretion, thus inhibiting normal responses of the gland to stress. Furthermore, since the response of the adrenal gland to stress is systemic in nature, it is also possible that the lack of a positive Vitamin C effect on bone growth is an indirect result of the atypical adrenal gland function previously proposed.

Discussion

Accurate interpretation of the effect exercise has on bone growth is dependent upon the results of both absolute and relative bone data. Evaluation of either absolute data or relative data alone would lead to misinterpretation of the exercise results as indicated by the opposite femoral growth trends previously reported in this chapter.

It is obvious from the body weight data that both groups of exercised animals, especially those on the sprint program, are significantly smaller in overall size when compared to the sedentary animals. This diversity in body weights between the three exercise treatment groups could be explained in several ways. One possibility is that the great increase in average body weight of the sedentary group could be attributed to excessive food intake. On the other hand, it could also be possible that the exercise programs could have decreased the appetites and thus lowered the food intake of the sprint and endurance animals resulting in their diminished overall body size. However, these are merely suppositions since food intake was not considered to be an integral part of the study and therefore was not monitored.

The most probable explanation for the body weight variations corresponds to the caloric expenditures characterized by the activity levels of each of the respective exercise treatment groups. Since the sedentary control animals were innately inactive throughout the 8-week treatment period,

it is not surprising that these animals may have merely taken in more calories than they were able to expend during their daily activities. As a result, these animals had higher body weights and increased overall size when compared to their exercised counterparts. Furthermore, it also follows that the animals placed on the relatively low intensity endurance running program would expend fewer calories than those animals on the relatively high intensity sprint running program. Thus, based on this body weight data, it can be concluded that overall body size is a function of the intensity of exercise.

Because of these significant between group body weight differences, it should be kept in mind that the interpretations of all subsequent findings will be greatly effected by this data. In particular, absolute data will especially be effected. Therefore, interpretations and conclusions of all subsequent exercise results will be based on the relative bone data which takes into account these body weight differences.

General bone growth changes are indicated by femur weight and length measurements. Both sets of relative data show the femurs of exercised animals to be significantly heavier in weight and longer in length proportional to their overall size than the femurs of sedentary animals. It is known from the findings of studies concerned with immobilization effects on bone growth, that optimal levels of both compressive and tensile forces are needed for normal, healthy long

bone growth. Adequate exposure to both of these types of forces results in balanced osteoblastic and osteoclastic activity. It is also known that running types of exercise exert both tensile and compressive types of forces at the proximal and distal ends of the femoral shaft, respectively. More specifically, it would appear that these exercise stressors had a positive effect on bone growth by enhancing epiphyseal plate maturation. This would account for the increased long bone length of the trained animals. However, exactly how cells of the epiphyses are effected by the various types of stressors imposed by running exercise is unknown. Since maturation of the epiphyseal plates depends, to some degree, on an adequate oxygen supply, it is possible that bone vascularity may also play a major role in the stress adaptability of long bones. However, vascular quantification and epiphyseal plate maturation was not determined in this investigation thus leaving these explanations, as to how exercise effects long bone growth, to pure supposition.

Evaluation of the various relative diameter measures also shows there was a positive exercise effect on growth in width of the femoral shaft of trained animals. Consistently, the relative data shows both trained groups to have femurs significantly larger in breadth than those of the unexercised group. In addition, although the relative diameter data was not statistically significant, the SPT animals also had femurs that were larger in width than those of END animals. Of further interest, is that in two of the three

relative diameter measures under study, the SPT animals showed a greater width increase at the proximal level of the femoral shaft than found at the same level in the END animals. Conversely, at the distal level there was less width increase in the femurs of SPT animals when compared to similar measurements on the END animals. Interestingly, these findings indicate that the bone response to running exercise varies along the length of the femoral shaft according to the type, intensity, and point of application of stress. Since the adductor muscles attach along the medial aspect of the proximal level of the femoral shaft, it is probable that a tensile type of force is created by contractions of these muscles. The result is an increase in bone width as noted in the SPT animals. Evidently, the higher intensity of the sprint program over that of the endurance program was great enough to stimulate additional osteoblastic activity at this particular area of bone. This bone increase would then act as a means of increasing the resistance of the bone to potential fatigue created by the tensile stress of the adductor muscles. Since exercise is known to increase the number, size, and strength of muscle fibers (95), it follows that bone tissue should show similar adaptations to exercise stress by also increasing in size. Therefore, it seems reasonable to assume that the bone size increase should at least be proportional to the increased strength of its attached muscles.

At the distal level of the femur, a compressive rather than tensile type of force seems to have been applied as a result of both sprint and endurance exercise programs. However, this time the greatest diameter increase occurred in END animals rather than SPT animals. It would appear that a great deal of compressive force is applied to the distal portion of the femur as an inherent part of any type of running exercise. In this study, the findings would indicate that the SPT animals have been exposed to an excessive amount of compressive force due to the high intensity of their exercise program. As a result, their femurs were not as readily adaptable to the compressive type of stress as they were to the tensile type of stress applied at the proximal portion of their femurs. A more optimal level of exercise intensity, allowing adequate adaptability to the compressive type of stress, appears to have been provided by the endurance program. Based on these findings, it can be concluded that compressive types of stress, such as that produced by running exercise, also enhance the appositional growth of long bones. However, it should be noted that unlike the tensile forces created at the proximal level of the femur, compressive forces acting at distal sites are very sensitive to exercise regimens of high intensity.

Evaluation of the various relative cross-sectional area determinations further indicates the positive effect that exercise has on appositional growth of the femur. Consistent with relative diameter data, a significant

increase in relative total cross-sectional area of the femurs of both SPT and END animals was also found. Again, this increase varied along the length of the femoral shaft. Also, consistent with the relative diameter data, is the finding that these size variations are reflective of the type, intensity, and point of application of the exercise stressors imposed by this study. It is interesting to speculate that the tensile force applied at the proximal level of the femur seems to have had a greater stimulatory effect on the osteoblastic activity on the periosteal surface of the femoral shaft than it did on the osteoclastic activity of the endosteal surface of this bone. However, at the distal level of the femur where compressive forces have their greatest effect, stimulation of both osteoblastic and osteoclastic activity was more balanced. These statements are based on the fact that at the proximal level of the femur approximately 76% of the total bone area was found to be composed of actual bone tissue with the remaining 24% of the total area consumed by the marrow cavity while at the distal portion of the femur only 58% of the relative total cross-sectional area was found to be composed of actual bone tissue leaving the marrow cavity to assume the remaining 42% of the total area. Thus, with respect to relative cross-sectional area determinations, it is apparent that appositional growth is enhanced in femoral bones exposed to both tensile and compressive forces as a result

of participation in either strenuous sprint or endurance running exercise programs.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This investigation was undertaken to study the effects of two strenuous running exercise regimens and Vitamin C supplementation on various morphological growth measurements of the femur. Eighty-four normal male rats (Sprague-Dawley strain) were used as subjects and randomly assigned to one of three exercise programs. The exercise programs consisted of high intensity sprint (SPT) and endurance (END) running regimens which were used in an attempt to selectively tax the anaerobic and aerobic metabolic pathways of the experimental animals, respectively. So that the specific intensity and duration of these exercise regimens could be regulated and the performance of each animal monitored, the experimental animals were trained on electronically controlled running wheels (CRW). The animals assigned to the sedentary (SED) group received no exercise and were used as controls.

In addition, one-half of the animals in each training group received a dietary supplement of Vitamin C (Vit-C) while the remaining animals received a sugar water placebo (No-C). Both dietary supplements were administered daily

by oral syringe. The Vitamin C dosage consisted of 2 mg of ascorbic acid in a .1 cc 5% sugar water solution per 100 gm of body weight.

At the start of the eight week treatment period all animals were 84 days of age. At the cessation of this treatment period, selected rats were sacrificed 72 to 96 hours after their last training session. The body weight of each animal was determined just prior to sacrifice. After being in frozen storage for approximately nine months, the animals were then thawed and the left femur removed. Wet weights and lengths were determined on the femurs prior to their processing for morphological studies.

As a result of performance criteria and sampling error, all morphological data analyses were based on an equal cell size of ten animals. All of these parameters were evaluated in terms of both absolute and relative values. In general terms, the absolute data showed the exercised animals, especially those on the sprint program, to be significantly smaller in overall size including all femoral size measurements, when compared to sedentary animals. However, when body size was taken into consideration, the relative data showed the exercised animals actually had femurs of larger magnitude proportional to their overall size than the unexercised animals. Thus, strenuous sprint and endurance running was found to enhance both the longitudinal and appositional growth of the femur.

The Vitamin C data was also analyzed in terms of absolute and relative values. However, no significant differences in femoral growth trends were noted between the Vit-C and No-C groups.

Conclusions

1) The average body weights of both SPT and END exercise groups are significantly less than those of the SED group with the SPT animals also significantly smaller in overall size than the END animals.

2) The effects of tensile and compressive forces, created by strenuous SPT and END running exercise regimens, vary along the length of the femoral shaft.

3) The greatest size and shape alterations, resulting from participation in strenuous SPT and END running programs, occur at the distal and proximal levels of the femoral shaft, respectively.

4) The middle portion of the femoral shaft is least affected by size and shape changes resulting from participation in strenuous SPT and END running programs.

5) The femurs of both SPT and END exercised animals are significantly longer in length and heavier in weight, relative to each group's average body weight, than the femurs of unexercised sedentary animals.

6) There were no significant differences between the relative femur weights and lengths of SPT and END animals.

7) The femurs of SPT and END exercised animals had significantly larger cross-sectional diameters, relative to body weight, than the femurs of SED animals.

8) There were no significant differences between the relative total cross-sectional diameters of femurs of SPT and END animals.

9) Foci of greater cross-sectional shape alterations were noted in the femurs of animals trained on SPT and END exercise programs when compared to the femurs of unexercised, sedentary control animals.

10) There were no significant bone shape alterations noted in the femurs of either SPT or END animals except at the distal portion of the bone where SPT animals had significantly greater shape alterations compared to END animals.

11) The relative femoral cortical wall areas of exercised animals were significantly larger than those of sedentary animals.

12) There were no significant differences between the relative femoral cortical wall areas of SPT and END exercised animals.

13) There were no significant differences between the relative femoral marrow cavity areas of any of the three exercise treatment groups except at the distal level of the femur where the relative marrow cavity area of the END group was significantly increased.

14) Dietary Vitamin C supplementation had no effect on either the longitudinal or appositional growth of femurs of any of the three exercise treatment groups.

Recommendations

1) Due to the significant body weight differences between the three exercise treatment groups, food intake should be monitored.

2) Due to the altered longitudinal growth patterns observed in the exercised animals, the processes of epiphyseal plate maturation should be studied.

3) Bone vascularity quantification should be determined due to the altered longitudinal as well as appositional growth patterns observed in SPT and END animals.

4) Femoral strength and deformation characteristics should be determined in further investigations to establish the true training effects of various strenuous running exercise regimens on bone growth.

5) Urinary Vitamin C excretion should be monitored in order to better determine the functional requirements of the vitamin in animals placed on strenuous running exercise programs.

6) Further investigations are needed to determine whether the sugar water placebo had any effect on altering the femoral growth patterns of trained animals.

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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.	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.	Acceler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repeti-tions per Bout	No. of Com-plete Bouts	Par-tial Bouts (min: sec)	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	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 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
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