THE EFFECT OF BORON SUPPLEMENTATION AND ORAL CONTRACEPTIVES ON MINERAL STATUS AND HORMONE STATUS OF COLLEGE FEMALE ATHLETES AND NON-ATHLETES

By

CANDICE LYN WARD

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APPROVED:

S.J. RITCHEY, Chairman

F.C. GWAZDAUSKAS

ELEANOR D. SCHLENKER

ELIZABETH A. THOMAS

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(ABSTRACT)

Twenty-nine college females, aged 18-29, participated in a six month boron supplementation protocol to evaluate mineral and hormone status of athletes and non-athletes. All subjects were classified into activity and oral contraceptive groups as follows: athletes (n=16), non-athletes (n=13), oral contraceptives (n=12), and no oral contraceptives (n=17). Subjects were randomly assigned to receive a placebo or 3 mg of boron per day. Subjects' maximal oxygen consumption (VO_{2MAX}) was assessed as a baseline measurement and was used to confirm a difference between activity groups. Body fat, height, bone mineral density, and serum calcitonin and parathyroid also were baseline measurements. Subjects were measured at baseline and six months for the following: dietary intake, body weight, serum levels of 17-ß-estradiol, progesterone, and testosterone, and plasma and urine levels of calcium, phosphorus, magnesium, and boron. Athletes had a significantly greater VO_{2MAX} ($p < 0.01$) than did non-

athletes. Subjects taking oral contraceptives had significantly lower serum estradiol ($p < 0.05$) as compared to subjects not taking oral contraceptives. Boron supplemented non-athletes had a significantly greater change in plasma calcium levels ($p < 0.05$) as compared to boron supplemented athletes, although the athletes had a significantly higher plasma calcium level ($p < 0.05$) at the baseline measurement. Control subjects (n=6) had a significantly greater change in dietary carbohydrate ($p < 0.01$) and protein intake ($p < 0.05$) as compared to subjects taking boron (n=23). Boron supplementation did not appear to significantly influence any of the parameters measured. However, boron supplementation combined with activity appeared to influence plasma calcium, while serum estradiol may be influenced by oral contraceptives.

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INTRODUCTION

Statement of the Problem

Intense exercise has become routine for many college-aged female athletes. However, this type of vigorous training has been associated with menstrual dysfunction, which may affect bone growth and metabolism (Drinkwater et al. 1986). Altered bone growth and metabolism in college-aged female athletes may lead to decreased bone mineral density (BMD), which may have drastic consequences later in life, such as increased susceptibility to bone fractures and osteoporosis (Cann et al. 1984; Drinkwater et al. 1984).

Lloyd and colleagues (1987) found that female athletes with exercise-induced menstrual dysfunction were more prone to reduced BMD and reduced levels of circulating progesterone and estradiol, and were more likely to receive musculoskeletal injuries as compared to eumenorrheic sedentary females. Matkovic (1991) suggested that female adolescents may be susceptible to developing skeletal inadequacy due, in part, to the imbalance between calcium intake and the body's demand for it during bone remodeling. According to Nielsen and associates (1990b), it is possible

that young females who intensely exercise may be categorized with postmenopausal women, for both have been found to experience loss in bone mineralization.

In postmenopausal women, boron supplementation has been reported to maintain bone mass via an effect on calcium metabolism (Nielsen et al. 1988, 1990b). If boron supplementation proves beneficial for postmenopausal women, it may also aid in preventing loss of BMD in premenopausal athletic females.

The purpose of this study was to determine whether boron supplementation use elicits changes in hormonal status or mineral status in college female athletes and non-athletes, as well as in college females who use oral contraceptives. A previous study (Darnton 1991; Volpe-Snyder 1991) of boron supplementation in female athletes at Virginia Polytechnic Institute and State University (VPI & SU) produced results that conflicted with those previously reported (Nielsen et al. 1987). Possible reasons for conflicting outcomes may have included differences in subject's ages and living conditions during the investigation, and the duration of the investigations. It is necessary to reproduce methodologies of other researchers in an attempt to verify results. The outcomes of this study will add to the knowledge base related to boron, and possibly provide a basis for future projects

exploring the use of boron supplementation in persons at risk for metabolic bone diseases, such as osteoporosis.

Research Hypotheses

The null hypotheses of this study are as follows:

HO: There is no effect of athletic training status on hormone status, mineral status, dietary intake, or BMD in subjects.

HO: There is no effect of oral contraceptive use on hormone status, mineral status, dietary intake, or BMD in subjects.

HO: Daily boron supplementation has no effect on subjects' serum concentrations of estrogen, progesterone, testosterone, calcitonin, and parathyroid hormone, as measured at baseline and six months.

HO: Daily boron supplementation has no effect on subjects' urinary excretion of boron, calcium, phosphorus, and magnesium, as measured at baseline and six months.

HO: Daily boron supplementation has no effect on subjects' plasma concentrations of boron, calcium, phosphorus, and magnesium, as measured at baseline and six months.

Significance of the Study

This study will contribute to the information concerning college-aged female athlete's susceptibility to bone demineralization. This is a recently recognized

problem since reduced bone mass has been recognized with intense athletic training. In addition, this study will provide information about the effects of boron supplementation and use of oral contraceptives on mineral status and hormone status in these college-aged female athletes and non-athletes.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The following will provide background material relevant to bone metabolism, osteoporosis, and menstrual dysfunction of female athletes. The processes involved with bone metabolism will be discussed, along with the hormones and minerals which participate in these processes. In order to comprehend metabolic bone disorders, bone metabolism must first be understood. Osteoporosis, a common metabolic bone disorder, will also be discussed in an attempt to provide additional information concerning the implications this type of disease may have on BMD. The mounting evidence which suggests that amenorrheic female athletes who intensely exercise are at risk for bone demineralization will be reviewed, as well as the suggested protective effect oral contraceptives, estrogen, progesterone, calcitonin, parathyroid hormone, vitamin D, calcium, and exercise may have on bone demineralization.

In addition, implications for the essentiality of boron will be discussed in relation to its postulated role in the prevention of bone demineralization. Finally, a review of several methods of sample preparation, mineral analysis, hormone analysis, and BMD will be included.

Bone Metabolism

The skeletal system is made up of three types of tissue: dense connective tissue, cartilage, and bone. Bone is a very dynamic tissue constantly undergoing change. There are four types of bone cells which function in maintaining bone homeostasis. They include: osteoprogenitor cells, which are derived from the mesenchyme and are unspecialized cells; osteoblasts, which secrete mineral salts and organic components and function in bone formation; osteocytes, which are mature bone cells that are the principle cells in bone; and finally osteoclasts, which develop from circulating monocytes and function in bone resorption or degradation (Tortora and Anagnostakos 1990). In addition, there are two regions of bone, both of which are affected by bone metabolism. They include the cortical or compact bone and trabecular or spongy bone (Fallon 1988; Marks and Popoff 1988; Tortora and Anagnostakos 1990). Cortical bone contains few spaces, is very dense, and is primarily located in the shafts of long bones. Trabecular bone is composed of many spaces which are filled with red bone marrow, and is found at the ends of long bones and in such flat bones as the vertebrae and pelvis (Tortora and Anagnostakos 1990; Chestnut 1991).

The activities of the osteoblasts and osteoclasts together compose the primary action of bone remodeling,

which may be described as the continuous activity of the bone tissue related to the maintenance of mineral homeostasis, or more simply, it is the formation of compact bone from spongy bone (Fallon 1988; Marks and Popoff 1988; Tortora and Anagnostakos 1990). Since trabecular bone is transformed into cortical bone, it is easy to understand that trabecular bone is lost at a more rapid rate than cortical bone (Avioli 1991; Marks and Popoff 1988). This also explains why the adult skeleton is composed of approximately 80% cortical bone (Weinerman and Bockman 1990). Fallon (1988) has found that peak cortical bone mass is attained by age 35, while peak trabecular bone mass occurs at a much earlier age. In general, a person can continue to build skeletal mass from ages 18-35 years, which is a period long after longitudinal bone growth has ceased (Heaney 1986; National Research Council 1989; Anderson 1990). Table 1 illustrates reported values of female BMD of the lumbar spine. Lohman (1985) suggested that BMD declines with increasing age, with the BMD of males decreasing from 1.064 to 1.036 g/cm^2 (0.028 g/cm^2) between the ages of twenty and seventy, while females in the same age range experience a decline from 1.034 to 1.013 g/cm^2 (0.021 g/cm^2). This difference between bone densities of males and females is present throughout all stages of life

TABLE 1
REPORTED FEMALE BASELINE VALUES OF BONE MINERAL DENSITY OF THE LUMBAR SPINE (L2-L4)

Population Measured	Age Range of Population (years)	Reported Bone Mineral Density (g/cm^2)
Eumenorrheic Athletes	18-24	1.30 ± 0.1[5]
	20-40	1.14 ± 0.02[3]
	25-52	1.18 ± 0.02[4]
Amenorrheic Athletes	20-43	1.07 ± 0.02[4]
	21-35	1.07 ± 0.01[3]
Premenopausal Women	18-24	1.19 ± 0.1[5]
	20-39	1.27 ± 0.16[2]
	21-35	1.11 ± 0.02[3]
	24-40	1.181 ± 0.116[1]
Oral Contraceptive Users	20-39	1.27 ± 0.13[2*] 1.28 ± 0.12[2**]
	24-40	1.179 ± 0.113[1]
	40-54	1.11 ± 0.137[6*] 1.125 ± 0.156[6**]
Postmenopausal Women	40-54	1.08 ± 0.165[6]
	61-65	0.990 ± 0.133[1]
	65 +	0.954 ± 0.09[1]

*Oral contraceptive use less than five years
**Oral contraceptive use greater than five years

[1]Laitinen et al., 1991
[2]Mazess and Barden, 1991
[3]Myerson et al., 1992
[4]Rutherford, 1993
[5]Volpe et al., 1993
[6]Zhang et al., 1992

(Aloia 1989; White and Hergenroeder 1990). During the life cycle, White and Hergenroeder (1990) suggested that females will have a net bone mineral loss of 35% cortical bone and 50% trabecular bone. This decline in bone mass, as well as lower total bone mass, place females at a higher risk of developing osteoporosis than males.

Functions of Hormones and Minerals as Relative to Bone Metabolism

Bone remodeling occurs even after bones reach their adult size. This process involves hormones, such as calcitonin, parathyroid hormone, progesterone, estrogen, testosterone, and vitamin D, and minerals, such as calcium, phosphorus, magnesium, and boron (Tortora and Anagnostakos 1990). Reported hormone and mineral concentrations in human samples may be found in Tables 2 and 3, respectively. Figure 1 illustrates estrogen and progesterone patterns associated with menstrual cycle phases. These hormone and mineral concentrations are of importance, for they indicate bodily functions, which are discussed as follows.

Calcitonin and parathyroid hormone are the two hormones directly involved in regulating plasma calcium, thus are directly responsible for bone metabolism. Calcitonin, which is synthesized by the parafollicular cells of the thyroid gland, has been associated with reducing the level of plasma

TABLE 2
REPORTED HORMONE CONCENTRATIONS IN HUMAN SERUM

Hormone	Human Serum Concentration
Estradiol (pg/ml)	120[9] 10-375[3] 11-460[5] 41-300[8] 50-700 or less[7]
Progesterone (ng/ml)	0.1-28[3] 0.1-0.3[3*] 1-11[7]
Testosterone (Female Values) (ng/ml)	0.6-8.6[3] 0.25-4[7]
Parathyroid Hormone (ng/ml)	0.01-0.055[5] 0.01-0.065[2,4] 0.04-0.2[8] 0-0.5[6] 0-2.7[3]
Calcitonin (ng/ml)	0.003-0.019[1] 0.005-0.1[8] 0-0.12[5] 0-0.5[3] 0.01-0.58[6]
Vitamin D (Plasma 1,25-dihydroxy vitamin D_3) (pmol/L)	38.4-156.0[2]

*Reported value for oral contraceptive users

[1]Body and Heath, 1984
[2]Dawson-Hughes et al., 1991
[3]Diagnostic Products, 1991-93
[4]Felsenfeld et al., 1991
[5]Grimston, 1993
[6]Heynen and Franchimont, 1974
[7]Kutsky, 1981
[8]Scharla, 1990
[9]Tepperman and Tepperman, 1987

TABLE 3
REPORTED MINERAL CONCENTRATIONS IN HUMAN SAMPLES

Mineral	Human Plasma Concentration (mg/dl)	Human Urine Concentration (mg/dl)
Calcium	8.5-10.2[7] 8.5-10.5[6] 8.5-10.6[5] 8.8-10.4[9] 9.0-11.0[10]	3.3-26.4[9] 23 mg/day[6]
Magnesium	1.0-3.0[9] 1.7-2.8[11]	1.96-11.2[9] 100 mg/day[6]
Phosphorus (Inorganic)	2.5-3.1[2] 2.5-4.5[4] 2.5-4.8[12] 3.1-4.9[9]	56[9] 340-1000 mg/day[12] 840 mg/day[6]
Boron	0.0183μg/ml[3] 0.02 μg/ml[5]	0.1-0.2 μg/ml[8] 0.75 μg/ml[1]

[1]Abou-Shakra et al., 1989
[2]Arnaud and Sanchez, 1990
[3]Clarke et al., 1987
[4]Combs, 1992
[5]Ferrando et al., 1993
[6]Free and Free, 1975
[7]Grimston, 1993
[8]Hunt, 1991
[9]Kutsky, 1981
[10]Marks and Popoff, 1988
[11]Perkin-Elmer, 1985
[12]Sigma Diagnostics, 1985

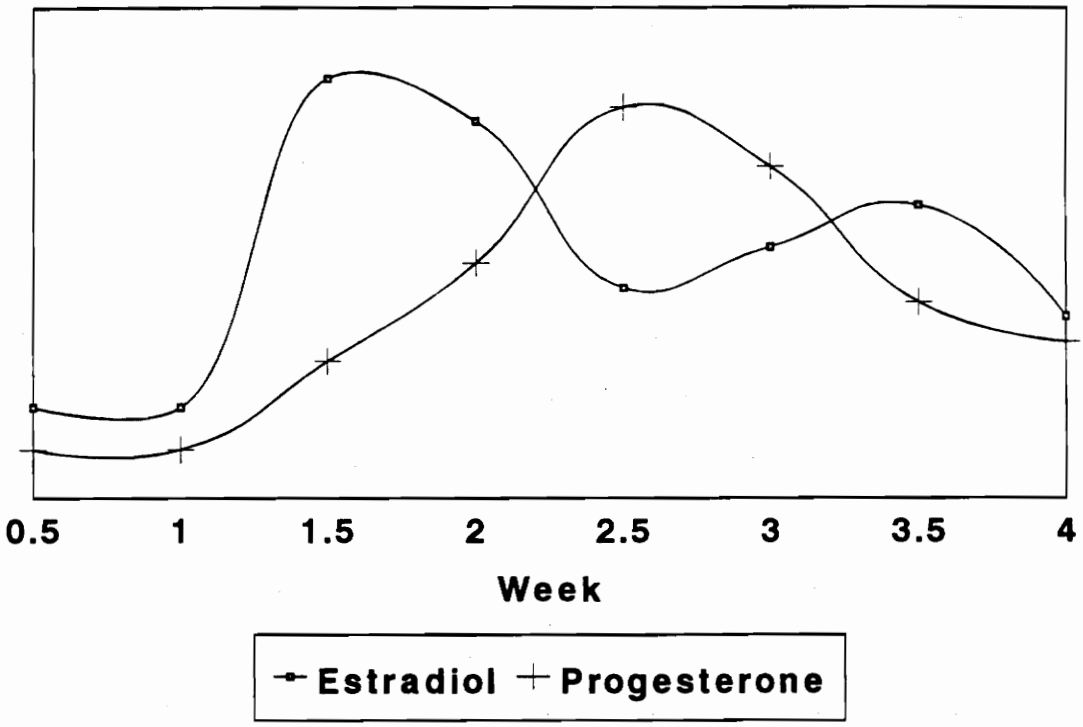


Figure 1 - Estrogen and Progesterone Patterns Associated with the Menstrual Cycle
***EST = Estradiol, PROG = Progesterone**

calcium through inhibition of osteoclastic activity, which then leads to an accelerated absorption of calcium by the bones, or an inhibition of calcium loss from the bones (Werner 1984; Villee et al. 1985; Tortora and Anagnostakos 1990; Raven and Johnson 1991). Parathyroid hormone is synthesized by the parathyroid gland, and functions in eliciting increases in the number and activity of osteoclasts and bone resorption (Tortora and Anagnostakos 1990; Peacock et al. 1984). This increase in osteoclasts causes an increase in blood calcium and phosphorus (Werner 1984; Villee et al. 1985; Tortora and Anagnostakos 1990; Raven and Johnson 1991).

The sex hormones, estrogen and testosterone primarily function in growth and development of reproductive organs, but have also been found to participate in promoting new bone growth. These hormones aid in osteoblastic activity, while simultaneously causing degeneration of the cartilage cells (Davies and Littlewood 1979; Tortora and Anagnostakos 1990; Raven and Johnson 1991). This process is the mechanism responsible for adolescents growth spurts (Tortora and Anagnostakos 1990). Progesterone, the other sex hormone is an important intermediate in steroid biosynthesis (Werner 1983). Along with estrogen, progesterone functions to develop and maintain female sexual characteristics, and with the gonadotropic hormones these female sex hormones regulate

the menstrual cycle, maintain pregnancy, prepare the mammary glands for lactation, and regulate oogenesis (Tortora and Anagnostakos 1990).

In addition to the previously mentioned hormones, vitamin D, which may be considered a hormone, due to its hormone-like characteristics, also plays a role in bone metabolism (Combs 1992). Vitamin D not only functions in bone formation, but also in the mobilization of bone mineral via interactions involving 1,25-dihydroxyvitamin D_3 (Combs 1992). According to DeLuca (1992), 1,25-dihydroxyvitamin D_3 has been found to "not only stimulate the intestine to absorb calcium and phosphorus, the bones to mobilize calcium and phosphorus, and the kidney to cause increased renal absorption of calcium, but also directly suppress the parathyroid hormone,... a developmental hormone necessary for the recruitment of cells for osteoclast formation, for female reproduction, for the development of skin, and for its treatment of certain malignant conditions." The recommended dietary allowance (RDA) for vitamin D for individuals aged 19-24 is 10 μg/day and for individuals aged 25 and older it is 5 μg/day (National Research Council 1989). Although there is an established RDA for vitamin D, the diet is not the only source of this vitamin. Animals and humans are capable of endogenously synthesizing vitamin D. Vitamin D may be formed by a reaction involving

ultraviolet light (at a wavelength of 285-315 nm) and 7-dehydrocholesterol in the skin. The efficiency of this reaction varies with individuals, time of day, season, and latitude (Combs 1992). According to Combs (1992), 7-dehydrocholesterol, the pro-vitamin sterol, is "both a precursor to and product of cholesterol (via different pathways); it is synthesized in the sebaceous glands of the skin and is secreted rather uniformly on to the surface where it is re-absorbed into the various layers of the epidermis".

Calcium, phosphorus, and magnesium are all components of bone, although each has its own function. Calcium physiologically functions as a component of bones and teeth, as well as participating in regulation of intracellular and hormonal secretions, muscle contractions, and blood clotting (Hunt and Groff 1990; Avioli 1988). Approximately 99% of the body's calcium is in the bones and teeth, with the remaining 1% participating in the above mentioned functions (National Research Council 1989; Hunt and Groff 1990). Plasma and urine calcium levels are tightly regulated via calcitonin, parathyroid hormone, and vitamin D, which affect bone metabolism, renal absorption, and intestinal absorption (Boden and Kaplan 1990). However, dietary factors may interfere with plasma and urine calcium levels. According to Boden and Kaplan (1990), dietary deficiencies of calcium,

phosphorus, magnesium, or vitamin D may alter the normal plasma calcium balance. Einhorn and colleagues (1990) suggested that diets high in protein are associated with increased calcium excretion. The RDA for calcium has been established for males and females, aged 25 and older, at 800 mg/day, while those 24 and younger, require additional calcium, 1200 mg/day, since during periods of growth, maximal bone mass has probably not been attained, and thus rapid bone mineralization occurs (National Research Council 1989; Hunt and Groff 1990).

In addition to a role as a component of bones and teeth, phosphorus also functions as a part of cell membranes, phospholipids, nucleic acids, coenzymes, phosphate energy transfer systems, and participates in regulation of pH and osmotic pressure of intracellular fluids (National Research Council 1989; Hunt and Groff 1990). Like calcium, the majority of phosphorus is contained in the skeleton. Eighty-five percent of the body's total phosphorus is in the skeleton, while the remaining 15% participates in the above mentioned functions (National Research Council 1989; Hunt and Groff 1990). Plasma and urine phosphorus levels are indicative of dietary intake of phosphorus. High dietary intake of phosphorus will increase plasma and urine levels of the mineral, and vice versa for low dietary intake of phosphorus. In addition, levels of

plasma and urine phosphorus vary with the time of day. Afternoon and evening levels may be double those of the morning, even during a fast (Arnaud and Sanchez 1990). The precise amount of phosphorus required by the body is unknown, although it is suggested that phosphorus be consumed at a ratio of 1:1 with calcium, so that individuals 19-24 years require 1200 mg and those 25 years and older need 800 mg/day (National Research Council 1989). Alterations of this calcium:phosphorus ratio, with phosphorus being greater, may lead to an increased risk of bone fractures (Jowsey 1976). This is of concern, for many young females may consume diets high in phosphorus, due to the high phosphorus content in soft drinks, and thus phosphorus may interfere with their calcium absorption (Jowsey 1976; Wyshak et al. 1989; Calvo et al. 1990).

Magnesium participates in nerve impulse transmission, protein synthesis, in enzyme activation of glycolysis, and as a component of bone (Hunt and Groff 1990; Shils 1988). Approximately 60% of the body's magnesium is found in bone, with the majority of the remaining 40% found intracellularly (National Research Council 1989; Hunt and Groff 1990). Dietary calcium and phosphorus may affect magnesium metabolism. Kim and Schuette (1991) suggested that high dietary intakes of calcium and phosphorus decrease magnesium absorption and excretion. Magnesium may play a role in

osteoporosis therapy. Abraham and Grewal (1990) reported that magnesium supplements were associated with increased bone mineral density in postmenopausal women, and thus may be beneficial as a treatment for osteoporosis. The RDA for magnesium for males aged 19 and older is 350 mg/day while females in the same age group require 280 mg/day (National Research Council 1989).

Although there is no established function for boron, it has been suggested that boron is essential in bone mineralization. Data supporting this statement will be further discussed in later sections of this thesis.

Osteoporosis

As previously stated, the adult skeleton is primarily composed of cortical bone, although trabecular bone is metabolically more active (Weinerman and Bockman 1990). For this reason, it seems that trabecular bone is most rapidly lost immediately after the menopause occurs (Weinerman and Bockman 1990). Marcus and colleagues (1983) found that trabecular bone volume is lost from the iliac crest throughout adult life. They suggested that factors in adolescence may impact skeletal mass.

As aging occurs there is a progressive loss of BMD. This loss leads to decreased bone strength, and thus an increased risk of bone fractures and metabolic bone

disorders, such as osteoporosis (National Institute of Health 1984; National Research Council 1989; Hunt and Groff 1990). Osteoporosis is a major health concern in the United States, with an estimated 20 to 25 million people over the age of 45 affected (Avioli 1991). Osteoporosis may be defined, according to Jowsey (1976) as "a disorder in which not enough bone is present to maintain skeletal strength, with the result that fractures occur with minimal stress". It appears that more women are affected by osteoporosis than men, with the highest prevalence found among postmenopausal women (Avioli 1984; Spencer and Kramer 1985). Since females have lower BMD than males, and experience hormonal changes during the menopause, they may be more susceptible to metabolic bone disorders than males (National Institute of Health 1984).

According to Aloia (1989), there are two types of osteoporosis which may affect elderly women: Postmenopausal (Type I) and Senile (Type II). Table 4 illustrates the factors which characterize both of these types of osteoporosis. Postmenopausal osteoporosis primarily occurs in women 10-20 years after the menopause and is characterized by increased bone resorption which affects trabecular bone, while senile osteoporosis usually occurs after 70 years of age and is characterized by a gradual loss of both trabecular and cortical bone due to aging (Aloia

TABLE 4
FACTORS INFLUENCING POSTMENOPAUSAL AND SENILE OSTEOPOROSIS*

Influential Factors	Postmenopausal Osteoporosis	Senile Osteoporosis
Age of Onset	55-75 years	> 70 years
Sex (Female:Male)	6:1	2:1
Main Causes	Menopausal-Related Factors	Age-Related Factors
Hormonal Cause	Estrogen Deficiency	Calcitriol Deficiency
Types of Bone Loss	Mainly Trabecular	Trabecular and Cortical
Fracture Types	Wrist/Vertebrae	Hip/Vertebrae
Importance of Dietary Calcium	Moderate	High
Calcium Absorption	Decreased	Decreased
Increased Parathyroid Hormone	No	Yes

*Adapted from Aloia (1989) and Riggs and Melton (1986)

1989; Chestnut 1991; Chapuy et al. 1992; Tolstoi et al. 1992; Wardlaw 1993). Tolstoi (1992) suggested that postmenopausal women may lose trabecular bone at three times the rate of normal bone loss, while also losing cortical bone at a slower rate. Risk factors affecting the development of osteoporosis may be classified into two categories: modifiable and non-modifiable. Table 5 depicts several risk factors for osteoporosis, as suggested by Aloia (1989), National Institute of Health (1984), and Hegsted (1986).

Dietary factors have also been associated with the development of osteoporosis. Excess dietary phosphorus, caffeine, or alcohol, and very low calorie diets have been associated with bone loss. In addition, increased urinary calcium losses, which are associated with bone loss, have been reported in subjects consuming excess protein or very low calorie diets (Aloia 1989). As previously stated, a person can continue to build skeletal mass from ages 18-35 (Heaney 1986; National Research Council 1989; Anderson 1990). Mundy (1994) stated that individuals are born with genes which determine their bone mineral density. However, it is this BMD achieved during the early adult years which may be closely associated with the risk of developing osteoporotic fractures (Morrison et al. 1994). A vitamin D gene which codes for vitamin D's receptor (VDR) has been

TABLE 5
MODIFIABLE AND NON-MODIFIABLE RISK FACTORS ASSOCIATED WITH THE DEVELOPMENT OF OSTEOPOROSIS

Modifiable Risk Factors	Non-Modifiable Risk Factors
•Estrogen deficiency	•Caucasian or Asian female
•Inactivity	•Family history of osteoporosis
•Low calcium diet	•Having light skin
•Cigarette smoking	•Having a small frame
•Excess consumption of protein, caffeine, alcohol, or phosphorus	•Experiencing previous bone loss due to immobilization, hyperparathyroidism, thyrotoxicosis, liver disease, malabsorption, rheumatoid arthritis, chronic illness, or glucocorticoids and other drugs
•Deficiency or altered metabolism of vitamin D	•Having scoliosis
•Low weight relative to height	•Suffering previous osteoporotic fractures

associated with fragile bones (Pennisi 1994).

Morrison and colleagues (1992) suggested that bone turnover may be predicted by allelic variation in the VDR. Two hundred and eighty-eight subjects participated in this investigation. Serum osteocalcin, a bone protein, was measured by radioimmunoassay procedures, while a 2.1-kilobase-pair fragment of the VDR cDNA was used to identify the restriction fragment length polymorphisms (RFLP). Results showed that of the 288 subjects, 91 had RFLP in the VDR gene which predicted the circulating osteocalcin levels. The investigators concluded that since the VDR gene and the osteocalcin gene are encoded on different chromosomes, common alleles of the VDR (the trans-acting factor), are functionally different and play a part in normal physiological variability of osteocalcin levels.

Based upon the results of Morrison and co-workers (1992), Morrison and associates (1994) examined the effect of VDR alleles on the bone density of human twins. Since monozygotic (MZ) twins share 100% of their genes and dizygotic (DZ) twins share 50% of their genes, the investigators suggested that if a gene contributes to an observed genetic effect, the DZ twins who both have the alleles for the gene will be more similar to each other than twins who do not share alleles for the gene, and thus will

be more comparable to MZ twins (Morrison et al. 1994).

Two hundred and fifty healthy caucasian Australian twins, 70 MZ and 55 DZ twin pairs, participated in the investigation by Morrison and co-workers (1994). Monozygotic subjects were aged 45 ± 13 years, and included seven male twin pairs, while the DZ participants were aged 44 ± 11 years, and included six male twin pairs. Dual photon or dual energy x-ray absorptiometry was used to measure the BMD of the lumbar spine and proximal femur. When comparing sets of DZ twins, they found that the BMD measurements of the lumbar spine and proximal femur were significantly less in twins with common VDR alleles as compared to twins with discordant alleles. However, when comparing the bone densities of DZ twins with common VDR alleles and MZ twins, there was no significant difference noted at the lumbar spine or proximal femur. Dizygotic twins with discordant VDR alleles had higher BMD measurements when compared to the MZ twins. In 21 of the 22 DZ twin pairs discordant for the VDR alleles, the b allele was correlated with higher bone mineral densities. When comparing all sets of twins, those with the bb genotype had higher BMD measurements, with a co-dominant effect. The authors concluded that the b allele on the VDR is associated with the genetic effect of BMD.

Hormone and Mineral Involvement with Bone Mineral Density and Osteoporosis

Estrogen and Progesterone

Although osteoporosis has been found to be responsible for much morbidity and death, Notelovitz (1993) suggested that it is preventable. The female hormones estrogen and progesterone were found to offer some resistance against osteoporosis. Clark and Schuttinga (1992) found that the use of estrogen/progesterone replacement therapy may play a role in reducing health care costs by reducing the treatment costs and lost productivity which are directly related to osteoporotic fractures. Early onset of the menopause has been implicated as a risk factor of osteoporosis, as these hormonal levels decline during menopause (Hunt and Groff 1990). According to Lloyd and colleagues (1989) estrogen status plays a lifetime role in the maintenance and status of BMD in women. Estrogen positively influenced bone mineralization, for it not only protects against bone loss, but also increases bone mass. However, once the estrogen level is naturally reduced during the menopause, bone degradation more rapidly occurs (Heaney 1986; Chernoff and Lipschitz 1988; Hunt and Groff 1990). For this reason, estrogen therapy has been suggested as a method of protection against osteoporosis (Avioli 1984; Hunt and Groff 1990; Weinerman and Bockman 1990; Gallagher 1988).

Progesterone therapy may accompany the estrogen treatments, for progesterone guards against the danger that elevated levels of estrogen may result in excess stimulation of the uterine lining, the endometrium, which, if not treated with progesterone will remain stimulated and increase the risk of endometrial cancer (Fardon 1985). In addition to the protective effect with estrogen, progesterone may also aid in the prevention of osteoporosis. According to Fardon (1985), bone receptors which normally bind glucocorticoids, a cortisone-like molecule which may actually aggravate osteoporosis, may be replaced by progesterone molecules, thus decreasing the aggravation of osteoporosis.

Felson and colleagues (1993) investigated the effect of prior estrogen therapy on BMD in 670 postmenopausal women, aged 68-96 years. Bone mineral density of the femur and lumbar spine (L2-L4) was measured by dual photon absorptiometry, and radius was measured by single photon absorptiometry. Women who had taken estrogen for seven or more years had significantly higher BMD than women who had not taken estrogen. The investigators concluded that BMD may be preserved when women take estrogen for at least seven years after the menopause.

Ng and associates (1993) studied the effect of one year of estrogen therapy on postmenopausal bone loss. Sixty-one

postmenopausal women were randomly assigned to one of three groups, receiving: 1.25 g of estradiol, 2.5 g of estradiol, or placebo. Bone mineral density of the vertebrae (T12-L3) was measured by quantitative computed tomography, while radioimmunoassay procedures were used to measure estrogen and testosterone. Women taking estrogen did not lose significant amount of BMD, while loss of BMD in the control group was not significant except for the four subjects with surgical menopause. The authors concluded that estrogen therapy may be used as a method of prevention against postmenopausal bone loss.

Civitelli and colleagues (1988a) investigated the effect of estrogen therapy on bone mass via a double-blind, placebo controlled study. Twenty-one postmenopausal osteoporotic women participated in this study. Estrogen, in the form of Premarin, was given at a level of 1.25 mg/day, while bone mineral content of the lumbar spine and femoral shaft was measured by dual photon absorptiometry. Serum levels of calcitonin and parathyroid hormone were measured by radioimmunoassay procedures. Results showed that bone mineral content of both the lumbar spine and femoral shaft increased, serum calcitonin increased, and no change was noted in serum parathyroid hormone in subjects taking estrogen. The investigators concluded that one year treatment of estrogens may improve the bone mineral content

of postmenopausal osteoporotic women.

Weiss and associates (1980) interviewed 327 women, aged 50-74 years at the time of hip or lower forearm fracture, to determine use of estrogen therapy prior to the bone fracture. Their responses were compared to those of 567 randomly selected women of the same age range to determine whether estrogen therapy had decreased the risk of fracture. The risk of fracture was 50-60 percent lower in the women who had used estrogen preparations for six years or more compared to the women who had used these hormones for a shorter time period, or not at all. The authors concluded that the use of estrogens may not only reduce the risk of bone fractures in the hip and forearm, but may also play a role in slowing the development of osteoporosis in postmenopausal women.

Lufkin and co-workers (1992) investigated the effect estrogen and progesterone plays as a treatment of postmenopausal osteoporosis. Seventy-eight postmenopausal women, aged 47-75 years, with at least one vertebral fracture due to osteoporosis participated in this one year study. Thirty-nine subjects received 0.1 mg of 17-ß-estradiol for days 1-21 and medroxyprogesterone acetate for days 11-21 of a 28 day cycle. The remaining subjects received placebo. Bone mineral density of the lumbar spine, femoral trochanter, femoral neck, and midradius were

measured. Subjects taking estrogen and progesterone had increased BMD of the lumbar spine, femoral trochanter, and midradius. Women in the treatment group also sustained less fractures than did subjects taking placebo. The investigators concluded that estradiol and progesterone treatment was beneficial for postmenopausal women with established osteoporosis.

Murphy and colleagues (1992) studied the relationship between sex hormones and BMD. Ninety postmenopausal women participated. Bone mineral density of the total body, spine, and hip was measured by dual energy x-ray absorptiometry, while radioimmunoassay procedures were utilized to measure serum estradiol and testosterone. A positive relationship was found between the endogenous free estradiol and the bone mineral densities of the total body, spine, and hip. The authors concluded that endogenous free estradiol may positively influence BMD of postmenopausal women.

Although estrogen and progesterone have been found to have beneficial effects with resisting the development of osteoporosis, some negative side effects have been indicated. According to Wardlaw (1993) and Fardon (1985), the use of estrogen therapy has been associated with increased risk of breast cancer, uterine cancer, endometrial cancer, stroke, heart attack, and blood clots, although

Martin and Freeman (1993) suggested that concurrent administration of progestins may possibly counteract the increased risk of endometrial cancer. In addition to the previously mentioned risks, another negative side effect of estrogen therapy is the periodic bleeding which may occur, although hormone replacement may be utilized to alleviate this inconvenience (Wardlaw 1993). Negative side effects of progesterone may include an affect on blood clotting, thrombophlebitis, fluid retention, hormone-dependent tumors, and induced feelings of depression (Fardon 1985).

As supported by the above mentioned investigations, estrogen and progesterone therapies have proven beneficial for reducing the risk of postmenopausal osteoporosis. However, along with this benefit are some negative side effects. When opting for a method to resist the development of osteoporosis, both the positive and negative aspects must be considered. Many times, it appears that the positive effects outweigh the negative ones.

Calcitonin and Parathyroid Hormone

Estrogen and progesterone are not the only hormones which may prove beneficial for slowing the development of osteoporosis. Calcitonin also has been implicated as a treatment when estrogens are not well tolerated or contraindicated (Wardlaw 1993). As a synthetic drug, salmon

calcitonin has been suggested as an alternative to estrogen rather than human calcitonin due to its greater potency, longer acting duration, and analgesic effects, such as reducing skeletal pain associated with osteoporosis (Wardlaw 1993). Parathyroid hormone also has been implicated as a method of treating osteoporosis. Treatment with daily injections of 1-34 human parathyroid hormone has been shown to increase axial cancellous bone mass, partially at the expense of peripheral cortical bone (Reeve et al. 1993).

Civitelli and co-workers (1988b) investigated the effect of calcitonin treatment on postmenopausal osteoporosis. Fifty-three postmenopausal osteoporotic women and 24 controls (6 premenopausal and 18 postmenopausal) were examined before and after treatment with synthetic salmon calcitonin. Participants had been given a dose of 50 IU every other day for one year. Bone mineral content of the lumbar spine (L2-L4) and femoral diaphysis were measured by dual photon absorptiometry. Treatment with salmon calcitonin significantly increased the vertebral bone mineral content of all subjects. The investigators concluded that calcitonin therapy is beneficial for patients with high-turnover osteoporosis, for bone loss may be slowed in the appendicular bones, while a gain in bone mineral mass in the axial skeleton may result.

MacIntyre and colleagues (1988) investigated the effect

of calcitonin on postmenopausal bone loss. Seventy postmenopausal women participated in a two year study. Subjects were randomly assigned to one of four groups: placebo, calcitonin (20 IU of synthetic human calcitonin injected three times a week), estradiol/progesterone (3 mg of 17-ß estradiol and 300 mg progesterone 12 days a month), or estradiol/progesterone and calcitonin (3 mg of 17-ß estradiol and 300 mg progesterone 12 days a month and 20 IU of synthetic human calcitonin injection three times a week). Vertebral BMD was measured by quantitative computed tomography. The amount of bone loss from the calcitonin group was equal to that lost by those in the estrogen group. The investigators concluded that calcitonin may be as effective as estrogen for reducing the incidence of bone loss in postmenopausal women.

Overgaard and associates (1992) investigated the effect of salmon calcitonin suppositories on calcium metabolism of postmenopausal osteoporotic women. Twenty-five subjects, aged 68-72 years, completed this two year investigation. During the first year of the study no treatment was implemented. The second year, all subjects were given 100 IU of salmon calcitonin rectally and 500 mg of calcium supplements. Bone mineral density of the lumbar spine (L2-L4) was measured by dual energy x-ray absorptiometry and bone mineral content of the arm was measured by single

photon absorptiometry. The BMD of the lumbar spine significantly increased and it was concluded that salmon calcitonin positively affects calcium metabolism of osteoporotic women.

Hesch and associates (1989) studied the effect of calcitonin in combination with 1-38 human parathyroid hormone (1-38hPTH) on vertebral density. Eight subjects (six males and two females) who had exhibited clinical and histological signs of osteoporosis participated in this 14 month investigation. Trabecular BMD was measured by quantitative computed tomography, while subjects were given 1-38hPTH and calcitonin (nasally) in a 105 day cycle. Participants were given 720-750 U of 1-38hPTH/day for 70 days straight. Every 14 days the 1-38hPTH treatment was complimented with 200 U calcitonin/day. After taking 1-38hPTH for 70 days, 14 more days of the calcitonin followed. After completion of 84 days, a three week wash-out period followed, which accounted for the 105 day cycle. Trabecular BMD of the vertebral spine increased in all subjects. The authors concluded that this combination therapy of 1-38hPTH and calcitonin leads to remineralization of the osteoporotic vertebrae.

Reeve and colleagues (1993) studied the effect of 1-34hPTH, in combination with estrogen, on peripheral bone resorption. They reported that spinal and cancellous bone

increased by 40-50% above initial values while no evident changes occurred with the radial cortical or cancellous bone. The authors concluded that 1-34hPTH and estrogen together may play a significant role in treating osteoporotic patients, who have already lost much spinal cancellous bone.

In contrast to the above mentioned positive effects of calcitonin and parathyroid hormone on bone mineralization in the osteoporotic individual, Kollerup and associates (1994) suggested otherwise. Fifty-four postmenopausal women, aged 55-84, with a history of hip fracture were randomly assigned to receive 100 IU of salmon calcitonin suppository six times/week, 200 IU of salmon calcitonin suppository three times/week, or placebo six times/week for a one year period. In addition, each group received 500 mg of calcium daily in the form of Calcium Sandoz effervescent tablets. Dual energy x-ray absorptiometry was used to measure the BMD of the lumbar spine (L2-L4) and femoral neck. There was no significant change in BMD of either treatment group. The authors suggested that treatment of postmenopausal osteoporosis with salmon calcitonin at the levels given was not beneficial.

Hurley and co-workers (1987) investigated the effect of calcitonin excess or deficiency on BMD. Twenty-one subjects who had undergone subtotal thyroidectomy at least 6.8 years

prior to the study, 11 patients with medullary thyroid carcinoma, and 187 controls participated. Dual photon absorptiometry was used to measure the BMD of the lumbar spine (L2-L4) while single photon absorptiometry was used to measure the radius. Plasma calcitonin was measured by radioimmunoassay procedures. There was no significant correlation between BMD and the duration of calcitonin excess or deficiency. It was concluded that bone mass is not affected by plasma calcitonin levels in adults.

When correlating calcitonin and parathyroid hormone therapies with a reduced risk of osteoporosis, conflicting results have been noted. The majority of researchers have found that calcitonin and parathyroid hormone treatments yield beneficial results with respect to bone mass, with no major side effects. However, there are those who have found no significant relationship between these hormones and increased bone mass.

Calcium and Vitamin D

Calcium plays a role in the prevention of osteoporosis. Calcium is primarily absorbed in the duodenum and proximal jejunum of the small intestine, and is generally completely absorbed within four hours of intake. Thirty to fifty percent of ingested calcium may be absorbed by adults, with the percent of absorption decreasing in the elderly

(Notelovitz 1993). Since vitamin D plays a significant role in calcium and bone metabolism, it has been associated with the prevention of osteoporosis.

Chapuy and associates (1992) studied the effect of vitamin D and calcium supplementation on the frequency of hip fractures and other non-vertebral fractures. Three thousand and seventy healthy women, aged 69-106, participated for 18 months. One thousand and thirty-four subjects received 20 μg (800 IU) of vitamin D_3 and 1.2 g of elemental calcium via tricalcium phosphate supplements. The remaining 1636 women received a double placebo. Serum parathyroid hormone was measured by immunochemoluminometric assay procedures while the BMD of the proximal femur was measured by dual-energy x-ray absorptiometry. Subjects in the treatment groups sustained 43% less hip fractures and 32% less non-vertebral fractures than the controls. The serum parathyroid hormone level of those in the treatment groups had decreased by 44% from the baseline measurement. The investigators concluded that supplementation with vitamin D and calcium reduces the risk of hip and non-vertebral fractures in elderly women.

Reid and colleagues (1993) studied the effect of calcium supplementation on bone loss in postmenopausal women. One hundred and twenty-two postmenopausal women, with a mean dietary calcium intake of 750 mg per day, were

randomly assigned to receive 1000 mg of a calcium supplement per day or placebo for two years. Dual-energy x-ray absorptiometry was used to measure the BMD of the lumbar spine (L2-L4), whole body, and proximal femur. The subjects in the calcium group had a 43% reduction in their loss of BMD as compared with the placebo group. The investigators concluded that calcium supplementation significantly slowed bone loss in normal, postmenopausal women.

The effects of calcium supplementation and exercise on bone density in elderly women was investigated by Lau and colleagues (1992). Fifty subjects, aged 62-92 years, were randomly assigned to one of four treatment groups: group 1 (daily 800 mg calcium supplementation [as calcium lactate gluconate]), group 2 (load-bearing exercise four times per week plus daily placebo), group 3 (daily calcium supplementation plus load-bearing exercise four times per week), and group 4 (daily placebo). The bone mineral density of the spine (L2-L4) and hip (femoral neck, Ward's triangle, and intertrochanteric area) was measured by dual x-ray densitometry. The BMD of the intertrochanteric area significantly increased in those subjects taking calcium supplements, while exercise had no effect of bone loss at any site. The authors concluded that calcium supplementation may prove beneficial for reducing bone loss in the hip.

Dawson-Hughes and associates (1987) assessed the effect of dietary calcium intake on BMD in postmenopausal women. Seventy-six healthy postmenopausal women, aged 40-70 years, participated in this investigation. Bone mineral density of the lower spine was measured by dual photon absorptiometry. Women with a calcium intake of 405 mg/day or less had lost BMD at a significantly greater rate than those subjects consuming 777 mg of calcium per day. The researchers concluded that there appears to be a "threshold of calcium intake below which increased calcium in the diet is likely to be beneficial in reducing spine mineral loss."

Polly and colleagues (1987) studied the effect of calcium supplementation on the BMD of the forearm. Two hundred and sixty-two postmenopausal women, with a mean age of 57 years, participated in a nine month investigation. Each subject had a daily calcium intake of less than 1000 mg. One hundred and thirty-six subjects were in the treatment group, who received 1000 mg of calcium supplements in the form of calcium gluconate, lactate, and carbonate, while the remaining 126 subjects were controls. Single photon absorptiometry was used to measure the BMD of the forearm. There was a significant reduction in the rate of bone loss in the treatment group. The investigators concluded that calcium supplementation reduces bone loss in the forearm of postmenopausal women.

Recker and Heaney (1985) studied the effects of milk supplementation on calcium balance, calcium metabolism, and bone metabolism. Twenty-two postmenopausal women, aged 45-70 years, participated in a two year investigation. Thirteen subjects were randomly assigned to receive milk and dairy supplementation in an amount equivalent to 24 ounces of lowfat milk per day, which was in addition to their normal calcium intake. The remaining nine participants were placed into the control group. There was a significant increase in absorbed calcium and a significant decrease in bone resorption in the treatment group. The authors concluded that milk and dairy products provide an appropriate level of calcium to sustain bone metabolism in postmenopausal women.

Pouilles and co-workers (1992) investigated the effect of vitamin D on bone loss prevention in postmenopausal women. Of thirty-six postmenopausal subjects, 25 completed two years of treatment. Subjects were given 1 μg of 1-α-hydroxyvitamin D_3 and 500 mg of calcium daily. Vertebral BMD was measured by dual photon absorptiometry. The BMD did not vary in the treated group, but decreased significantly in the control group. The authors concluded that supplementation with 1-α-hydroxyvitamin D_3 may be beneficial for preventing postmenopausal bone loss.

Lidor and colleagues (1993) found that women with

subcapital fracture of the femur may have decreased bone levels of 1,25-dihydroxyvitamin D_3. Subjects included 19 women, with a mean age of 78 ± 2 years, with subcapital fracture of the femur, which is a bone failure caused by osteoporosis. Serum and bone 1,25-dihydroxyvitamin D_3 was measured indirectly in the subjects, while bone levels were directly measured in female cadavers. Serum 1,25-dihydroxyvitamin D_3 was found to be within normal levels, while bone levels were greatly reduced compared to the levels obtained in the cadavers and reported levels of non-osteoporotic elderly women. The investigators concluded that 1,25-dihydroxyvitamin D_3 may be used to treat elderly osteoporotic women to reduce the incidence of subcapital fracture of the femur.

Palmieri and colleagues (1989) investigated the role of vitamin D and calcitonin in the prevention of osteoporosis. Thirteen postmenopausal women, aged 54-73, who had radiologic osteopenia participated in this study. All subjects received 50,000 IU of vitamin D, 40 units of salmon calcitonin, and 1 g of calcium carbonate three days a week for two to six years. Bone mineral density of the radius and iliac crest was measured by single photon absorptiometry. There was a significant increase in BMD of the iliac crest but not of the radius. The authors concluded that vitamin D in combination with calcitonin may

prove beneficial in the treatment of postmenopausal osteoporosis.

Villareal and co-workers (1991) investigated the effect of vitamin D deficiency on low vertebral bone mass. Ninety-eight postmenopausal women, with a mean age of 64 years, participated in this investigation. Forty-nine women with low serum levels of 25-hydroxyvitamin D_3 were placed into one group, while 49 other women with normal serum levels of 25-hydroxyvitamin D_3 were placed into the control group. Vertebral bone mass was measured by quantitative computed tomography. Women with low levels of 25-hydroxyvitamin D_3 had significantly reduced bone mass as compared to the controls. The researchers concluded that low serum 25-hydroxyvitamin D_3 is associated with decreased vertebral bone mineral density.

Khaw and associates (1992) examined the relationship between BMD and 25-hydroxyvitamin D and parathyroid hormone, which are indices of calcium metabolism. One hundred and thirty-eight women, aged 45-64, participated in this investigation. Dual energy x-ray absorptiometry was used to measure the BMD of the lumbar spine and femur neck. Immunoradiometric assay procedures were used to measure parathyroid hormone. A positive relationship was found between serum 25-hydroxyvitamin D and the BMD of the lumbar spine and femur, and an inverse relationship existed between

serum parathyroid hormone and the BMD of the lumbar spine and femur neck. The investigators concluded that vitamin D plays a role in increasing BMD of middle aged women, and may possibly play a role in the prevention of osteoporosis.

Results of the previously mentioned investigations have generally suggested that calcium and vitamin D may be useful in the prevention of osteoporosis. Although these nutrients may play a role in prevention, they are not the only factors involved in this process.

Exercise Involvement with Bone Mineral Density and Osteoporosis

According to Notelovitz (1993), "Exercise is a known initiator of the bone-remodeling cycle because mechanical loading, muscular activity, and gravity stimulate the bone cells to differentiate and grow." Normal exercise may increase BMD, except when exercise becomes excessive. Overly-intensive exercise may induce exercise-induced amenorrhea, which, in turn, may reduce the BMD. The effects of excessive exercise on BMD of female athletes will be discussed in later sections. However, in this section, exercise will be discussed as it relates to the positive effects on BMD.

Hatori and associates (1993) studied the effects of exercise on the BMD of postmenopausal women. Thirty-five

postmenopausal women, aged 45-67, participated in this seven month investigation. Twelve subjects were placed into the high intensity exercise group (110% of the heart rate at anaerobic threshold), nine were in the moderate intensity exercise group (90% of the heart rate at anaerobic threshold), and the remaining 12 were controls. Prior to the exercise intervention, each subject performed a treadmill exercise test, in which the anaerobic threshold was measured by expired gas analysis. The exercise intervention consisted of stretching and walking for 30 minutes, at the specified heart rate level at anaerobic threshold, three times a week. Bone mineral density of the lumbar spine (L2-L4) was measured by dual energy x-ray absorptiometry. The high intensity exercise group had significantly increased BMD, while the moderate intensity exercise group and controls had a slight decrease in BMD. The authors concluded that short-term high intensity exercise above the anaerobic threshold is a safe and effective method of preventing postmenopausal BMD.

The effect of moderate physical exercise on BMD of perimenopausal women was studied by Zhang and colleagues (1992). Three hundred and fifty-two women, aged 40-54 years, participated in this investigation. Current levels of physical activity were measured by a portable accelerometer which estimated the daily energy expenditure

of the subjects. Dual photon absorptiometry was used to measure the BMD of the lumbar spine (L2-L4), while single photon absorptiometry was used to measure the bone mineral density of the midradius and distal radius. Postmenopausal women had lower bone mineral densities than premenopausal women, and women who exercised had significantly higher bone mineral densities than those who did not exercise. The researchers concluded that moderate levels of physical activity positively affects the BMD of perimenopausal women.

Grove and Londeree (1992) studied the effect of high impact and low impact exercise on BMD of postmenopausal women. Fifteen postmenopausal women participated in a one year investigation. Subjects were randomly assigned to one of three groups: low impact exercise group, high impact exercise group, and non-exercising control group. The exercise intervention consisted of walking on a treadmill for 20 minutes, three days per week. Dual photon absorptiometry was used to measure the BMD of the spine (L2-L4). No difference was found between the intensity of exercise on BMD of the subjects, for both groups maintained their current level of BMD. The investigators concluded that both types of exercise may prove effective for maintaining BMD of early postmenopausal women.

The effect of non-weight bearing exercise on BMD was studied by Bloomfield and co-workers (1993). Fourteen

postmenopausal women participated in an eight month study. Seven subjects exercised regularly at moderate intensities (60-80% of the maximum heart rate) on a bicycle ergometer, while the remaining seven were placed in the sedentary control group. Bone mineral density of the lumbar spine and femoral neck was assessed by dual photon absorptiometry. Subjects in the exercise group significantly increased their BMD of the lumbar spine, and had no change in the BMD of the femoral neck. The authors concluded that non-weight bearing exercise may prove beneficial for reversing bone loss in healthy postmenopausal women.

Caplan and associates (1993) investigated the effects of exercise on BMD of postmenopausal women. Thirty postmenopausal women participated in a two year exercise investigation. Nineteen subjects, with a mean age of 66.4 ± 1.2 years, exercised aerobically twice a week, while the remaining 11 subjects, with a mean age of 65.4 ± 1.5 years, were controls who did not engage in any aerobic exercise program. Bone mineral density of the lumbar spine and hip were measured. Bone mineral densities of the exercise group significantly increased, while there was a decrease in the bone mineral densities of the controls. The authors concluded that aerobic weight bearing exercise has a protective effect on the BMD of postmenopausal women.

Each of these investigations provided evidence that

normal exercise may prove beneficial in protecting the BMD of postmenopausal women, thus may aid in the prevention of osteoporosis. However, as previously discussed, Lau and associates (1992) indicated that moderate weight-bearing exercise was not effective for preventing bone loss.

Oral Contraceptives and Bone Mineral Density

There is controversy as to whether oral contraceptives provide protection against loss of BMD. According to Mishell and associates (1972), persons taking oral contraceptives have lower circulating estrogen levels than their normal cycling counterparts, in the early follicular phase of the menstrual cycle, and higher levels than postmenopausal women. Since a reduction in circulating estrogen levels at menopause has been associated with loss of cortical and trabecular bone density, estrogen replacements have been routinely used as a therapy for postmenopausal osteoporosis (Chernoff and Lipschitz 1988; Lloyd et al. 1989). Since estrogen status appears to play a major role in BMD status of women, estrogen administration in premenopausal females, through oral contraceptives, may positively affect BMD.

According to Kritz-Silverstein and Barrett-Connor (1993), oral contraceptives significantly increased bone mineral density. Two hundred and thirty-nine postmenopausal

women participated in this study. Upon completion of a medical history questionnaire, subjects were categorized into one of three categories based on previous use of oral contraceptives: never, 1-5 years, or six or more years. Dual x-ray absorptiometry was used to measure BMD of the lumbar spine (L1-L4) and femoral neck (hip). Bone densities were correlated with length of previous oral contraceptive use. Women who had used oral contraceptives for six or more years had significantly higher bone mineral densities of femoral neck and lumbar spine than women who had never taken oral contraceptives. The authors concluded that oral contraceptive use may enhance BMD since estrogens may decrease calcium loss and increase calcium absorption. By using oral contraceptives, women may enter menopause with greater bone mass, thus be less susceptible to osteoporosis development.

The effect of oral contraceptives on bone mass was studied by Recker and colleagues (1992). One hundred and fifty-six healthy women, aged 19-26 years, participated in a five year study. Thirty-four subjects reported use of oral contraceptives for the duration of the investigation, while another 43 subjects reported use of oral contraceptives part of the time. Bone mineral density of the forearm was measured by single photon absorptiometry, while dual photon absorptiometry was used to measure the lumbar spine (L2-L4)

and total body. Forearm and spine bone mineral densities were higher for those using oral contraceptives. The researchers concluded that the use of oral contraceptives positively affects bone mass in young adult women.

Kleerekoper and co-workers (1991) reported that oral contraceptives may be protective against low bone mass. These investigators recruited 2297 women to participate in their investigation. Of the total subjects, 1746 were postmenopausal. Medical history questionnaires, which provided information about oral contraceptive use and menstrual cycles, were completed. Dual photon absorptiometry was used to measure the BMD of the lumbar spine, while single photon absorptiometry was used to measure the forearm. Thirty percent of the postmenopausal women had reported a history of oral contraceptive use, while 48% of the premenopausal women reported use of oral contraceptives. A history of oral contraceptive use was protective against low BMD in both the forearm and lumbar spine. It was concluded that the degree of protection against low BMD was related to the length of time the oral contraceptives were used.

In contrast to the positive benefits associated with oral contraceptive use, Lloyd and colleagues (1989) suggested otherwise. Twenty-five premenopausal women, 14 of whom used oral contraceptives for at least 67 months.

Single-energy computerized tomography of the lumbar spine (L1-L3) was used to measure trabecular BMD. There was no significant difference between the bone densities of oral contraceptive users and non-users. The authors concluded that long-term oral contraceptive use among premenopausal women has no effect on vertebral BMD.

Mazess and Barden (1991) reported that oral contraceptives do not affect BMD of premenopausal women. Approximately half of the 300 premenopausal women who participated in this investigation were past or current users of oral contraceptives. Dual-photon absorptiometry was used to measure BMD of the lumbar spine (L2-L4), femur, and humerus, while single-photon absorptiometry was used to measure the radius. The authors concluded that there was no significant relationship between the bone mineral densities of oral contraceptive users and non-users.

Hreshchyshyn and associates (1988) found no relationship between BMD measurements of oral contraceptive users and non-users. Five hundred and eighty-eight subjects participated by completing medical history questionnaires. In addition to oral contraceptive use, parity and breast-feeding were associated with bone density measures. Dual photon absorptiometry was used to measure the BMD of the lumbar spine (L2-L4) and femoral neck. No significant relationship was found between the use of oral

contraceptives and bone mineral densities. The authors concluded that oral contraceptives did not affect BMD.

The use of oral contraceptives has a conflicting effect on BMD. Many agree that oral contraceptives, like estrogen and progesterone therapies, may have a protective effect against bone demineralization for postmenopausal women, although others disagree with the possible preventative effect of oral contraceptives.

Bone Mineral Density and Female Athletes

Generally, aerobic exercise is beneficial for the body. However, for some female athletes the duration and intensity of the exercise may be so great that exercise-induced amenorrhea develops, which then may lead to decreased BMD (McArdle et al. 1986). This was demonstrated in an investigation by Lindberg and associates (1984). Eumenorrheic controls were compared to eumenorrheic, amenorrheic, and oligomenorrheic athletes. Single photon absorptiometry was used to assess BMD. Trabecular bone loss occurred most often in women who had exercise-induced amenorrhea.

In order to determine if exercise would induce menstrual dysfunction, Bullen and colleagues (1985) studied 28 athletically untrained college women participating in strenuous exercise for a period of eight weeks. Subjects

were randomly assigned to a weight loss or weight maintenance group, and were expected to participate in moderately intense sports for three and one-half hours per day. Subjects were to run four miles per day, which was increased to ten miles per day by the fifth week. Subjects kept menstrual records in order to determine if exercise would induce menstrual dysfunction. Menstrual cycles were disrupted in 24 of the 28 subjects. The majority of women were in the weight loss group, and lost an average of four pounds. The investigators concluded that intense exercise along with weight loss induced menstrual dysfunction in college females.

Cann and co-workers (1984) suggested that amenorrheic, premenopausal females may be at a higher risk than their eumenorrheic counterparts of developing osteoporosis. An investigation in which 36 amenorrheic females and 50 eumenorrheic controls participated showed that amenorrheic cortical bone was slightly less dense, and trabecular BMD was 20-30% lower than that of the controls. It should be noted that the duration of subject's exercise was not controlled. These investigators concluded that increased skeletal stress, which was due to exercise, along with decreased bone mass placed amenorrheic women at an increased risk for stress fractures and osteoporosis.

Menstrual function and bone mass was studied by Marcus

and associates (1985) in 17 athletic female subjects, 11 of whom were amenorrheic. The amenorrheic and eumenorrheic athletes were matched for age of menarche, aerobic capacity, body fat, and exercise intensity with controls. Computed tomography was used to measure BMD of the lumbar spine (L1-L2) and radius, while radioimmunoassay was used to measure plasma hormones. The BMD in amenorrheic athletes was significantly lower than the eumenorrheic athletes, and again, lower than the non-athlete controls. Plasma estradiol was lower in amenorrheic athletes than in eumenorrheic athletes and controls. In addition, more frequent exercise-related fractures were reported in the amenorrheic than eumenorrheic athletes. It was concluded that menstrual function of athletes does affect BMD.

The effect of amenorrhea on BMD in female athletes was studied by Myerson and associates (1992). Thirteen amenorrheic runners, 13 eumenorrheic runners, and 12 sedentary controls participated. All subjects were between the ages of 21-35 years. Dual photon absorptiometry was used to measure the bone mineral content and BMD of the total body. The amenorrheic athletes had significantly lower levels of bone mineral content and BMD than their eumenorrheic counterparts. The authors concluded that the amenorrheic athletes have lower values of total body bone mineral density.

Myburgh and colleagues (1990) investigated the effect of low bone density on stress fractures in athletes. Twenty-five athletes (19 women) with confirmed stress fractures and 25 control athletes participated in this investigation. Of the 19 female athletes, four were oligomenorrheic and three were amenorrheic. Bone mineral density of the lumbar spine (L2-L4) and left proximal femur was measured by dual energy x-ray absorptiometry. Athletes with stress fractures had significantly lower bone densities than athletes without stress fractures. The authors concluded that athletes with stress fractures were more likely to have lower bone mineral densities than athletes without stress fractures.

Drinkwater and co-workers (1984) conducted a study to determine whether a difference in BMD existed between amenorrheic and eumenorrheic athletes. Fourteen of 28 athletic participants were amenorrheic. The amenorrheic athletes were age, height, weight, sport, and training regimen matched with the eumenorrheic athletes. Radioimmunoassay confirmed that 14 subjects were amenorrheic, since a lower mean estradiol concentration, and progesterone peak was noted in the amenorrheic group. Single and dual photon absorptiometry were used to measure BMD of the lumbar spine (L1-L4). Amenorrhea was associated with decreased bone mineral density. The authors concluded

that a difference in BMD exists between athletes with different menstrual patterns.

Rutherford (1993) studied the effect of menstrual status on BMD in female athletes. Thirty-one athletes, aged 20-52 years, participated. Sixteen athletes were eumenorrheic, while the remaining 15 were amenorrheic. In addition, bone mineral densities were compared to an age and weight matched reference group, as reported by the Lunar British database. Dual energy x-ray absorptiometry was used to measure the BMD of the lumbar spine (L1-L4) and total body. Amenorrheic athletes had significantly lower bone mineral densities than their eumenorrheic counterparts and the reference controls. The eumenorrheic group had significantly higher total and regional bone mineral densities than the reference group. The author concluded that weight-bearing activity may increase BMD in eumenorrheic athletes at sites stressed by the exercise, but this was not true for amenorrheic athletes.

Lloyd and colleagues (1987) investigated the difference in bone mass between oligomenorrheic and eumenorrheic athletes. These subjects were matched for age, height, and weight with sedentary eumenorrheic controls. Computed tomography was utilized to determine trabecular BMD of the lumbar spine (L1-L3). The researchers found that oligomenorrheic athletes had significantly lower BMD, and

concluded that menstrual status does affect bone mass.

When Lloyd and co-workers (1988) repeated the study protocol of Lloyd and associates (1987) the relationship between BMD and irregular menstrual function was significant. Oligomenorrheic athletes had mean bone densities that were 69% of the mean bone densities of eumenorrheic athletes. In addition, oligomenorrheic athletes had lower plasma estrogen levels than the eumenorrheic athletes. The researchers again made the same conclusion.

Drinkwater and associates (1986) investigated whether amenorrheic athletes who regained menses would have an alteration in BMD. Amenorrheic athletes who regained menses were compared to amenorrheic athletes who did not regain menses, and to eumenorrheic athletes. Single and dual photon absorptiometry were used to assess BMD of the lumbar spine (L1-L4) and radius. Women who regained menses had a significant increase in BMD, while those who remained amenorrheic continued to experience a decline in bone mass. Investigators concluded that the primary factor responsible for the increase in BMD in amenorrheic athletes was the resumption of menses.

Since a relationship between bone demineralization and athletic menstrual dysfunction has been recognized via several studies as previously discussed, future research

must be directed at aleviating this problem. Mineral supplementation studies are being conducted to address the issue. Boron has been postulated to aid in bone metabolism (Hunt and Nielsen 1988; Hunt 1989; Nielsen et al. 1988, 1992b). If boron functions in bone metabolism, dietary boron may play a role in prevention of the bone demineralization experienced by many female athletes.

Boron

Boron and Plants

The essentiality of boron in vascular plants dates back to the early 1920's (Sommer and Lipman 1926; Warington 1923). Although the exact mechanism of how boron functions is not quite understood, it is proposed to be involved in regulating plant hormones, such as gibberellic acid, cytokinin, and auxin, and controlling calcium, which is a second messenger, at the cell membrane level (Parr and Loughman 1983; Duggar 1983). Cytokinin, auxin, and gibberellic acid have been shown to stimulate plant growth (Davies and Littlewood 1979; Villee et al. 1985). Lovatt and Dugger (1984) suggested that boron metabolism may be hard to study for two reasons: (1) Boron does not have a heavy or radioactive isotope, thus localization and transport investigations are difficult to conduct, which means that only comparative studies may be employed and;

(2) a zero boron concentration is hard to establish, for traces of boron are found in hard glass, distilled water, and chemicals. For these reasons it is difficult to investigate boron in animals and humans.

Boron in Animal Growth and Bone Mineralization

There is a conflict as to whether boron is essential in animals. In 1939, Hove and co-workers reported that boron was required by the rat during periods of growth, while McCoy (1967) suggested otherwise. More recently, Hunt and Nielsen (1981, 1988) support the claim by Hove and colleagues (1939), that boron is needed by animals.

According to Hunt and Nielsen (1981), a relationship exists between boron and cholecalciferol. The methodologies utilized by these investigators included the use of cockerel chicks who were either fed diets adequate (250 IU/kg) or inadequate (125 IU/kg) in cholecalciferol, while a boron supplement of 3 μg/g was given. Chicks that consumed a cholecalciferol deficient diet and given boron supplements had a 38% greater growth rate than chicks on the same diet, without boron supplements. It was suggested that cholecalciferol deficiency may have actually enhanced the body's need for boron, and since a possible interaction between the two exists, it was postulated that boron is essential for animals.

Hunt and Nielsen (1988) conducted a similar study in which cockerel chicks were provided a diet with inadequate dietary cholecalciferol (125 IU). The investigators found that chicks fed inadequate levels of magnesium in addition to the deficient cholecalciferol, exhibited enhanced growth when given dietary boron supplements, and that chicks fed adequate levels of magnesium experienced depressed growth when supplemented with boron. The amount of boron given was 3 mg/kg for 28 days. These results suggested that boron may play a significant role in bone metabolism. They stated that "the interaction between boron and cholecalciferol may be of importance in such bone diseases as osteoporosis."

King and colleagues (1991a,b) conducted a study similar to that of Hunt and Nielsen (1988), except that the animal model studied were embryos. Cholecalciferol adequate and inadequate hatching eggs were used. At eighth day of embryogenesis, the cholecalciferol adequate chick embryos were injected with 0.1 mg, 0.5 mg, or 1.0 mg boron, while cholecalciferol deficieny embryos were injected with 0.5 mg boron, 0.5 mg boron and 0.3 μg cholecalciferol, or 0.3 μg or 1.5 μg cholecalciferol. Boron and cholecalciferol in ovo administration enhanced the hatchability of the cholecalciferol deficient embryos, while in cholecalciferol adequate embryos boron was associated with decreased hatchability. These results support the findings by Hunt

and Nielsen (1988) that an interaction exists between boron and cholecalciferol.

Hunt (1989) investigated the effect of boron supplementation on bone mineralization via a study similar to that of Hunt and Nielsen (1988). Cholecalciferol deficient cockerel chicks were given 3 mg/kg of boron, and were given inadequate (300 mg/kg) or adequate (500 mg/kg) magnesium, and zero or 20 mg/kg of molybdenum. Results of this study seemed to support the notion that boron may be beneficial in bone mineralization, for the cholecalciferol induced deficiency was significantly reduced by giving the chicks dietary boron. Other speculations from this investigation were that during cholecalciferol and magnesium induced deficiencies, boron suppressed bone anabolism, while during periods of magnesium adequacy, bone catabolism was suppressed by boron. This suggested that boron may function in modifying mineral metabolism during periods of cholecalciferol deficiency.

Hegsted and colleagues (1991) investigated the effect of boron with cholecalciferol deficiency. Weanling Harlan Sprague-Dawley rats were fed diets which lacked cholecalciferol, but contained either 0.158 μg of boron/g of diet, (unsupplemented) or 2.72 μg of boron/g of diet (supplemented). After 12 weeks on the diet, the unsupplemented group had significantly lower calcium and

phosphorus ash than the boron supplemented group. It was speculated that since supplemental boron did not result in greater body weight, organ mineral content, or bone density that other nutrient interaction were occurring.

Nielsen and Shuler (1992) performed an experiment to document that a boron-calcium interaction exists in animals. Weanling Harlan Sprague-Dawley rats were fed supplements of boron (zero or 3 μg), calcium (2.5 or 5 mg), and magnesium (100 or 400 μg) in factorial arrangement. Plasma boron was prepared by the wet-ashing procedure of Hunt and Shuler (1989), and analyzed by inductively coupled argon plasma (ICP) spectroscopy. Both boron and magnesium supplementation increased rat growth. A significant interaction resulted between calcium and boron, which affected the hematocrits. When given inadequate calcium and boron supplements, the rats had higher hematocrits than when deprived boron. The exact mechanism of the boron-calcium interaction was not elucidated, but these data implied that both minerals acted on similar systems in the rat (Nielsen and Shuler 1992).

Weanling Harlan Sprague-Dawley rats were used in another study by Nielsen and associates (1992b) to determine whether dietary boron would elicit an alteration in bone composition. Boron (zero or 3 μg), potassium (1.0, 1.4, 1.8, and 3.6 mg), and a L-arginine free-base of 10 mg or a

L-methionine base of 2.5 mg supplements were given. Boron supplementation alone did not significantly alter bone composition. However, dietary boron was found to elicit changes in bone composition by interactions with potassium and amino acids, or with potassium alone. The investigators concluded that boron has an essential physiological function, for it was able to affect the metabolism of nutrients that were required for calcium and bone metabolism.

Shuler and Nielsen (1988) conducted a rat study to observe the effect of boron on bone mineral status. Boron (zero or 3 μg/g), magnesium (100 or 400 μg/g), and aluminum (zero or 1.0 mg/g) supplements were given in a factorial arrangement. Boron supplementation enhanced bone phosphorus while the reverse occurred with boron deprivation, as elicited by a high dietary intake of aluminum. The authors suggested that boron is an essential nutrient that is involved in mineral metabolism.

These animal studies indicated the potential essentiality of boron, as suggested via possible interactions with cholecalciferol, calcium, phosphorus, and magnesium, which are nutrients that have been established to function in bone metabolism. Inferences from many of the previous studies are made unto the human population. It is due to these inferences that more research is being

conducted with human subjects.

Boron in Human Tissues

Boron has been found in several human tissues, blood, and urine. Studies dating back to the 1950's give well established values of the boron concentration in human tissues, many of which are consistent with levels reported today, as illustrated in Table 6.

Boron and Bone Mineralization in Humans

Current research indicates that boron may be essential in humans. Boron may help prevent bone demineralization or aid in maintaining normal bones (Nielsen 1990). Newnham (1981) found that boron bone content in subjects with either osteo- or rheumatoid arthritis was much lower than the content of normal healthy bone. It was also discovered that the arthritic subjects who received a boron supplement had harder bone tissue than the unsupplemented arthritic group. This is of significance since bone demineralization may be the early stage of metabolic bone disorders, such as osteoporosis (Newnham 1981).

Nielsen and associates (1987) investigated the effect of dietary boron (3 mg/day) on hormone and mineral status, and bone metabolism in 13 postmenopausal women, two of whom were on estrogen therapy. They were housed in a metabolic

TABLE 6
REPORTED BORON CONCENTRATIONS IN HUMAN SAMPLES

HUMAN SAMPLE	REPORTED BORON CONCENTRATION (ppm)
Blood	0.03[2] 0.0567[1] 0.1[3] 0.141[4] 0.2[8] 0.4[6]
Plasma	0.0183[2] 0.0201[3] 0.5-0.6 (at birth)[5]
Serum	0.0223[1] 0.18-0.21[2]
Brain	0.06 (fresh weight)[1]
Breast Milk	0.06-0.08[5]
Kidney	0.248 (fresh weight)[6] 0.6 (fresh weight)[4]
Liver	0.2 (fresh weight)[6] 0.114 (fresh tissue)[4]
Lung	0.6 (fresh weight)[6]
Muscle	0.1 (fresh weight)[2]
Rib (Varies with type of water)	6.2 ± 2 (ash, soft)[6] 10.2 ± 5 (ash, hard)[6]
Skin	0.119 (fresh weight)[4]
Urine	0.1 - 0.2[7] 0.753[1]

[1]Abou-Shakra et al., 1989
[2]Clarke et al., 1987
[3]Ferrando et al., 1993
[4]Forbes et al., 1954
[5]Friis-Hansen et al., 1982
[6]Hamilton et al., 1972-1973
[7]Hunt, 1991
[8]Panteliadis et al., 1975

unit for 167 days. Boron supplementation significantly reduced urinary calcium, phosphorus, and magnesium when the magnesium intake was low. In addition, boron supplementation significantly increased serum concentrations of 17-ß estradiol and testosterone when dietary magnesium was low. The investigators suggested that "supplementation of a low-boron diet with an amount of boron commonly found in diets high in fruits and vegetables induces changes in postmenopausal women consistent with the prevention of calcium loss and bone demineralization."

In a similar study, Nielsen and associates (1988) implicated the essentiality of dietary boron in prevention of calcium loss and bone demineralization. Thirteen postmenopausal women were fed conventional foods and supplemented with boron (zero or 3 mg/day) and aluminum (zero or 1000 mg/day). Dietary boron affected major mineral metabolism. Boron supplementation significantly increased serum ionized calcium and decreased urinary calcium excretion when subjects consumed a diet low in magnesium.

Another investigation by Nielsen (1989) suggested that boron may not only affect calcium metabolism, but also copper metabolism. Five males, nine postmenopausal females, five of whom were on estrogen therapy, and one premenopausal female participated, and received a mixed Western diet for 63 days with 3 mg of boron per day. Plasma ionized calcium

and copper, erythrocyte enzymatic ceruloplasmin, serum superoxide dismutase, and serum 25-hydroxycalciferol were lower while serum calcitonin and glucose were higher during periods of boron depletion. These results indicated that dietary boron may play a role in calcium and copper metabolism.

Nielsen and associates (1990) recruited subjects who fit the same criteria and were fed the same type of diet as previously mentioned (Nielsen 1989). Boron was supplemented at a level of 3 mg/day. It was shown that serum calcitonin and osteocalcin were significantly higher while plasma-ionized calcium and serum 25-hydroxycholecalciferol were significantly lower in men and postmenopausal women not taking estrogen during boron depletion. It was concluded that boron deprivation elicited the above mentioned results, and these changes were consistent with those found in postmenopausal women who have osteoporosis. For this reason it was suggested that boron supplementation is required for optimum calcium metabolism and the prevention of bone loss.

In contrast to the reported positive effect of boron on bone mineral density, a study by Darnton (1991) and Volpe-Snyder (1991) suggested otherwise. They investigated college-aged female athletes during a ten month boron supplementation (3 mg/day) period, and observed no significant changes in BMD due to the supplementation.

Darnton (1991) found that the boron supplemented athletes had lower serum magnesium and increased boron excretion. Possible reasons for conflicting outcomes in the human studies reviewed may include differences in subject's ages and living conditions during the investigations, and the duration of the investigations. A long-term study with college-aged female athletes is needed to examine the possibility that boron may indeed play a significant role in prevention of bone demineralization as suggested by the findings of Nielsen and associates (1987, 1988, 1990) and Nielsen (1989) with postmenopausal women. In addition, none of the subjects in previously mentioned studies were taking oral contraceptives, which may be protective of BMD. A study with college-aged female athletes who take oral contraceptives is needed to investigate the effects of boron and oral contraceptives on mineral status and BMD.

Other Potential Functions of Boron in Humans

Since boron supplementation has been associated with calcium metabolism (Nielsen et al. 1988, 1989), which may be associated with blood cell counts and composition, a human study was conducted to determine if dietary boron would affect human blood cell counts and hemoglobin. Nielsen and co-workers (1991) recruited four males, four postmenopausal females on estrogen therapy, and one additional female, who

initially believed she was postmenopausal. Each was fed a mixed Western diet for 63 days and supplemented with 3 mg of boron per day. Blood hemoglobin, red blood cell (RBC) count, and platelet count concentrations were determined weekly. During periods of boron supplementation, hemoglobin was significantly higher, while during boron deprivation, RBC and platelet counts were elevated. These findings suggested that boron may affect cellular membrane function by affecting the formation of hemoglobin and RBC at this level.

Boron has been suggested to enhance and even mimic some of the effects of estrogen therapy. If this were true, it may be more beneficial to consume boron supplements rather than estrogen therapy, since there are numerous side effects with estrogen therapy. A study was performed in which four men, nine postmenopausal women, five of whom were on estrogen therapy, and one premenopausal women, were given 3 mg of boron per day (Nielsen et al. 1992a). During boron supplementation, women on estrogen therapy had significantly higher levels of serum 17-B estradiol and plasma copper than subjects not on estrogen therapy. They concluded that boron enhanced and even mimicked some of the effects of estrogen therapy.

Methodology Review

Sample Preparations for Mineral Analysis

Sample preparation methods prior to mineral analysis vary. Three of the more common methods include open-vessel, wet-ash, low-temperature, Teflon-tube digestion (WALTTT), microwave digestion, and nitric acid digestion. The WALTTT method of digestion was introduced by Hunt and Shuler in 1989, and is a sample preparation for inductively coupled argon plasma spectroscopy (ICP) analysis. A sandbath was used in order to heat samples digested in nitric acid and hydrogen peroxide. The use of Teflon tubes was implicated as an attempt to eliminate potential sample contamination of boron which is present in the form of borosilicate in glass tubes. This method has been validated for the use of boron digestion, since 99.7% of spiked boron standard samples were recovered.

Microwave digestion has also been implicated as a sample preparation for ICP analysis. Microwave digestion can offer rapid and precise digestion in addition to limited exposure to hazardous materials and limited contamination (Ferrando et al. 1993). White and Douthit (1985) obtained a recovery rate of 91.3 to 114% of nine elements wet-ashed in a microwave. Abu-Samra and colleagues (1975) found that twelve biological samples, 0.5 g or less, can be digested in about 15 minutes.

A final sample preparation methodology has been performed by Zarcinas and associates (1987). This procedure involves wet-ashing at temperatures below 140°C in conditioned glassware with nitric acid. This temperature constraint is given so that samples do not volatilize.

Mineral Analysis

The most commonly used methods of mineral determinations include ICP and atomic absorption spectroscopy (AAS). Other methods, such as x-ray fluorescence spectroscopy, emission spectrometry, neutron activated analysis, and spark source mass spectrometry have also been used for mineral determinations. However, these procedures are very costly, and have various problems with sample preparation and size (Williams et al. 1986).

According to Williams and co-workers (1986), ICP is the fastest method for multi-element analysis, and involves the use of argon as the gas carrier and coolant which carries the nebulized sample. During this process, the plasma, which has a great fraction of its atoms in the ionized form, is created into a gas when the argon is made conductive by its exposure to an electrical charge (Williams et al. 1986). A nebulizer changes the initially liquid sample into an aerosol which is then carried into a very hot plasma region by the argon gas. It is within this region that the various

elements are excited to their emission temperatures. The intensity of the light emitted is proportional to the amount of and wavelength characteristic of each element, which is then compared to the individual element wavelengths in the spectrographic library (Williams et al. 1986; Hunt and Shuler 1989).

A slight alteration of the ICP method is the use of inductively coupled plasma-mass spectrometry (ICP-MS). This method is the same as above, with the exception that the data output appear in the form of mass spectrometry output (Vandecasteele et al. 1990). Vandecasteele and associates (1990) and Ward and colleagues (1990) suggested that ICP-MS is a very accurate method of mineral determination in serum.

The AAS method of mineral analysis relies on measuring the amount of light that is absorbed, while energy is applied to a specific atom (Williams et al. 1986). During this process, an electron in the outer configuration is excited from the ground state to a less stable excited state. The light is produced when this change from the ground state to the excited state occurs. It is the ground state atom that absorbs the light energy at a specific wavelength (Williams et al. 1986).

Hormone Analysis

Several methods of hormonal analysis have been

identified. Table 7 illustrates the various assay methods used in endocrinology. According to O'Sullivan (1984), radioimmunoassay is a very sensitive method of hormone analysis. This method generally does not involve a purification step, even when the assay requires the use of complex biological fluids. Bevan (1981) reported that this procedure involves a saturation analysis, in which the end point is to determine the relative proportion of free and bound antigen. There are, however, a few disadvantages to using this procedure, which include the short half-lives of most radioisotopes which are used in this procedure, and the potentially hazardous levels of radiation which may be encountered (Bevan 1981; O'Sullivan 1984). Enzyme immunoassay procedures may be useful when analyzing hormones, for enzymes do not have the potential dangers associated with the radioactivity of the radioimmunoassay procedures, and are stable for a longer period of time. Enzymes may be used as labels, for their catalytic nature allows them to detect and quantify very small amounts of samples. Procedures involved here are very similar to those of the radioimmunoassay (Bevan 1981; O'Sullivan 1984). Gas-liquid chromatography is a common method of measuring such hormones as closely related steroids, for slight differences in compound structure may be detected by this method of analysis. However, a drawback is that positive

TABLE 7
ASSAY METHODS USED IN ENDOCRINOLOGY*

Assay Method	Hormones Analyzed
Radioimmunoassay	Polypeptides Steroids
Enzyme Immunoassay	Steroids
Gas-Liquid Chromatography	Steroids
Gas Chromatography/ Mass Spectrometry	Steroids
Colorimetry	Steroids
Fluorimetry	Steroids Adrenal Medullary Hormones

*Adapted from Bevan, 1981 and O'Sullivan, 1984.

identification of the components is not readily available as they are eluted from the column (Bevan 1981). Gas chromatography/mass spectrometry is a very useful method of steroid analysis, while fluorimetry and colorimetry have been found to be useful in analyzing estrogens (Bevan 1981).

Radioimmunoassay procedures have been found, and suggested to be effective for analyzing estrogen, testosterone, progesterone, calcitonin, and parathyroid hormone in human plasma or serum samples due to the simple and rapid methods involved (O'Riordan et al. 1976; Hesch et al. 1976; Leyendecker et al. 1976; Van der Molen et al. 1976; Nieschlag 1976; Verneullen 1976; Abraham 1976).

Assessment of Bone Mineral Density

Several methods exist for assessing BMD. These measurements may be divided into two categories: indirect and direct methods. Several indirect methods, which involve biochemical analyses, are used to estimate bone loss, and may be found in Table 8. Sokoll and Dawson-Hughes (1989) suggested that plasma ionized calcium is routinely used since it is not bound to protein and thus is a measure of circulating calcium, while serum total calcium may be analyzed by atomic absorption spectrophotometry or a NOVA 7 Total Calcium/Ionized Calcium Analyzer. Delmas (1988) and Notelovitz (1993) suggested that serum measurements of

TABLE 8 INDIRECT MEASURES OF BONE DENSITY
Biochemical Measures of Bone Density
Plasma ionized calcium
Serum alkaline phosphatase
Serum osteocalcin
Urinary hydroxyproline
Hydroxyproline:creatinine ratio
Calcium:phosphorus ratio
Serum 1,25-dihydroxyvitamin D_3
Serum 25-hydroxyvitamin D
Serum parathyroid hormone
Serum calcitonin

alkaline phosphatase, which is the most frequently used biomarker, and osteocalcin may be used to determine bone metabolism. Normal values of serum or plasma alkaline phosphatase may be used to indicate bone formation, while values above normal indicate fracture or bone loss. Notelovitz (1993) suggested that serum alkaline phosphatase values above or below normal indicate bone turnover. Osteocalcin is a bone matrix protein which is synthesized by proliferating osteoblasts, which is why serum osteocalcin levels have been associated with the formation of trabecular bone (Delmas 1988; Karlsson et al. 1992). Delmas (1988) indicated that an increased level of urinary hydroxyproline has been associated with an increased level of serum immunoreactive parathyroid hormone. Horowitz and colleagues (1984) suggested that a low ratio of hydroxyproline to creatinine indicates a reduction in bone resorption, while Draper and Scythes (1981) reported that a calcium:phosphorus ratio below 1:1 may initiate bone resorption. DeLuca (1988) suggested that low serum 1,25-dihydroxyvitamin D_3 or 25-hydroxyvitamin D_3 may indicate bone loss. Finally, parathyroid hormone and calcitonin have been used as biochemical markers of bone loss. Diagnostics Products Corporation (1993) indicated an inverse relationship between plasma calcium and parathyroid hormone. High levels of plasma parathyroid hormone seen in response to hypocalcemia

are associated with bone loss. Calcitonin secretion may be regulated by rising and falling plasma calcium levels, with an increase in circulating calcium prompting in increase in calcitonin (Diagnostics Products Corporation 1992). Chestnut and associates (1980) suggested that a deficiency of calcitonin may result in bone resorption.

More accurate measures of BMD have been noted by use of direct measurements. Hansen and associates (1990) suggested that measurements of BMD (g/cm^2) and bone mineral content (g/cm) more accurately represent the type of bone remodeling which is occurring in bone. Methods of directly measuring bone density may be found in Table 9. Single and dual photon absorptiometry are commonly used techniques. According to Mazess and Wahner (1988) and Mazess and Barden, (1989), single photon absorptiometry is generally used to measure the bone mineral density of the shaft of a long bone, although it may also be used to measure such regions as the distal radius or os calcis (heel).

Dual photon absorptiometry may be used to measure such bones as the lumbar spine (L2-L4), hip, proximal femur, or whole body, and is useful for measuring both cortical and trabecular BMD (Mazess and Wahner 1988; Mazess and Barden 1989). Mazess and Barden (1989) stated that an analysis of the spine or femur may take up to 20 minutes, while a total body scan may take 60-70 minutes. In addition, dual photon

TABLE 9 DIRECT MEASURES OF BONE DENSITY
Radiological Measures of Bone Density
Single photon absorptiometry
Dual photon absorptiometry
Quantitative Computed Tomography
Dual Energy X-Ray Absorptiometry
Radiographic Absorptiometry
Dual Energy Roentgenogram Absorptiometry

absorptiometry may be used more often in research or clinical practice due to the low radiation dosage and low cost (Mazess and Barden 1989). Quantitative computed tomography may be used to measure cross-sections of the lumbar spine, but it uses a higher amount of radiation and is more costly than dual photon absorptiometry (Mazess and Barden 1989). According to Genant and colleagues (1988), quantitative computed tomography may be used to measure both trabecular and cortical bone, in addition to its ability to spatially separate the highly responsive cancellous bone from the less responsive compact bone. Dual energy x-ray absorptiometry is another method for measuring BMD. Hansen and associates (1990) and Laitinen and colleagues (1991) indicated that dual energy x-ray absorptiometry has greater precision, spatial resolution, and reduced scan time (five minutes for spine or femur and ten minutes for total body) as compared to dual photon absorptiometry. Radiographic absorptiometry has been used to measure the BMD of the hand. Only three to five minutes are required for this procedure. According to Johnston and colleagues (1991), dual energy roentgenogram absorptiometry has been used as a measure of bone density. The spine, hip, and total body may be measured in three to seven minutes (Johnston et al. 1991).

CHAPTER III

METHODOLOGY

Subjects and Study Design

Subjects

The Institutional Review Board for Research Involving Human Subjects at VPI & SU (#92-104) granted permission to conduct this study prior to subject recruitment. This form may be found in Appendix A. Criteria for subject selection included:

1. College females, ages 18-29 years, athletes and non-athletes.
2. Nonsmokers (either never smoked or refrained at least six months).
3. Nulliparous.
4. Free from health problems.
5. Free from orthopedic problems.
6. No history of chronic illness.
7. Free from use of chronic medications, with the exception of oral contraceptive agents

Initially 18 athletes and 15 non-athletes from VPI & SU volunteered to participate in this investigation. Within two months of beginning the investigation, two athletes and two non-athletes dropped out of the study, all of whom were placed in the placebo treatment group. Table 10 illustrates

subject classification and treatment assignment for the investigation.

During recruitment, athletes were defined as exercising greater than one hour per day, five days a week, and non-athletes were defined as being inactive or exercising less than one hour per day, five days a week. However, upon completion of an aerobic capacity test athletes were defined as being in excellent physical condition, while non-athletes were in either average, fair, or poor physical condition. All atheltes were on a varisty athletic team, intramural team, or exercised independently. This test confirmed the physical status of the athletes. In return for participation in this six month investigation, subjects received one credit of undergraduate research. An undergraduate research credit form may be found in Appendix B.

All subjects were given an oral and written explanation of the study, which included general information about the study, sample collection methods, and general procedures which were to be followed. A medical history questionnaire and written consent form were completed by those who volunteered. These forms may be found in Appendices C and D, respectively. In addition, each participant was confirmed to be in good health prior to participation by a physician, as indicated by having had a physical examination

TABLE 10
SUBJECT GROUPS (n=29)

Group	Boron	Placebo	OC*	NOC*
Athletes (n=16)	14	2	8	8
Non-Athletes (n=13)	9	4	4	9
Totals	23	6	12	17

*OC = Oral Contraceptives; NOC = No Oral Contraceptives

within the last year.

Once volunteers meeting the above criteria for participation in the study were selected, they were asked to sign a contract to confirm that they were serious about participating in this study, that they were willing to participate through the duration of the study (unless dropping from the study was seen by the student or investigator as in the best interest of the student), and that they were to follow procedures for taking supplements or placebo, specimen collections, and for recording exercise patterns, menstrual histories, and dietary information. The contract may be found in Appendix E. Subjects completed exercise records for seven consecutive days at baseline so that the investigator could monitor the level of activity performed by the athletes. The exercise records may be found in Appendix F. Subjects completed monthly menstrual histories to assess menstrual status and to schedule the six month blood collection. It was important to be consistent when collecting the samples, for hormone analysis at different phases of the menstrual cycle yield various results. Menstrual records may be found in Appendix G.

Experimental Design

This study was conducted over a six month period. The subjects within each group were randomly assigned to receive

a daily placebo (corn starch) or boron supplement (3 mg Tri-Boron capsule, Twin Laboratories, Inc., Ronkonkoma, New York 11779). The supplement dosage, according to Nielsen and colleagues (1987) was consistent with the amount of boron obtained from a diet high in fruits and vegetables. Subjects were instructed to take one capsule daily and to consume self-selected diets throughout the duration of the investigation. Table 11 summarizes all measurements made at the baseline and six months.

Analytical Methods

Dietary Analysis

Dietary analysis of three-day dietary records were completed at baseline and six months. Food records may be found in Appendix H. Each diet was analyzed for protein, fat, carbohydrate, total energy, calcium, magnesium, phosphorus, and fiber using the Nutritionist IV™ Computer Program (N-squared Company, Analytic Software, Silverton, Oregon: 503-873-5906). A code book for Nutritionist IV™ was prepared by the investigator which listed default food codes which were used when the subjects were not specific about the type of food consumed. This code book was used to minimize the inconsistencies associated with coding several diets, and may be found in Appendix I, along with the procedures for using Nutritionist IV™.

TABLE 11
ANALYTICAL METHODS OF DATA COLLECTION

Data Collection Method	Time*	
	1	2
NUTRIENT INTAKE Nutritionist IV™, N-squared Company, Analytic Software, Silverton, Oregon) (total calories, protein, carbohydrate, fat, calcium, magnesium, phosphorus, dietary fiber)	X	X
HEIGHT (Meter stick)	X	
WEIGHT (Physician's scale)	X	X
% BODY FAT (Futrex, Futrex, Inc., Gaithersburg, MD)	X	
PLASMA BORON, CALCIUM, MAGNESIUM, AND PHOSPHORUS (Prepared by: Open-Vessel, Wet-Ash, Low-Temperature Teflon Tube Digestion Procedure [WALTTT] according to Hunt and Shuler, 1989; Boron analyzed by: ICP, Calcium and Magnesium analyzed by: AAS, Phosphorus analyzed by: Colorimetric Method)	X	X
URINARY BORON, CALCIUM, MAGNESIUM, AND PHOSPHORUS (WALTTT and ICP)	X	X
SERUM HORMONES (Radioimmunoassay kits, Diagnostic Products Corporation, Los Angeles, CA) (Estradiol, Progesterone, Testosterone, Calcitonin, Parathyroid Hormone)	X	X**
BONE MINERAL DENSITY (Lunar DP3 Dual Photon Absorptiometer, Madison, WI)	X	
AEROBIC CAPACITY (Physical Work Capacity 170 Test)	X	
MENSTRUAL RECORDS (Calendars)	X	X
EXERCISE JOURNALS (Nutritionist IV™)	X	

*Time 1 = baseline; Time 2 = six months
**Only estradiol, progesterone, and testosterone were measured at six months time due to nondetectable levels of calcitonin and parathyroid hormone measured at baseline.

Anthropometric Data

Anthropometric data were obtained at baseline. After subjects had their blood drawn, they were weighed on a physician's scale. Subjects were weighed and had their heights measured in light clothing, no shoes, and after a 12-14 hour fast. In addition, a Futrex™ infrared spectrophotometer was used to measure percent body fat. A trained, designated person (Bryan Cobb) measured the body fat of all subjects to minimize investigative error.

Aerobic Work Capacity

A Physical Work Capacity Test (PWC_{170}) (Wahlund 1948) was used to determine aerobic capacity of each subject. This test was used to assign subjects to the athlete and non-athlete groups, based upon subjects' maximal oxygen consumption (VO_{2MAX}) and physical condition. This procedure was performed on a Monarch Bicycle Ergometer (Monark 818 E, Vargerg, Sweden) in the Exercise Physiology Laboratory in the War Memorial Hall at VPI & SU. A PWC_{170} subject information sheet may be found in Appendix J. Prior to testing, the bicycle was calibrated via pedal weights and the seat height was adjusted for each subject. Also, each subject had her resting heart rate (RHR) determined by radial or carotid palpitation. Borg's Rating of Perceived Exertion (RPE) scale was explained to each subject prior to

the test so that during the test, each subject described how the exercise felt on a numeric scale (Borg 1978). This scale may be found in Appendix K.

The actual test consisted of cycling at a constant speed of 50 revolutions per minute in testing stages of four minutes a piece. As time went on, each stage presented a greater resistance. At several points during the testing session, subject heart rate was recorded by carotid or radial palpitation, and the subject was asked to assess how the exercise felt according to Borg's RPE scale. Those subjects exercising the longest had greater aerobic capacity and reached higher workloads than those who exercised for shorter amounts of time. Information for predicting maximal oxygen uptake from heart rate and work load, may be found in Appendix L. After finishing the test, each subject was required to "cool down", or continue pedaling at a low intensity for several minutes until the heart rate returned to RHR.

Bone Mineral Density

A dual photon absorptiometer (DPA) (Lunar Model DP3, Lunar Radiation Corporation, Madison, WI 608-274-2663) at Montgomery Regional Hospital, Blacksburg, VA, was used by qualified personnel (Gretchen Price, Lead Technologist in Nuclear Medicine) to measure BMD of the lumbar spine

(L2-L4). Subjects reported to the Nuclear Medicine Division (703-953-5131) of the hospital to have this procedure performed. Each subject was instructed to remove all metal (belts and jewelry) prior to having a lower back scan. The procedure took about 20 minutes. A sample data sheet may be found in Appendix M.

Sample Collection

Twelve-hour fasting blood samples were drawn into trace element-free vacutainers (Becton Dickinson VACUTAINER™ System, Becton Dickinson and Co., Rutherford, NJ 07070) by qualified personnel (Janet Rinehart, MLT [ASCP]) at baseline and six months. Thirty milliliters of blood were taken on the designated day between 7:00-9:00 am after a brief rest period. Blood samples were collected and kept on ice. Human waste disposal procedures may be found in Appendix N. The researchers provided juice and bagels following each blood collection.

The first tube drawn contained the anticoagulant, sodium heparin, and was later used to obtain plasma samples, while the remaining three vacutainers were used to obtain serum samples. Plasma and red blood cells were separated by centrifugation (IEC Model DPR-6000 Centrifuge, Damon/IEC Division, International Equipment Co., Needham Heights, MA 02194) at 2800 rpm for 30 minutes. The plasma was then

aliquoted and stored at -20°C for mineral analysis. Serum and red blood cells were separated by centrifugation at 3200 rpm for 30 minutes. The serum was aliquoted and stored at -20°C for hormone analysis.

Urine samples were collected for a twenty-four hour period in acid-washed polyethylene containers, and returned to the investigators during a scheduled time. The collection period was defined as beginning with the second voiding of day one and included all voids for twenty-four hours, including the first void of day two. Urine samples were measured for total volume, then aliquoted and stored at -20°C for mineral analysis. Urine collection instructions may be found in Appendix O.

Sample Processing and Mineral Analysis

Duplicate plasma samples were wet ashed according to the open-vessel wet-ash, low-temperature, Teflon-tube (WALTTT) procedure of Hunt and Shuler (1989) (Appendix P). Digested samples were analyzed for boron using Inductively Coupled Plasma Spectroscopy (ICP) (Perkin-Elmer Plasma 400 ICP Emission Spectrometer, Rockville, MD 301-984-4700). Table 12 illustrates mineral detection limits for the Perkin-Elmer Plasma 400 Spectrometer. Atomic Absorption Spectroscopy (AAS) was used to analyze samples for calcium and magnesium content (Perkin-Elmer Atomic Absorption

TABLE 12
MINERAL DETECTION LIMITS FOR PERKIN-ELMER PLASMA 400 SPECTROMETERS*

Element	Mineral Detection Limit
Boron	3 ppb
Calcium	5.7 ppb
Phosphorus	45 ppb
Magnesium	20 ppb

*Reported by Perkin-Elmer, 1994

Spectrophotometer 2100, Rockville, MD 301-984-4700) (Appendix Q), while phosphorus was determined by a colorimetric procedure (Appendix R) using a spectrophotometer (Phosphorus Kit 670-A, Sigma Diagnostics, St. Louis, MO 63103) (Spectronic 501 Spectrophotometer, Milton Roy Co., Rochester, NY 14625). All ICP, AAS, and colorimetric procedures were performed in Wallace Hall, VPI & SU, in the laboratories of Dr. Ryland Webb (703-231-6784) and Dr. Forrest Thye (703-231-6620). Table 13 illustrates mineral detection limits for the Perkin-Elmer 2100 Atomic Absorption Spectrophotometer.

Serum Hormone Analysis

Radioimmunoassay (RIA) procedures (Diagnostic Products Corporation, Los Angeles, CA) (Appendix S) were used to assess the following hormones: estrogen (estradiol), progesterone, testosterone, calcitonin, and parathyroid hormone. Due to nondetectable levels of calcitonin and parathyroid hormone at baseline measurements, these two hormone assays were not repeated at six months. Table 14 illustrates the kit, amount of serum, and the calibration range for each hormone test kit. Standard radioimmunoassay procedures were followed as given in each kit. All hormone analyses were performed in Litton-Reaves Hall, VPI & SU in the laboratory of Dr. F. C. Gwazdauskas (703-231-4756).

TABLE 13
MINERAL DETECTION LIMITS FOR PERKIN-ELMER 2100 ATOMIC ABSORPTION SPECTROPHOTOMETER*

Element	Mineral Detection Limit
Calcium	0.001 ppm
Magnesium	0.0001 ppm

*Reported by Perkin-Elmer, 1982

TABLE 14
CHARACTERISTICS OF EACH HORMONE KIT USED*

Hormone Kit	Amount of Serum Per Assay	Kit Calibration Range
Estradiol (TKE21)	100 μL	20-3600 pg/ml
Progesterone (TKPG1)	100 μL	0.10-40 ng/ml
Testosterone (TKTT1)	50 μL	0.20-16 ng/ml
Calcitonin (KCLD1)	200 μL	0.03-1.2 ng/ml
Parathyroid Hormone (KPMD2)	50 μL	0.20-10 ng/ml

*Adapted from Diagnostic Products Corporation, 1991-1993

Statistical Analysis

Data were expressed as mean ± standard deviation. Analysis of Variance (ANOVA) was used to test for main effects and interaction of the two by two factorial treatments, while Tukey's multiple range test was utilized when differences were found. SAS™ (SAS, SAS Institute, Inc., Cary, NC 27512) statistics program was used to perform these analyses. The significance level was set a priori at 0.05. Data were analyzed, and correlations drawn at each sampling period. The Statistics Consulting Center at VPI & SU was contacted prior to data analysis to confirm statistical procedures.

CHAPTER IV

RESULTS

Twenty-nine college female subjects, aged 18-29, completed the six month supplementation protocol. Supplementation compliance was assumed. Uneven distribution of subject groups was the result of subject withdrawal prior to completion of the investigation (Table 10). All measurements were compared between activity, supplementation, and oral contraceptive groups, as well as between the following interaction groups: activity/supplement, activity/oral contraceptive, and oral contraceptive/supplement. Statistical analysis was not performed on the interactions of all three subject groups together (activity status, supplementation, and use of oral contraceptives) due to the small number of subjects within each cell. The general linear model procedures of SAS™ were used to adjust for unequal cell size when each cell had more than two subjects. Parameters not shown in table form were non-significant, although raw data and statistical analyses for all data may be found in Appendicies T and U, respectively.

Of the 29 subjects in this study, 7% had reported menstrual dysfunction. However, monthly menstrual records (Appendix T) indicated that 3% of subjects in this

investigation had menstrual dysfunction (oligomenorrhea). Figures 2, 3, and 4 illustrate estrogen and progesterone patterns associated with menstrual cycle phases for all subjects, subjects taking oral contraceptives, and subjects not taking oral contraceptives, respectively.

Table 15 illustrates that all baseline BMD, mineral and hormone measurements were within reported ranges (Tables 1, 2, and 3), except for calcitonin and parathyroid hormone which were non-detectable. Plasma boron values, for subjects with detectable levels (n=14) (Appendix T), and urinary boron values were slightly higher than reported in the literature (Clarke et al. 1987, Ferrando et al. 1993, Abou-Sharka et al. 1989, Hunt 1991). Table 16 shows that baseline dietary intake of all nutrients had met the RDA goals.

Table 17 illustrates urinary boron values. The only significant value found was at the baseline measurement between the boron and placebo supplemented groups. The boron group was found to have a significantly greater ($p < 0.01$) excretion of boron.

Table 18 illustrates significant baseline parameters for athlete and non-athlete groups. As expected, athletes had a significantly greater predicted maximal oxygen consumption (VO_{2MAX}) than did their non-athlete counterparts, as determined via the PWC_{170} test. Dietary fat, as analyzed

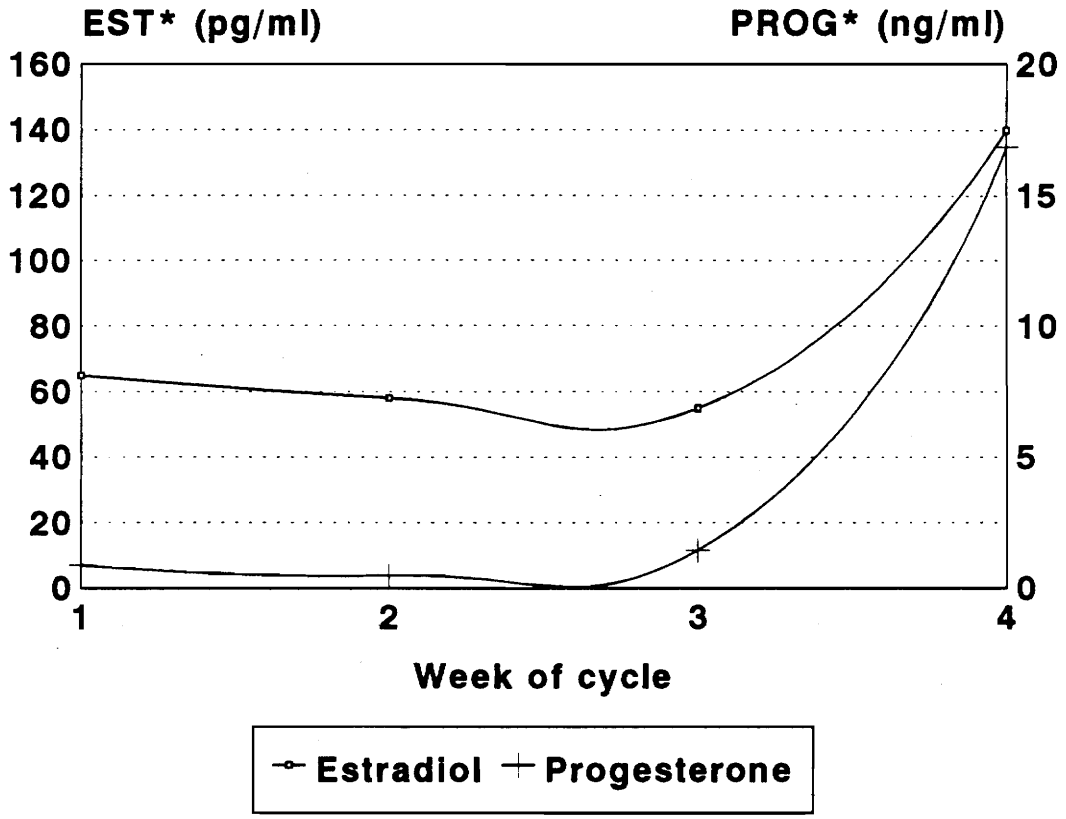


Figure 2 - Estrogen and Progesterone Patterns Of All Subjects
***EST = 17-B-Estradiol, PROG = Progesterone**

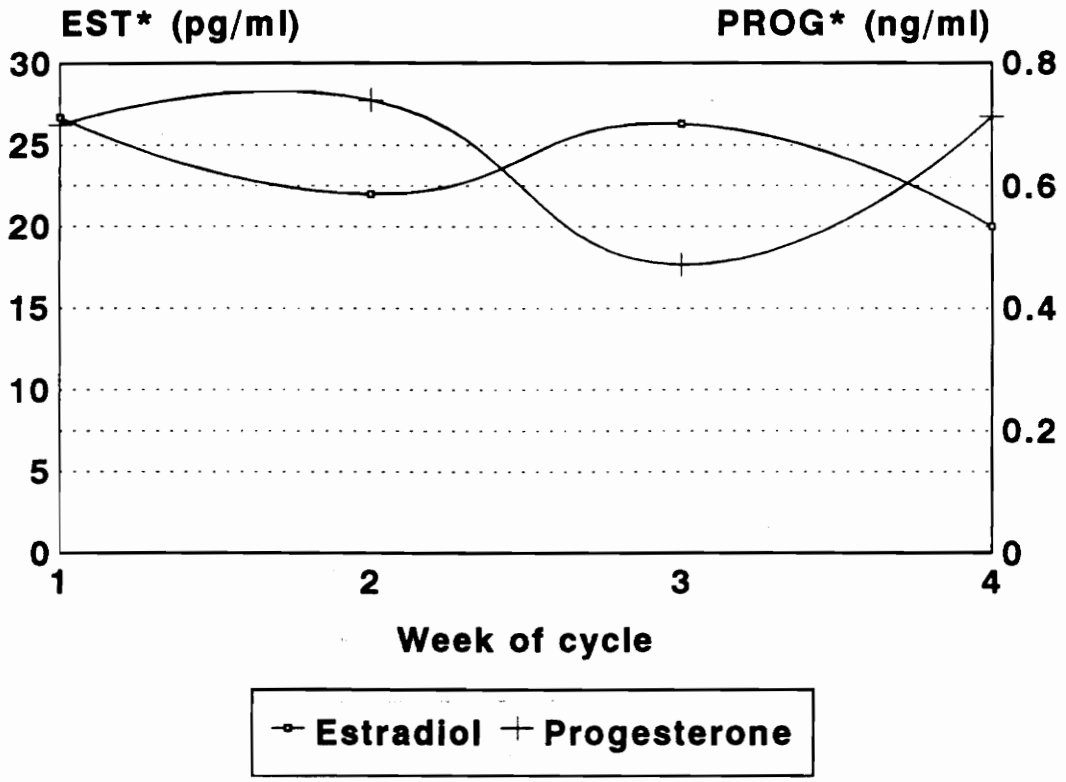


Figure 3 - Estrogen and Progesterone Patterns Of Subjects Taking Oral Contraceptives
***EST = 17-B-Estradiol, PROG = Progesterone**

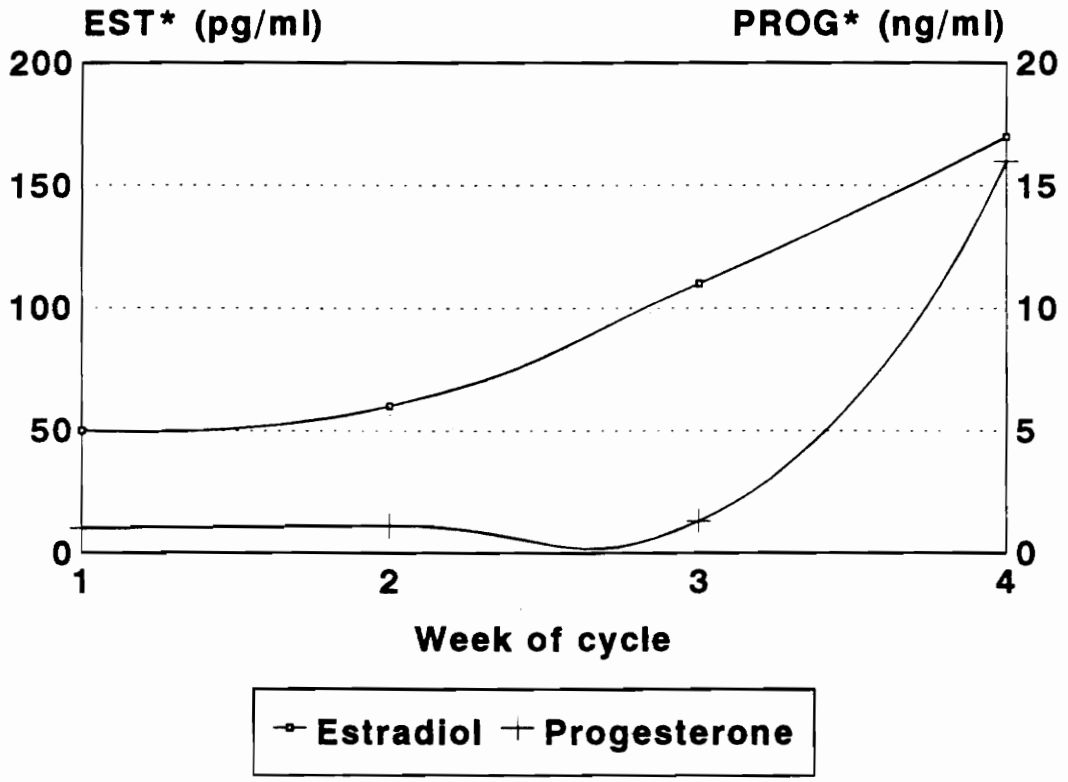


Figure 4 - Estrogen and Progesterone Patterns Of Subjects Not Taking Oral Contraceptives
***EST = 17-B-Estradiol, PROG = Progesterone**

TABLE 15
BASELINE MINERAL, HORMONE, AND BONE DENSITY MEASUREMENTS FOR ALL SUBJECTS*

Parameter		Measurement
Plasma Minerals	Calcium	8.59 ± 0.95 mg/dl
	Magnesium	1.72 ± 0.19 mg/dl
	Phosphorus	3.48 ± 0.43 mg/dl
	Boron	0.05 ± 0.04 μg/ml
Urine Minerals	Calcium	13.37 ± 6.61 mg/dl
	Magnesium	6.42 ± 3.03 mg/dl
	Phosphorus	0.74 ± 0.50 g/24 hours
	Boron	3.21 ± 2.32 μg/ml
Serum Hormones	Estradiol	74.8 ± 70.0 pg/ml
	Progesterone	4.0 ± 6.9 ng/ml
	Testosterone	0.4 ± 0.2 ng/ml
	Calcitonin	Non-detectable
	Parathyroid Hormone	Non-detectable
Bone Mineral Density	L2 Region of Spine	1.24 ± 0.13 g/cm^2
	L3 Region of Spine	1.26 ± 0.12 g/cm^2
	L4 Region of Spine	1.21 ± 0.12 g/cm^2

*Values represent means ± standard deviation

TABLE 16
BASELINE RESULTS OF DIETARY ANALYSIS FOR ALL SUBJECTS*

Nutrient	Three-Day Average
Total Energy	2016 ± 753 kcal
Protein	14.9 ± 4.5 % of total kcal
Carbohydrate	58.8 ± 11.1 % of total kcal
Fat	26.0 ± 10.7 % of total kcal
Calcium	977.7 ± 574.0 mg
Magnesium	282.6 ± 122.3 mg
Phosphorus	1440.5 ± 724.1 mg
Dietary Fiber	18.5 ± 10.2 g

*Values represent means ± standard deviation

TABLE 17
URINARY BORON VALUES*

Group	Time**	Urinary Boron (μg/ml)
Athletes (n=16)	1	3.8 ± 2.5
	2	2.8 ± 2.5
Non-Athletes (n=13)	1	2.5 ± 1.9
	2	2.6 ± 2.5
Boron (n=23)	1	3.8 ± 2.3
	2	3.0 ± 2.7
Placebo (n=6)	1	0.9 ± 0.2[a]
	2	1.4 ± 0.5
Oral Contraceptives (n=12)	1	4.1 ± 2.9
	2	2.9 ± 3.0
No Oral Contraceptives (n=17)	1	2.6 ± 1.5
	2	2.5 ± 2.0

*Values represent means ± standard deviation
**Time 1 = baseline; Time 2 = six months
[a]$p < 0.01$ for boron vs. placebo supplemented groups at Time 1

TABLE 18
SIGNIFICANT BASELINE PARAMETERS FOR ATHLETES AND NON-ATHLETES*

Parameter	Athletes (n=16)	Non-Athletes (n=13)
Predicted Maximal Oxygen Consumption (VO_{2MAX}) (ml/kg/minute)	48.6 ± 5.9	34.6 ± 4.6[a]
Dietary Fat (% Total Kcal)	23.1 ± 9.6	29.6 ± 6.6[b]
Serum Progesterone (ng/ml)	5.8 ± 8.4	1.8 ± 4.0[c]
Serum Estradiol (pg/ml)	81.0 ± 77.7	67.1 ± 64.8[a]

*Values represent means ± standard deviation
[a]$p < 0.01$ for athlete vs. non-athlete groups
[b]$p < 0.05$ for athlete vs. non-athlete groups
[c]$p < 0.01$ for athlete vs. non-athlete groups

by Nutritionist IV™, was significantly lower for the athletes as compared against the non-athletes. Serum progesterone and estradiol were significantly higher for the athletes as compared to the non-athletes.

Significant baseline parameters for boron and placebo supplemented groups may be found in Table 19. Dietary protein and magnesium were significantly higher in the boron group as compared to the placebo group. Serum testosterone was significantly lower in the boron supplemented group when compared against the placebo group.

Table 20 illustrates significant baseline parameters for oral contraceptive and no oral contraceptive groups. Subjects not taking oral contraceptives had significantly higher percentages of body fat. In addition, subjects taking oral contraceptives had significantly lower levels of serum estradiol and testosterone. Plasma phosphorus levels were significantly lower in subjects taking oral contraceptives.

Significant baseline parameters for activity and supplement groups are found in Table 21. Serum progesterone and estradiol levels were significantly lower in the boron supplemented athletes as compared to the athletes taking placebo. When comparing serum progesterone andestradiol levels between athletes on placebo to non-athletes on placebo, the athletes on placebo were found to

TABLE 19
SIGNIFICANT BASELINE PARAMETERS FOR BORON AND PLACEBO SUPPLEMENTED SUBJECTS*

Parameter	Boron (n=23)	Placebo (n=6)
Dietary Protein (% Total Kcal)	15.5 ± 2.9	12.5 ± 2.4[a]
Dietary Magnesium (mg)	298.8 ± 80.9	220.5 ± 69.2[a]
Serum Testosterone (ng/ml)	0.4 ± 11.5	0.6 ± 0.2[a]

*Values represent means ± standard deviation
[a]$p < 0.05$ for boron vs. placebo groups

TABLE 20
SIGNIFICANT BASELINE PARAMETERS FOR ORAL CONTRACEPTIVE AND NON ORAL CONTRACEPTIVE GROUPS*

Parameter	Oral Contraceptives (n=12)	No Oral Contraceptives (n=17)
Body Fat (%)	25.5 ± 6.2	30.4 ± 5.4[a]
Serum Estradiol (pg/ml)	39.3 ± 54.0	99.8 ± 72.7[a]
Serum Testosterone (ng/ml)	0.3 ± 0.2	0.5 ± 0.2[a]
Plasma Phosphorus (mg/dl)	3.2 ± 0.3	3.7 ± 0.4[a]

*Values represent means ± standard deviation
[a]$p < 0.05$ for oral contraceptive vs. no oral contraceptive groups

TABLE 21
SIGNIFICANT BASELINE PARAMETERS FOR ACTIVITY AND SUPPLEMENT GROUPS*

Parameter	Athletes		Non-Athletes	
	Boron (n=14)	Placebo (n=2)	Boron (n=9)	Placebo (n=4)
Serum Progesterone (ng/ml)	4.0 ± 7.2	18.2 ± 5.3[a]	2.3 ± 4.8	0.6 ± 0.3[b]
Serum Estradiol (pg/ml)	57.9 ± 46.6	242.5 ± 51.6[c]	71.6 ± 77.0	57.0 ± 27.9[d]
Plasma Calcium (mg/dl)	8.9 ± 0.4	8.0 ± 1.0[a]	8.1 ± 1.4	9.0 ± 0.6[e]

*Values represent means ± standard deviation
[a]$p < 0.05$ for athlete/boron vs. athlete/placebo groups
[b]$p < 0.01$ for athlete/placebo vs. non-athlete/placebo groups
[c]$p < 0.01$ for athlete/boron vs. athlete/placebo groups
[d]$p < 0.01$ for athlete/placebo vs. non-athlete/placebo groups
[e]$p < 0.05$ for athlete/boron vs. non-athlete/boron groups

have significantly higher levels of both hormones. Plasma calcium was significantly higher in the boron supplemented athletes, when compared against the athletes on placebo and the non-athletes on boron.

Table 22 illustrates the significant parameters for athletes and non-athletes at the beginning of the study and following an intervention of six months. All significant values represent the change in scores from baseline to six months time. Non-athletes had a significantly greater change in serum estradiol levels, while athletes had a significantly greater change in serum progesterone and testosterone levels. However, serum estradiol increased in both the athlete and non-athlete groups, while progesterone decreased in both groups. Serum testosterone decreased in the athletes, but did not change from the baseline measure in the non-athletes.

Significant parameters for boron and placebo supplemented groups are found in Table 23. All significant values represent the change in scores from baseline to six months time. Subjects taking placebo had a significantly greater change in dietary carbohydrate and dietary protein, as compared to the boron supplemented subjects. A reduction was noted with the dietary carbohydrates while there was an increase in dietary protein.

Significant parameters for oral contraceptive groups

TABLE 22
SIGNIFICANT PARAMETERS FOR ATHLETES AND NON-ATHLETES*

Parameter	Time**	Athletes (n=16)	Non-Athletes (n=13)
Serum EST*** (pg/ml)[a]	1	81.0 ± 77.7	67.1 ± 64.8
	2	90.1 ± 69.5	109.7 ± 92.7
Serum PROG*** (ng/ml)[b]	1	5.8 ± 8.4	1.8 ± 4.0
	2	4.0 ± 5.1	1.1 ± 1.0
Serum TEST*** (ng/ml)[a]	1	0.5 ± 0.2	0.4 ± 0.2
	2	0.4 ± 0.2	0.4 ± 0.2

*Values represent means ± standard deviation
**Time 1 = baseline; Time 2 = six months
***EST = Estradiol; PROG = Progesterone; TEST = Testosterone
[a]$p < 0.05$ for athlete vs. non-athlete change scores from baseline to six months time
[b]$p < 0.01$ for athlete vs. non-athlete change scores from baseline to six months time

TABLE 23
SIGNIFICANT FOR BORON AND PLACEBO SUPPLEMENTED SUBJECTS*

Parameter	Time**	Boron (n=23)	Placebo (n=6)
Dietary CHO*** (% kcal)[a]	1	58.5 ± 9.3	59.9 ± 5.1
	2	60.9 ± 8.5	52.1 ± 4.2
Dietary Protein (% kcal)[b]	1	15.5 ± 2.9	12.4 ± 2.4
	2	15.4 ± 3.8	14.9 ± 3.7

*Values represent means ± standard deviation
**Time 1 = baseline; Time 2 = six months
***CHO = Carbohydrate
[a]$p < 0.01$ for boron vs. placebo change scores from baseline to six months time
[b]$p < 0.05$ for boron vs. placebo change scores from baseline to six months time

are found in Table 24. All significant values represent a significance in the change scores from baseline to six months time. Serum estradiol had increased in both groups, with those subjects not taking oral contraceptives having a significantly greater change.

Table 25 illustrates the significant parameters for activity and supplement groups. All significant values represent the change in scores from baseline to six months months time. When comparing boron supplemented athletes with athletes taking placebo, the athletes taking placebo had a significantly greater change in serum progesterone, although both groups had decreased levels. Athletes taking placebo had a significantly greater change in serum progesterone than did non-athletes taking placebo. The change in serum estradiol was significantly greater in the athletes taking placebo as compared against the boron supplemented athletes. The change in plasma calcium was significantly greater in boron supplemented non-athletes when compared against boron supplemented athletes, although both groups had increased calcium levels.

TABLE 24
SIGNIFICANT PARAMETERS FOR SUBJECTS TAKING AND NOT TAKING ORAL CONTRACEPTIVES*

Parameter	Time**	Oral Contraceptives (n=12)	No Oral Contraceptives (n=17)
Serum Estradiol (pg/ml)[a]	1	39.3 ± 54.0	99.8 ± 72.7
	2	41.9 ± 39.6	139.2 ± 84.2

*Values represent means ± standard deviation
**Time 1 = baseline; Time 2 = six months
[a]$p < 0.05$ for oral contraceptive vs. no oral contraceptive change scores from baseline to six months time

TABLE 25
SIGNIFICANT PARAMETERS FOR ACTIVITY AND SUPPLEMENT GROUPS*

Parameter	Time**	Athletes		Non-Athletes	
		Boron (n=14)	Placebo (n=2)	Boron (n=9)	Placebo (n=4)
Serum PROG*** (ng/ml)[a,b]	1	4.0 ± 7.2	18.2 ± 5.3	2.3 ± 4.8	0.6 ± 0.3
	2	3.6 ± 4.9	7.0 ± 5.8	1.3 ± 1.1	0.7 ± 0.3
Serum EST*** (pg/ml)[a]	1	57.9 ± 46.6	242.5 ± 51.6	71.6 ± 77.0	57.0 ± 27.9
	2	85.3 ± 65.4	125.5 ± 85.5	105.3 ± 99.4	106.5 ± 75.5
Plasma Calcium (mg/dl)[c]	1	8.9 ± 0.4	8.0 ± 1.0	8.1 ± 1.4	9.0 ± 0.6
	2	9.4 ± 0.4	9.5 ± 0.2	9.5 ± 0.2	9.1 ± 0.2

*Values represent means ± standard deviation
**Time 1 = baseline; Time 2 = six months
***PROG = Progesterone; EST = Estradiol
[a]$p < 0.05$ for athlete/boron vs. athlete/placebo change scores from baseline to six months time
[b]$p < 0.01$ for athlete/placebo vs. non-athlete/placebo change scores from baseline to six months time
[c]$p < 0.05$ for athlete/boron vs. non-athlete/boron change scores from baseline to six months time

CHAPTER V

DISCUSSION

Menstrual Status

College-aged female athletes were initially targeted for this investigation for it was expected that many of them would have exercise-induced menstrual dysfunction. This expectation was based upon the findings of previous studies (Bullen et al. 1985, Drinkwater et al. 1986, Lindberg et al. 1984, McArdle et al. 1986), which reported that vigorous exercise is associated with menstrual dysfunction. In eumenorrheic individuals, estrogen has been associated with bone growth via an effect on calcium metabolism. However, this association is diminished with menstrual dysfunction and menopause, for estrogen levels decline. A decline in estrogen increases susceptibility to metabolic bone disorders (Cann et al. 1984, Davies and Littlewood 1979, Drinkwater et al. 1984, Lloyd et al. 1987, Mishell et al. 1972, Raven and Johnson 1991, Tortora and Anagnostakos 1990).

Bone Mineral Density

It was expected that subjects taking oral contraceptives would have significantly denser bones, as reported in the literature (Kleerekoper et al. 1991, Kritz-

Silverstein and Barrett-Connor 1993, Rucker et al. 1992). However, upon further investigation, subjects in the present investigation were taking oral contraceptives for only one to 30 months at the time of the BMD measurement (Appendix T), while previous studies (Kleerekoper et al. 1991, Kritz-Silverstein and Barrett-Connor 1993) indicated that benefits were seen in subjects taking oral contraceptives for at least six years.

Dietary Analysis

Baseline analysis of the diet revealed that intake of fat was significantly different between athletes and non-athletes, while protein and magnesium were significantly different between boron and placebo supplemented groups. Non-athletes consumed more fat than did the athletes, and those in the boron supplemented group consumed more protein and magnesium than did the placebo group. Although subjects consumed self-selected diets, subjects taking placebo had a significantly greater change in dietary intake of carbohydrate and protein than did boron supplemented subjects. Recker and associates (1992) reported that college-females consumed 77 ± 22 g fat/day and 65 ± 15 g protein/day, which were very similar to the levels consumed by all subjects in the present investigation. Contrary to the results of the present study, Lloyd and colleagues

(1987) reported that eumenorrheic athletes consumed more fat (80 ± 5 g/day) than non-athletes (67 ± 5 g/day). According to Lloyd and associates (1987) and Recker and co-workers (1992), college females have been reported to consume 48% of total calories as carbohydrate, while all subjects in the present study consumed diets higher in carbohydrate.

Calcium and phosphorus should be consumed in a ratio of 1:1, as stated by the National Research Council (1989), while the ratio for the present investigation was 0.76 for (Appendix T) all subjects. Alterations in this ratio, with phosphorus being greater, may lead to an increased risk of bone fracture (Jowsey 1976). This is of concern, for many young females consume diets high in phosphorus, due to the high phosphorus content in soft drinks, and thus phosphorus interferes with calcium absorption (Jowsey 1976, Wyshak et al. 1989, Calvo et al. 1990).

Plasma Mineral Analysis

Other boron supplementation studies have assessed the effects of boron on plasma mineral status (Nielsen et al. 1987, Nielsen et al. 1988, Nielsen 1989, Nielsen et al. 1990, Darnton 1991). Nielsen and associates (1987, 1988) have reported that boron supplementation significantly influenced serum ionized calcium when dietary intake of magnesium was low. Boron supplementation has been found to

significantly lower plasma ionized calcium and copper during periods of boron depletion (Nielsen 1989, Nielsen et al. 1990). Darnton (1991) reported that boron supplemented athletes had significantly lower serum magnesium.

From the mineral analyses previously mentioned, plasma magnesium (Appendix T) was the only similar measurement made during the present investigation. However, similar results were not observed. Analysis of baseline plasma calcium between boron supplemented athletes and non-athletes, as well as athletes taking boron and placebo revealed significant differences between the groups prior to treatment. Changes in plasma calcium were significant between boron supplemented athletes and non-athletes after supplementation in the present study. Since differences were not seen between supplementation groups, it may be suggested that the differences are due to the level of activity. However, since vitamin D interacts with calcium metabolism, and vitamin D synthesis varies with season, it is possible that changes in plasma calcium are related to seasonal changes associated with vitamin D (Combs 1992, DeLuca 1992, Boden and Kaplan 1990). Nielsen (1990) reported that plasma total calcium may be elevated under conditions of boron deprivation, which may cause depressed levels of plasma ionized calcium and calcitonin, but similar results were not found in the present investigation.

Darnton (1991) found changes in serum phosphorus levels associated with supplementation, with the boron supplemented sedentary subjects having the lowest levels, although similar changes were not observed in the present investigation (Appendix T). The only significant difference observed with plasma phosphorus was a baseline measurement between oral contraceptive groups.

Urinary Mineral Analysis

Previous boron investigations have assessed the effect of boron on urinary mineral levels (Nielsen et al. 1987, Nielsen et al. 1988, Nielsen 1989, Nielsen et al. 1990, Darnton 1991). Nielsen and associates (1987, 1988) reported that boron supplementation significantly reduced urinary calcium, phosphorus, and magnesium when dietary intake of magnesium was low. Similar results were not found in the present investigation (Appendix T). Boron supplemented subjects in the present investigation did not have increased urinary excretion of boron as expected, and reported by Darnton (1991). Although Darnton (1991) reported an increase in urinary boron excretion after supplementation, the observation from the present investigation suggests that boron may be excreted in fecal matter, possibly by a similar mechanism as with copper and manganese (Cikrt 1972, Klaassen 1974).

Serum Hormone Analysis

Estrogen and progesterone patterns associated with menstrual cycle phases for subjects in the present investigation do not correspond to established hormone patterns, as reported by Raven and Johnson (1990). Estrogen levels are expected to peak just before week two of the menstrual cycle, while progesterone is expected to peak at two and one-half weeks. Serum hormone analyses revealed low estrogen levels in ten subjects (Appendix T), but these subjects were taking oral contraceptives. This observation was consistent with that reported by Mishell and colleagues (1972), except that subjects were not in the early follicular phase, but rather in the luteal phase of the cycle. As previously mentioned, hormonal changes occur with each phase of the menstrual cycle. It is important to note that both baseline and six month blood samplings were taken from subjects during the same phase of the their menstrual cycles (Appendix T), so that a subject who was at week one of her cycle at baseline collection was also at week one for her six month collection. However, as a group, not all subjects were at the same phase of the cycle during the blood collections, which may account for the various hormone ranges observed (Appendix T). It appears that most changes observed in the present study are due to the individual differences of the menstrual cycle, rather than to activity,

or supplementation.

When comparing subjects who do and do not use oral contraceptives, baseline analysis of serum estradiol and testosterone revealed significant differences between these groups. Also, there was a significant change in scores from baseline to six months time for estradiol between the oral contraceptive groups. These differences may be due to the oral contraceptives, for both groups were at similar phases of the menstrual cycle (Appendix T) when blood samples were collected, and no significance was found between oral contraceptive and activity groups or between oral contraceptive and supplement groups.

Oral contraceptive interactions between activity level and/or supplementation did not yield significant results with any hormone analysis (Table U), which eliminated this variable as a reason for the significant differences. With the remaining significant hormone observations in the present investigation, the significance may be attributed to group variation of menstrual cycle phases, rather than to an effect of activity or supplement, for these group differences with the menstrual cycle phases may be the reason for the wide variation in hormone levels.

Nielsen and colleagues (1987) reported increases in serum 17-ß-estradiol in boron supplemented postmenopausal women. Since estradiol levels were within normal ranges for

subjects in the present study, the effects of boron may not have been as noticeable as with investigations with postmenopausal women, who have low levels of estradiol.

Conclusions

1. Boron supplementation did not appear to affect any of the parameters measured.
2. Activity level significantly affected plasma calcium, with athletes having higher levels. However, the non-athletes experienced a greater change in plasma calcium than did athletes.
3. Oral contraceptive use significantly influenced serum estradiol. Subjects taking oral contraceptives had significantly lower levels of serum estradiol compared to subjects not taking oral contraceptives.

Although no significant differences were found with boron supplementation, the present investigation had too many overlapping variables, which may have hidden any effects due to boron supplementation. Had there been more subjects, statistical analyses could have been performed on the interactions between activity, supplementation, and oral contraceptive groups. These additional analyses would have allowed any effects due to boron to be seen more clearly. In addition, better control of the menstrual cycle phases

between subjects was needed, for this factor may have also hidden any effects due to boron supplementation. Since it was not too difficult to schedule additional blood collections based upon menstrual records once the initial blood collection was taken, there may have been more control by attempting to schedule the initial blood collection during a certain phase of the cycle so that all subjects would be near the same phase of the cycle, or even schedule weekly blood collections for one month at baseline and six months so that individual hormone patterns could be better identified.

CHAPTER VI

RECOMMENDATIONS FOR FUTURE RESEARCH

From the results of the present investigation, it may be suggested that more research is needed with boron to determine any essential effects it may have as a nutrient. The following are recommendations for future research:

1. Studying the effects of boron supplementation with any of the following subject groups: young females with menstrual dysfunction, young and older females who use oral contraceptives, young and older women with varying levels of activity, young and older men with varying levels of activity, and children. The above subject groups are of interest, for most current research focuses on postmenopausal women (Nielsen et al. 1987, 1988, 1989, 1990). The female groups mentioned above may also be at risk of metabolic bone disorders, as are postmenopausal women, due to menstrual dysfunction and extreme exercise patterns (Cann et al. 1984, Myerson et al. 1992, Myburgh et al. 1990, Drinkwater et al. 1984, 1986, Rutherford 1993, Lloyd et al. 1987, 1988). Men and children have yet been investigated for any effect of boron supplementation.
2. Studying the effects of boron supplementation on

hormone status with subjects who do and do not use oral contraceptives. These subject groups may be of interest since oral contraceptives may be protective of bone mass, thus possibly slowing the process of bone demineralization (Kritz-Silverstein and Barrett-Connor 1993, Recker et al. 1992, Kleerekoper et al. 1991). The phases of the menstrual cycle need to be as controlled as possible. In addition, various lengths of time that oral contraceptives are used could be observed.

3. A long-term investigation is needed with any of the above mentioned subject groups to determine the role of boron in bone metabolism, since the mechanisms of action are unknown.

REFERENCES

Abou-Shakra FR, Havercroft J, Ward N. Lithium and boron in biological tissues and fluids. Trace Elements in Medicine. 1989;6:142-146.

Abraham GE. Radioimmumoassay of estradiol-17-ß in plasma. In: Breuer H, Hamel D, Kruskemper HC, eds. Methods of Hormone Analysis. Stuttgart, Germany: Georg Thieme Verlag; 1976.

Abraham GE, Grewal H. A total dietary program emphasizing magnesium instead of calcium. Journal of Reproductive Medicine. 1990;35:503-507.

Abu-Samra A, Morris JS, Koirtyhann S. Wet ashing of some biological samples in a microwave oven. Analytical Chemistry. 1975;47:1475-1477.

Alexander GV, Nusbaum RE, MacDonald NS. The boron and lithium content of human bones. Journal of Biological Chemistry. 1951;192:489-496.

Aloia JF. Osteoporosis: A Guide to Prevention and Treatment. Champaign, IL: Leisure Press; 1989.

Anderson JJB. Dietary calcium and bone mass through the life cycle. Nutrition Today. 1990;25:9-14.

Arnaud CD, Sanchez SD. The role of calcium in osteoporosis. Annual Review of Nutrition. 1990;10:397-414.

Avioli LV. Calcium and osteoporosis. Annual Review of Nutrition. 1984;4:471-491.

Avioli LV. Calcium and phosphorus. In: Shils ME and Young VR, eds. Modern Nutrition in Health and Disease. 7th ed. Philadelphia, PA: Lea and Febiger; 1988;142-158.

Avioli LV. Significance of osteoporosis: a growing international health problem. Calcified Tissue International. 1991;49(Suppl):S5-S7.

Bevan BR. General survey of techniques of analysis for hormonal substances. In: Pennington GW, Naik S, eds. Hormone Analysis: Methodology and Clinical Interpretation. Volume I. Boca Raton, FL: CRC Press, Inc.; 1981.

Bloomfield SA, Williams NI, Lamb DR, Jackson RD. Non-weightbearing exercise may increase lumbar spine bone mineral density in healthy postmenopausal women. American Journal of Physiology. 1993;72:204-209.

Boden SD, Kaplan FS. Calcium homeostasis. Orthopedic Clinics of North America. 1990;21:31-42.

Body JJ, Heath H. Nonspecific increases in plasma immunoreactive calcitonin in healthy individuals. Clinical Chemistry. 1984;30:511-514.

Borg G. Subjective effort in relation to physical performance and working capacity. In: Pick HL, ed. Psychology: From Research to Practice. New York, NY: Plenum Publishing Corporation; 1978:331-361.

Bullen BA, Skrinar GS, Beitins IZ, von Mering G, Turnbull BA, McArthur JW. Introduction of menstrual disorders by strenous exercise in untrained women. The New England Journal of Medicine. 1985;312:1349-1353.

Calvo MS, Kumar R, Heath H. Persistently elevated parathyroid hormone secretion and action in young women after four weeks of ingesting high phosphorus, low calcium diets. Journal of Clinical Endocrinology and Metabolism. 1990;70:1334-1340.

Cann CE, Martin MC, Genant HK, Jaffe RB. Decreased spinal mineral content in amenorrheic women. Journal of the American Medical Association. 1984;251:626-629.

Caplan GA, Ward JA, Lord SR. The benefits of exercise in postmenopausal women. Australian Journal of Public Health. 1993;17:23-26.

Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaue S, Delmas PD, Meunier PJ. Vitamin D_3 and calcium to prevent hip fractures in elderly women. The New England Journal of Medicine. 1992;327:1637-1642.

Chernoff R, Lipschitz DA. Nutrition and Aging. In: Shils ME, Young VR, eds. Modern Nutrition in Health and Disease. 7th ed. Philadelphia, PA: Lea and Febiger; 1988.

Chestnut CH. Theoretical overview: bone development, peak bone mass, bone loss, fracture risk. American Journal of Medicine. 1991;91(Suppl 5B):2S-4S.

Chestnut CH, Baylink DJ, Sisom K, Nelp WB, Roos BA. Basal plasma immunoreactive calcitonin in postmenopausal osteoporosis. Metabolism. 1980;29:559-562.

Cikrt M. Biliary excretion of ^{203}Hg, ^{64}Cu, ^{52}Mn, and ^{210}Pb in the rat. British Journal of Industrial Medicine. 1972;29:74-80.

Civitelli R, Agnusdei D, Nardi P, Zacchei F, Avioli LV, Gennari C. Effects of one-year treatment with estrogens on bone mass, intestinal calcium absorption, and 25-hydroxyvitamin D-1α-Hydroxylase reserve in postmenopausal osteoporosis. Calcified Tissue International. 1988a;42:77-86.

Civitelli R, Gonnelli S, Zacchei F, Bigazzi S, Vattimo A, Avioli LV, Gennari C. Bone turnover in postmenopausal osteoporosis. Journal of Clinical Investigation. 1988b;82:1268-1274.

Clark AP, Schuttinga JA. Targeted estrogen/progesterone replacement therapy for osteoporosis: calculation of health care cost savings. Osteoporosis International. 1992;2:195-200.

Clarke WB, Koekebakker M, Barr RD, Downing RG, Fleming RF. Analysis of ultratrace lithium and boron by neutron activation and mass-spectrometric measurement of ^{3}He and ^{4}He. Applied Radiation and Isotopes. 1987;38:735-743.

Combs GE. The Vitamins: Fundamental Aspects in Nutrition and Health. San Diego, CA: Academic Press, Inc.; 1992.

Cosman F, Shen V, Xie F, Seibel M, Ratcliffe A, Lindsay R. Estrogen protection against bone resorbine effects of parathyroid hormone infusion. Assessment by use of biochemical markers. Annals of Internal Medicine. 1993;118:337-343.

Darnton SM. The effects of boron supplementation on bone mineral density, blood and urinaty calcium, magnesium, phosphorus and urinary boron in female athletes. (dissertation). 1991.

Davies J, Littlewood BS. Elementary Biochemistry: An Introduction to the Chemistry of Living Cells. Englewood Cliffs, NJ: Prentice-Hall, Inc.; 1979.

Dawson-Hughes B, Harris S, Dallal GE. Serum ionized calcium, as well as phosphorus and parathyroid hormone, is associated with the plasma 1,25-dihydroxyvitamin D_3 concentration in normal postmenopausal women. Journal of Bone and Mineral Research. 1991;6:461-468.

Dawson-Hughes B, Jacques P, Shipp C. Dietary calcium intake and bone loss from the spine in healthy postmenopausal women. The American Journal of Clinical Nutrition. 1987;46:685-687.

Delmas PD. Biochemical markers of bone turnover in osteoporosis. In: Riggs BL, Melton LJ, III, eds. Osteoporosis: Etiology, Diagnosis, and Management. New York, NY: Raven Press, Inc.; 1988.

Deluca HF. New concepts of vitamin D functions. Annuals of New York Academy of Science. 1992;669:59-68.

Deluca HF. Vitamin D and its metabolites. In: Shils ME, Young VR, eds. Modern Nutrition in Health and Disease. 7th ed. Philadelphia, PA: Lea and Febiger; 1988.

Diagnostic Products Corporation. Calcitonin Double Antibody Package Insert. Los Angeles, CA: 1992.

Diagnostic Products Corporation. Estradiol Coat-A-Count Package Insert. Los Angeles, CA: 1991.

Diagnostic Products Corporation. Progesterone Coat-A-Count Package Insert. Los Angeles, CA: 1993.

Diagnostic Products Corporation. PTH Double Anitbody Package Insert. Los Angeles, CA: 1993.

Diagnostic Products Corporation. Total Testosterone Coat-A-Count Package Insert. Los Angeles, CA: 1992.

Draper HH, Scythes CA. Calcium, phosphorus, and osteoporosis. Federation Proceedings. 1981;40:2434-2438.

Drinkwater BL, Nilson K, Chestnut CH, Bremner WJ, Shainholtz S, Southworth MB. Bone mineral content of amenorrheic and eumenorrheic athletes. The New England Journal of Medicine. 1984;311:277-281.

Drinkwater BL, Nilson K, Ott S, Chestnut CH. Bone mineral density after resumption of menses in amenorrheic athletes. Journal of The American Medical Association. 1986;256:380-382.

Dugger WM. Boron in plant metabolism. In: Lauchli A, Bieleski RL, eds. Inorganic Plant Nutrition, Encyclopedia of Plant Physiology. Vol. 15. Springer-Verlag, Heidelberg; 1983:626-650.

Einhorn TA, Levine B, Michel P. Nutrition and bone. Orthopedic Clinics of North America. 1990;21:43-50.

El-Hajj-Fuleihan G, Brown EM, Curtis K, Berger MJ, Berger BM, Gleason R, LeBoff MS. Effect of sequential and daily continuous hormone replacement therapy on indexes of mineral metabolism. Archives of Internal Medicine. 1992;152:1904-1909.

Fallon MD. Assessment of bone structure. In: Shils ME, Young VR, eds. Modern Nutrition in Health and Disease. 7th ed. Philadelphia, PA: Lea and Febiger; 1988;861-886.

Fardon DF. Osteoporosis: Your Head Start on the Prevention and Treatment of Brittle Bones. New York, NY: Macmillan Publishing Company; 1985.

Felsenfeld AJ, Ross D, Rodriguez M. Hysteresis of the parathyroid hormone repsponse to hypocalcemia in hemodialysis patients with low turnover aluminum bone disease. Journal of the American Society of Nephrology. 1991;2:1136-1143.

Felson DT, Zhang Y, Hannan MT, Kiel DP, Wilson PW, Anderson JJ. The effect of postmenopausal estrogen therapy on bone density in elderly women. The New England Journal of Medicine. 1993;329:1141-1146.

Ferrando AA, Green NR, Barnes KW, Woodward B. Microwave digestion preparation and ICP determination of boron in human plasma. Biological Trace Element Research. 1993;37:17-25.

Forbes RM, Cooper AR, Mitchell HH. The occurrence of beryllium, boron, cobalt, and mercury in human tissue. Journal of Biological Chemistry. 1954;209:857-865.

Free AH, Free HM. Urinalysis in Clinical Laboratory Practice. Cleveland, OH: CRC Press, Inc.; 1975.

Friis-Hansen B, Aggerbeck B, Aas Jansen J. Unaffected blood boron levels in newborn infants treated with a boric acid ointment. Food and Chemical Toxicology. 1982;20:451-454.

Gallagher JC. Drug therapy of osteoporosis: calcium, estrogen, and vitamin D. In: Riggs BL, Melton LJ, III, eds. Osteoporosis: Etiology, Diagnosis, and Management. New York, NY: Raven Press; 1988.

Genant HK, Vogler JB, Block JE. Radiology of osteoporosis. In: Riggs BL, Melton LJ, III, eds. Osteoporosis: Etiology, Diagnosis, and Management. New York, NY: Raven Press; 1988.

Golding L, Meyerc C, Sinning W. The Y's Way to Physical Fitness. 2nd ed. Rosemont, IL: YMCA; 1982.

Grimston SK, Tanguay KE, Gundberg CM, Hanley DA. The calcitropic hormone response to changes in serum calcium during exercise in female lone distance runners. Journal of Clinical Endocrinology and Metabolism. 1993;76:867-872.

Grove KA, Londeree BR. Bone density in postmenopausal women: high impact vs. low impact exercise. Medicine and Science in Sports and Exercise. 1992;24:1190-1194.

Hamilton EL, Minski MJ, Cleary JJ. The concentration and distribution of some stable elements in healthy human tissues from the United Kingdom: an environmental study. Science of the Total Environment. 1972-73;1:341-344.

Hansen MA, Hassager C, Overgaard K, Marslew U, Riis BJ, Christiansen C. Dual-energy x-ray absorptiometry: a precise method of measuring bone mineral density in the lumbar spine. The Journal of Nuclear Medicine. 1990;31:1156-1162.

Hatori M, Hasegawa A, Adachi H, Shinozaki A, hayashi R, Okano H, Mizunuma H, Murta K. The effects of walking at the anerobic threshold level on vertebral bone loss in postmenopausal women. Calcified Tissue International. 1993;52:411-414.

Heaney RP. Calcium, bone health, and osteoporosis. In: Peck WA, ed. Bone and Mineral Research. New York, NY: Elsevier Science Publishers; 1986:255-301.

Hegsted DM. Calcium and osteoporosis. Journal of Nutrition. 1986;116:2316-2319.

Hegsted M, Keenan MJ, Saver F, Wozniak P. Effect of boron on vitamin D deficient rats. Biological Trace Element Research. 1991;28:243-255.

Hesch RD, Busch U, Prokop M, Delling G, Rittinghaus EF. Increase of vertebral density by combination therapy with pulsatile 1-38hPTH and sequential addition of calcitonin nasal spray in osteoporotic patients. Calcified Tissue International. 1989;44:176-180.

Hesch RD, Hufner M. von zue Muhlen A. Radioimmunoassay of calcitonin (HCT) in serum. In: Breuer H, Hamel D, Kruskemper HC, eds. Methods of Hormone Analysis. Stuttgart, Germany: Georg Thieme Verlag; 1976.

Heynen G, Franchimont P. Human calcitonin radioimmunoassay in normal and pathological conditions. European Journal of Clinical Investigation. 1974;4:213-222.

Horowitz M, Need AG, Philcox JC, Nordin BE. Effect of calcium supplementation on urinary hydroxyproline in osteoporotic postmenopausal women. The American Journal of Clinical Nutrition. 1984;39:857-859.

Hove E, Elvehjem CA, Hart EB. Boron in animal nutrition. American Journal of Physiology. 1939;127:689-701.

Hreshchyshyn MM, Hopkins A, Zylstra S, Anbar M. Associations of parity, breast-feeding, and birth control pills with lumbar spine and femoral neck bone densities. American Journal of Obstetrics and Gynecology. 1988;159:318-322.

Hunt CD. Dietary boron modified the effects of magnesium and molybdenum on mineral metabolism in the cholecalciferol-deficient chick. Biological Trace Element Research. 1989;22:201-220.

Hunt CD. Personal communication via Darnton SM. 1991.

Hunt CD, Nielsen FH. Dietary boron affects bone calcification in magnesium and cholecalciferol deficient chicks. Proceeding of the 6th International Symposium on Trace Element Metabolism in Man and Animals. In: Hurley LS, Keen CL, Lonnerdal B, Rucker RB, eds. Trace Elements in Man and Animals. New York, NY: Plenum Press; 1988.

Hunt CD, Nielsen FH. Interaction between boron and cholecalciferol in the chick. Proceedings of the 4th International Symposium on Trace Element Metabolism in Man and Animals. In: McCHowell J, Gawthrone LM, White CL, eds. Trace Element Metabolism in Man and Animals. Canaba City, Australia: Australian Academy of Science; 1981.

Hunt CD, Shuler TR. Open-vessel, wet-ash, low-temperature digestion of biological materials for inductively coupled argon plasma spectroscopy (ICAP) analysis of boron and other elements. Journal of Micronutrient Analysis. 1989;6:161-174.

Hunt SA, Groff JL. Advanced Nutrition and Human Metabolism. St. Paul, MN: West Publishing Co.; 1990.

Hurley DL, Tiegs RD, Wahner HW, Heath H. Axial and appendicular bone mineral density in patients with long-term deficiency or excess of calcitonin. The New England Journal of Medicine. 1987;317:537-541.

Johnston CC Jr, Slemenda CW, Melton LJ III. Clinical use of bone densiometry. The New England Journal of Medicine. 1991;324:1105-1109.

Jowsey J. Osteoporosis: its nature and the role of the diet. Postgraduate Medicine. 1976;60:75-79.

Karlsson R, Eden S, von Schoultz B. Oral contraception affects osteocalcin serum profiles in young women. Osteoporosis International. 1992;2:118-121.

Khaw KT, Sneyd MJ, Compston J. Bone density parathyroid hormone and 25-hydroxyvitamin D concentrations in middle aged women. British Medical Journal. 1992;305:273-277.

Kim Y, Schutte S. Magnesium metabolism as affected by the levels of calcium and phosphorus intake in the young adult male. FASEB Proceedings. 1991;A1313.

Klaassen CD. Biliary excretion of manganese in rats, rabbits, and dogs. Toxicology and Applied Pharmacology. 1974;29:458-468.

Kleerekoper M, Brienza RS, Schultz LR, Johnson CC. Oral contraceptive use may protect against low bone mass. Archives of Internal Medicine. 1991;151:1971-1976.

King N, Odom TW, Sampson HW, Pardue SL. In ovo administration of boron alters bone mineralization of the chick embryo. Biological Trace Element Research. 1991a;30:47-58.

King N, Odom TW, Sampson HW, Yersin AG. The effect of in ovo boron supplementation of bone mineralization of the vitamin D-deficient chick embryo. Biological Trace Element Research. 1991b;31:223-233.

Kollerup G, Hermann AP, Brixen K, Lindblad BE, Mosekilde L, Sorensen OH. Effects of salmon calcitonin suppositories on bone mass and turnover in established osteoporosis. Calcified Tissue International. 1994;54:12-15.

Kritz-Silverstein D, Barrett-Connor E. Bone mineral density in postmenopausal women as determined by prior oral contraceptive use. American Journal of Public Health. 1993;83:100-102.

Kutsky RJ. Handbook of Vitamins, Minerals, and Hormones. 2nd ed. New York, NY: Van Nostrand Reinhold, Co.; 1981.

Laitinen K, Valimaki M, Keto P. Bone mineral density measured by dual-energy x-ray absorptiometry in healthy Finnish women. Calcified Tissue International. 1991;48:224-231.

Lau EMC, Woo J, Leung PC, Swaminathan R, Leung D. The effects of calcium supplementation and exercise on bone density in elderly Chinese women. Osteoporosis International. 1992;2:168-173.

Leyendecker G, Wardlaw S, Nocke W. Radioimmunoassay of 17-hydroxyprogesterone in serum. In: Breuer H, Hamel D, Krusemper HC, eds. Methods of Hormone Analysis. Stuttgart, Germany: Georg Thieme Verlag; 1976.

Lidor C, Dagiv P, Amdur B, Gepstein R, Otremski I, Hallel T, Edelstein S. Decrease in bone levels of 1,25-dihydroxyvitamin D in women with subcapital fracture of the femur. Calcified Tissue International. 1993;52:146-148.

Lindbereg JS, Fears WB, Hunt MM, Powell MR, Boll D, Wade C. Exercise-induced amenorrhea and bone density. Annals of Internal Medicine. 19984;101:747-648.

Lloyd T, Buchanan JR, Bitzer S, Waldman CJ, Myers C, Ford BG. Interrelationships of diet, athletic activity, menstrual status, and bone denisty in collegiate women. American Journal of Clinical Nutrition. 1987;46:681-684.

Lloyd T, Myers C, Buchanan JR, Demers LM. Collegiate women athletes with irregular menses during adolescence have decreased bone density. Obstetrics and Gynecology. 1988;72:639-642.

Lloyd T, Buchanan JR, Ursino GR, Myers C, Woodward G, Halbert DR. Long-term oral contraceptive use does not affect trabecular bone density. American Journal of Obstetrics and Gynecology. 1989;160:402-404.

Lohman TG. Research relating to assessment of skeltal status. In: Roche AF, ed. Body-Composition Assessments in Youth and Adults. Report of the 6th Ross Conference on Medical Research. Columbus, OH: Ross Laboratories; 1985.

Lovatt CJ, Dugger WM. Boron. In: Frieden E, ed. Biochemistry of the Essential Ultratrace Elements. New York, NY: Plenum Press; 1984:389-421.

Lufkin EG, Wahner HW, O'Fallon WM, Hodgson SF, Kotowicz MA, Lane AW, Judd HL, Caplan RH, Riggs BL. Treatment of postmenopausal osteoporosis with transderman estrogen. Archives of Internal Medicine. 1992;117:1-9.

Lukert B, Higgins J, Stoskopf M. Menopausal bone loss is partially regulated by dietary intake of vitamin D. Calcified Tissue International. 1992;51:173-179.

MacIntyre I, Whitehead MI, Banks LM, Stevenson JC, Wimalawansa SJ, Healy MJR. Calcitonin for prevention of postmenopausal bone loss. The Lancet. 1988;1:900-902.

Marcus R, Cann C, Madvig P, Minkoff J, Goddard M, Bayer M, Martin M, Gaudiani L, Haskell W, Genant H. Menstrual function and bone mass in elite women distance runners. Annals of Internal Medicine. 1985;102:158-163.

Marcus R, Kosek J, Pfefferbaum A, Horning S. Age-related loss of trabecular bone in premenopausal women: a biopsy study. Calcified Tissue International. 1983;35:406-409.

Marks SC, Popoff SN. Bone cell biology: the regulation of development, structure and function in the skeleton. The American Journal of Anatomy. 1988;183:1-44.

Martin KA, Freeman MW. Postmenopausal hormone-replacement therapy. The New England Journal of Medicine. 1993;328:1115-1117.

Matkovic V. Diet, genetics, and peak bone mass of adolescent girls. Nutrition Today. 1991;26:21-24.

Mazess RB, Barden HS. Bone densiometry for diagnosis and monitoring osteoporosis. Society for Experimental Biology and Medicine. (Lunar Corporation) Madison, WI. 1989.

Mazess RB, Barden HS. Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills. American Journal of Clinical Nutrition. 1991;53:132-142.

Mazess RB, Wahner HM. Nuclear medicine and densiometry. In: Riggs BL, Melton LJ, III, eds. Osteoporosis: Etiology, Diagnosis, and Management. New York, NY: Raven Press; 1988.

McArdle WD, Katch FI, Katch VL. Exercise Physiology: Energy, Nutrition, and Human Performance. 2nd ed. Philadelphia, PA: Lea and Febiger; 1986.

McCoy RH. Dietary requirements in the rat. In: Farris RJ, Griffeth JQ, eds. The Rat in Laboratory Investigation. New York, NY: Hafner Publishing Co.; 1967:68-103.

Michell DR, Throneycroft IH, Nakamura RM. Serum estradiol in women ingesting combination oral contraceptive steroids. American Journal of Obstetrics and Gynecology. 1972;114:923-928.

Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. Nature. 1994;367:284-287.

Morrison NA, Yeoman R, Kelly PJ, Eisman JA. Contributing of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphisms and circulating osteocalcin. Proceedings of the National Academy of Science. 1992;89:6665-6669.

Mundy GR. Boning up on genes. Nature. 1994;367:216-217.

Murphy S, Khaw KT, Sneyd MJ, Compston JE. Endogenous sex hormones and bone mineral density among community-based postmenopausal women. Postgraduate Medical Journal. 1992;68:908-913.

Myburgh KH, Hutchins J, Fataar AB, Hough SF, Noakes TD. Low bone density is an etiologic factor for stress fractures in athletes. Annals of Internal Medicine. 1990;113:754-759.

Myerson M, Gutin B, Warren MP, Wang J, Lichtman S, Pierson RN. Total body bone density in amenorrheic runners. Obstetrics and Gynecology. 1992;79:973-978.

National Institute of Health. Osteoporosis - Consensus Conference. Journal of The American Medical Association. 1984;252:799-802.

National Research Council. Recommended Dietary Allowances. 10th ed. Washington, DC: National Academy Press; 1989.

Newnham RE. Mineral imbalance and boron deficiency. Proceedings of the 4th International Symposium on Trace Element Metabolism in Man and Animals. In: McCHowell J, Gawthrone LM, White CL, eds. Trace Element Metabolism in Man and Animals. Canberra City, Australia: Australian Academy of Science; 1981.

Ng HT, Chang SP, Yang TS, Cho MP, Wei TC. Estradiol administered in a percutaneous gel for the prevention of postmenopausal bone loss. Asia Oceania Journal of Obstetrics and Gynaecology. 1993;19:115-119.

Nielsen FH. Effect of boron depletion and repletion on calcium and copper status indices in humans fed a magnesium-low diet. FASEB Journal. 1989;3:A760.

Nielsen FH. New essential trace elements for the life sciences in Nuclear Analytical Methods in the Life Sciences. Biological Trace Element Research. 1990;26-27:599-611.

Nielsen FH, Hunt CD, Mullen LM, Hunt JR. Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. FASEB Journal. 1987;1:394-397.

Nielsen FH, Gallagher SK, Johnson LK, Nielsen EJ. Boron enhances and mimics some effects of estrogen therapy in postmenopausal women. The Journal of Trace Elements in Experimental Medicine. 1992a;5:237-246.

Nielsen FH, Mullen LM, Gallagher SK. Effect of boron depletion and repletion on blood indicators of calcium status in humans fed a magnesium-low diet. The Journal of Trace Elements in Experimental Medicine. 1990;3:45-54.

Nielsen FH, Mullen LM, Gallagher SK, Hunt JR, Hunt CD, Johnson LK. Effects of dietary boron, aluminum, and magnesium on serum alkaline phosphatase, calcium and phosphorus, and plasma cholesterol in postmenopausal women. Proceeding of the 6th International Symposium on Trace Element Metabolism in Man and Animals. In: Hurley LS, Keen CL, Lonnerdal B, Rucker RB, eds. Trace Elements in Man and Animals. New York, NY: Plenum Press; 1988.

Nielsen FH, Mullen LM, Nielsen EJ. Dietary boron affects blood cell counts and hemoglobin concentrations in humans. The Journal of Trace Elements in Experimental Medicine. 1991;4:211-223.

Nielsen FH, Shuler TR. Studies of the interaction between boron and calcium, and its modification by magnesium and potassium in rats: effects on growth, blood variables, and bone mineral composition. Biological Trace Element Research. 1992;35:225-237.

Nielsen FH, Shuler TR, Uthus EO. Dietary arginine and methionine effects and their modification by dietary boron and potassium, on the mineral element comopsition of plasma and bone in the rat. The Journal of Trace Elements in Experimental Medicine. 1992b;5:247-259.

Nieschlag E. Radioimmunoassay of testosterone in plasma. In: Breuer H, Hamel D, Kruskemper HC, eds. Methods of Hormone Analysis. Stuttgart, Germany: Georg Thieme Verlag; 1976.

Notelovitz M. Osteoporosis: screening, prevention, and management. Fertility and Sterility. 1993;59:707-725.

O'Riordan JLH, Woodhead JS. Immunoradiometric assay of parathyroid hormone in serum. In: Breuer H, Hamel D, Kruskemper HC, eds. Methods of Hormone Analysis. Stuttgart, Germany: Georg Thieme Verlag; 1976.

O'Sullivan MJ. Enzyme immumoassay. In: Butt WR, ed. Practical Immunoassay: The State of the Art. New York, NY: Marcel Dekker, Inc.; 1984.

Overgaard K, Hansen MA, Dirksen KL, Christiansen C. Rectal salmon calcitonin for the treatment of postmenopausal osteoporosis. Calcified Tissue International. 1992;51:184-188.

Palmieri GMA, Pitcock JA, Brown P, Karas JG, Roen LJ. Effect of calcitonin and vitamin D in osteoporosis. Calcified Tissue International. 1989;45:137-141.

Parr AJ, Loughman BC. Boron and membrane function in plants. Annual Proceedings of the Phytochemical Society of Europe. 1983;21:87-107.

Panteliadis C, Boenigk HE, Janke W. Uber das Spektrogramm der Spurenelemente bei Neugeborenen und Sauglingen. Infusiontherapie. 1975;2:377-382.

Peacock M, Horsman A, Aaron JE, Marshall DH, Selby PL, Simpson M. The role of parathyroid hormone in bone loss. Proceedings of the Copenhagen International Symposium on Osteoporosis. In: Christiansen C, Arnaud CD, Nordin BEC, Parfitt AM, Peck WA, Riggs BL, eds. Osteoporosis. Denmark: Department of Clinical Chemistry, Glostrup Hospital; 1984.

Pennisi E. Fragile bones linked to vitamin D gene. Science News. 1994;145:55.

Polley KJ, Nordin BE, Baghurst PA, Walker CJ, Chatterton BE. Effect of calcium supplementation on forearm bone mineral content in postmenopausal women: a prospective, sequential controlled trial. Journal of Nutrition. 1987;117:1929-1935.

Pollock ML, Wilmore JL, Fox SM. Exercise in Health and Disease: Evaluation and Prescription for Prevention and Rehabilitation. Philadelphia, PA: WB Saunders, Co.; 1984.

Pouilles JM, Tremollieres F, Ribot C. Prevention of post-menopausal bone loss with 1-α-hydroxyvitamin D_3. A three year prospective study. Clinical Rheumatology. 1992;11:492-497.

Raven PH, Johnson GB. Understanding Biology. 2nd ed. St. Louis, MO: Mosby Year-Book, Inc.; 1991.

Recker RR, Davies M, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. Journal of the American Medical Association. 1992;268:2403-2408.

Recker RR, Heaney RP. The effect of milk supplements on calcium metabolism and calcium balance. American Journal of Clinical Nutrition. 1985;41:254-263.

Reeve J, Arlot ME, Bradbeer JN, Hesp R, Mcally E, Meunier PJ, Zanelli JM. Human parathyroid peptide treatment of vertebral osteoporosis. Osteoporosis International. 1993;3(Suppl 1):199-203.

Reid IR, Ames RW, NZCS, Evans MC, Gamble GD, Sharpe SJ. Effect of calcium supplementation on bone loss in postmenopausal women. The New England Journal of Medicine. 1993;328:360-464.

Riggs BL, Melton LJ. Involutional osteoporosis. The New England Journal of Medicine. 1986;314:1676-1684.

Riggs BL, Wahner HW, Melton LJ, Richelson LS, Judd HL, Offord KP. Rates of bone loss in the appendicular and axial skeletons of women. Journal of Clinical Investigation. 1986;77:1487-1491.

Rutherford OM. Spine and total body bone mineral density in amenorrheic endurance athletes. Journal of Applied Physiology. 1993;74:2904-2908.

SAS Institute, Inc. SAS/STAT™ User's Guide. Version 6, 4th ed, Volume 2. Cary, NC: SAS Institute; 1990.

Scharla SH, Minne HW, Waibel-Treber S, Schaible A, Lempert UG, Wuster C, Leyendecker G, Ziegler R. Bone mass reduction after estrogen deprivation by long-acting gonadotropin-releasing hormone agonists and its relation to pretreatment serum concentrations of 1,25-dihydroxyvitamin D_3. Journal of Clinical Endocrinology and Metabolism. 1990;70:1055-1061.

Shils ME. Magnesium. In: Shils ME and Young VR, eds. Modern Nutrition in Health and Disease. 7th ed. Philadelphia, PA: Lea and Febiger; 1988;159-192.

Shuler TR, Nielsen FH. Boron and methionine status of the rat affects the plasma and bone mineral response to high dietary aluminum. Proceeding of the 6th International Symposium on Trace Element Metabolism in Man and Animals. In: Hurley LS, Keen CL, Lonnerdal B, Rucker RB, eds. Trace Elements in Man and Animals. New York, NY: Plenum Press; 1988.

Sinning WE. Experiments and Demonstrations in Exercise Physiology. Philadelphia, PA: W.B. Saunders, Co.; 1975.

Sokoll LJ, Dawson-Hughes B. Effect of menopause and aging on serum total and ionized calcium and protein concentrations. Calcified Tissue International. 1989;44:181-185.

Sommer AL, Lipman CB. Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiology. 1926;1:231-249.

Spencer H, Kramer LB. Nutritional and other factors influencing skeletal status. In: Roche AF, ed. Body-Composition Assessments in Youth and Adults. Report of the 6th Ross Conference on Medical Research. Columbus, OH: Ross Laboratories; 1985;33-37.

Tepperman J, Tepperman HM. Metabolic and Endocrine Physiology. 5th ed. Chicago, IL: Year Book Medical Publishers; 1987.

Tolstoi LG, Levin RM. Osteoporosis - the treatment controversy. Nutrition Today. 1992;July/August:6-29.

Tortora GJ, Anagnostakos NP. Principles of Anatomy and Physiology. 6th ed. New York, NY: Harper and Row, Publishers; 1990.

Vandecasteele C, Vanhoe H, Dams R, Versieck J. Determination of trace elements in human serum by inductively coupled plasma-mass spectrometry: comparison with nuclear analytical techniques in Nuclear Analytical Methods in the Life Sciences. Biological Trace Element Research. 1990;26-27:553-560.

van der Molen HJ, Dejong FH. Determination of progesterone in plasma by gas-liquid chromatography. In: Breuer H, Hamel D, Kruskemper HC, eds. Methods of Hormone Analysis. Stuttgart, Germany: Georg Thieme Verlag; 1976.

Verneullen A. Determination of testosterone in plasma by gas-liquid chromatography. In: Breuer H, Hamel D, Kruskemper HC, eds. Methods of Hormone Analysis. Stuttgart, Germany: Georg Thieme Verlag; 1976.

Villareal DT, Civitelli R, Chines A, Avioli LV. Subclinical vitamin D deficiency in postmenopausal women with low vertebral bone mass. Journal of Clinical Endocrinology and Metabolism. 1991;72:628-634.

Villee CA, Solomon EP, Davis PW. Biology. Philadelphia, PA: CBS College Publishing; 1985.

Volpe SL, Taper LJ, Meacham S. The effect of boron supplementation on bone mineral density and hormonal status in college female athletes. Medicine, Exercise, Nutrition and Health. 1993;2:323-330.

Volpe-Snyder SL. The effect of boron supplementation on bone mineral density and hormonal status in college female athletes. (dissertation). 1991.

Wahlund H. Determination of the physical working capacity: a physiological and clinical study with special reference to standardization of cardiopulmonary functional test. Acta Medica Scandinavica. 1948;132(Suppl 215).

Ward NI, Abou-Shakra FR, Durrant SF. Trace element content of biological materials: a comparison of NAA and ICP-MS analysis in Nuclear Analytical Methods in the Life Sciences. Biological Trace Element Research. 1990;26-27:177-187.

Wardlaw GM. Putting osteoporosis in perspective. Journal of the American Dietetic Association. 1993;93:1000-1006.

Warrington K. The effect of boric acid and borax on the broad bean and certain other plants. Annals of Botany. 1923;37:629-671.

White CM, Hergenroeder AC. Amenorrhea, osteopenia, and the female athlete. Pediatric Clinics of North America. 1990;37:1125-1141.

Weinerman SA, Bockman RS. Medical therapy of osteoporosis. The Orthopedic Clinics of North America. 1990;21:109-124.

Weiss NS, Ure CL, Jude MA, Ballard JH, Williams AR, Daling JR. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. The New England Journal of Medicine. 1980;303:1195-1198.

Werner R. Essentials of Modern Biochemistry. Boston, MA: Jones and Bartlett Publishers, Inc.; 1983.

White RT, Douthit GE. Use of microwave oven and nitric acid-hydrogen peroxide digestion to prepare botanical materials for elemental analysis by inductively coupled argon plasma emission spectroscopy. Journal of the Association of Official Analytical Chemists. 1985;68:766-769.

Williams TR, Van Doren JB, Smith BR, McElvany BW, Zink H. ICP analysis of biological materials. American Biotechnology Laboratory. 1986;Sept/Oct:52-57.

Zarcinas BA, Cartwright B, Spouncer LR. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. Communications in Soil Science and Plant Analysis. 1987;18:131-146.

Zhang J, Feldblum PJ, Fortney JA. Moderate physical activity and bone density among perimenopausal women. American Journal of Public Health. 1992;82:736-738.

APPENDIX A

APPROVAL FROM THE INSTITUTIONAL REVIEW BOARD FOR RESEARCH INVOLVING HUMAN SUBJECTS AT VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Virginia Tech
VIRGINIA POLYTECHNIC INSTITUTE
AND STATE UNIVERSITY

Research Division
306 Burruss Hall
Blacksburg, Virginia 24061-0244
(703)231-9359 FAX (703)231-4384

April 13, 1992

TO: L. Janette Taper
Human Nutrition and Foods
Campus

FROM: E. R. Stout
Associate Provost for Research

SUBJECT: IRB APPROVAL /"Investigations Related to Boron, A Potential
Essential Nutrient"
Ref. 92-104

I have reviewed your request to the IRB for the above referenced project. I concur with Dr. Schlenker that the experiments are of minimal risk to the human subjects who will participate and that appropriate safeguards have been taken. Therefore, on behalf of the Institutional Review Board for Research Involving Human Subjects, I have given your request expedited approval.

Best wishes.

ERS/php

cc: Dr. Schlenker

P.S. Please send copy of Informed Consent for the file.

REQUEST FOR APPROVAL OF
INVESTIGATION INVOLVING HUMAN SUBJECTS

92-104

Principal Investigator(s) L J TAPER Department HUMAN NUTRITION

Project Title: INVESTIGATIONS RELATED TO BORON A POTENTIAL ESSENTIAL NUTR IE

Source of Support: Departmental Research [ ] Sponsored Research [✓] Proposal No. _____

1. The criteria for "expedited review" by the Institutional Review Board for a project involving the use of human subjects and with minimal risk* is one or more of the following. Please initial all applicable conditions and provide a substantiating statement of protocol.

[ ] a. Collection of:

1) hair or nail clipping in a non-disfiguring manner;
2) deciduous teeth;
3) permanent teeth if patient care indicates need of extraction.

[✓] b. Collection of excreta and external secretions: sweat, uncanulated saliva, placenta removed at delivery, amniotic fluid obtained at time of rupture of the membrane.

[✓] c. Recording of data from subjects 18 years or older, using noninvasive procedures routinely employed in clinical practice. Exemption does not include exposure to electromagnetic radiation outside the visible range.

[✓] d. Collection of blood samples by venipuncture (not exceeding 150 ml/8 week period, and no more than twice a week) from subjects 18 years or older, in good health and not pregnant.

[ ] e. Collection of supra- and subgingival dental plaque and calculus, provided the procedure is no more invasive than routine scaling of the teeth.

[ ] f. Voice readings.

[ ] g. Moderate exercise by healthy volunteers.

[ ] h. Study of existing data, documents, records, pathological specimens or diagnostic specimens.

[ ] i. Research on drugs or devices for which an investigational exemption is not required.

2. If the project involves human subjects who are exposed to "more than minimal risk" and are not covered by the criteria above (1 to 9), the IRB review must involve the full IRB board. Please check if the research involves more than minimal risk** __ and provide a substantiating statement of protocol.

3. Human subjects would be involved in the proposed activity as either: Minors and/or Children*___, Fetuses___, Abortuses___, Pregnant Women___, Prisoners___, Mentally Retarded___, Mentally Disabled___.

Note that if children are involved in the research as human subjects, they may have to provide consent as well as their parents.

Whether or not the project may undergo "expedited review" or must be reviewed by the full Institutional Review Board, it is necessary that the required informed consent forms also be reviewed. These should be submitted with the proposal. However, if there is insufficient time to meet the sponsor's deadline, submittal can be delayed up to thirty days after submittal of the proposal without jeopardizing the IRB certification to the prospective sponsor.

*Minimal risk means that the risks of harm anticipated in the proposed research are not greater, considering the probability and magnitude, than those encountered in daily life or during performance of routine physical or psychological examinations or tests.

**Subject at risk is an individual who may be exposed to the possibility of injury as a consequence or participation as a subject in any research, development or related activity which departs from the application of those established and accepted methods necessary to meet his needs, or which increases the ordinary risks of daily life, including the recognized risks inherent in a chosen occupation or field of science.

This is to certify that the project identified above will be carried out as approved by the Human Subject Review Board, and will neither be modified nor carried out beyond the period approved below without express review and approval by the Board.

L Janette Taper
Principal Investigator/Date

E Clare O Schlenker 3/25/92
Departmental Reviewer/Date

The Human Subjects Review Board has reviewed the protocol identified above, as it involves human subjects, and hereby approves the conduct of the project for 1a months, at which time the protocol must be resubmitted for approval to continue

4/13/92

APPENDIX B

UNDERGRADUATE RESEARCH CREDIT FORM

College of Human Resources

Virginia Polytechnic Institute and State University

Independent Study and Undergraduate Research (IS/UR) Request Form
(X974, X994)

STUDENT INFORMATION

1). Name: _____
2). Student #: _____
3). Local Address: _____
_____
_____
4). Major: _____
5). College: _____
6). Total Hours Passed: _____
7). Overall QCA[1]: _____
8). In Major QCA[1]: _____
9). Previously completed _____ hours of IS and _____ hours of UR.
10). Hours of IS/UR this term: _____
11). Total hours this term: _____
12). Use of this course for:
Free elective: _____, Other: _____,
Dept/College Elective: _____, Required: _____,
Substitution for Department: _____
Course Number[2]: _____

COURSE INFORMATION

1). Department: _____
2). Course #: _____ Index #: _____
3). Term/Year: _____
4). Instructor: _____
5). Instructor's SSN: _____
6). Date Request Submitted: _____
7). Credit Hours: _____, P/F: _____, A/F: _____
8). Title of Proposed Study (limit to 21 characters):
_____
9). Related courses already completed by student:
(attach list if necessary -- OMIT FOR ENGINEERING)
_____
_____
_____
10). Does student satisfy departmental standards for IS or UR?
Yes ( ) No ( )

[1] Student must meet the minimum QCA of his/her College and of the department offering the course for approval.

[2] Substitution form must be submitted if IS/UR is to be used for a required course.

ATTACH ADDITIONAL INFORMATION AS NEEDED

Give brief description of the study, objectives, materials and methods, justification *and method of evaluation.*

THIS FORM DOES NOT CONSTITUTE REGISTRATION FOR THE COURSE. THE STUDENT MUST REGISTER THROUGH NORMAL CHANNELS (OP-SCAN FORM, TERMINAL, ETC.).

APPROVALS:

Student _____
Advisor _____
Instructor _____
Dept. Head of Instructor _____
Academic Dean of Instructor _____
Academic Dean of Student _____

Original and 5 copies to be forwarded to the student's Academic Dean's Office prior to the end of classes of the preceding term.

APPENDIX C

MEDICAL HISTORY QUESTIONNAIRE AND ORAL CONTRACEPTIVE QUESTION SHEET

MEDICAL HISTORY QUESTIONNAIRE

PLEASE COMPLETE AND RETURN TO CANDICE WARD IN 340 WALLACE HALL. IF YOU HAVE ANY QUESTIONS CALL 231-7708 OR 951-0711.

SECTION I

LAST NAME _____

FIRST NAME _____ MIDDLE INITIAL _____

DATE OF BIRTH _____ AGE _____ SEX _____

SOCIAL SECURITY NUMBER _____

LOCAL PHONE NUMBER _____ WORK PHONE _____

LOCAL ADDRESS _____

PERMANENT PHONE NUMBER _____

PERMANENT ADDRESS _____

FAMILY PHYSICIAN _____

CLASS STANDING (CIRCLE): FRESHMAN SOPHOMORE JUNIOR SENIOR

SECTION II

DATE OF LAST PHYSICAL EXAMINATION? _____

PLEASE LIST ANY ALLERGIES (MEDICATIONS, FOODS, OTHER)

_____

PLEASE LIST ANY CHRONIC OR SERIOUS ILLNESSES, AS CONFIRMED BY A PHYSICIAN

_____

PLEASE LIST INFORMATION ABOUT LAST THREE HOSPITALIZATIONS (IF THIS DOES NOT APPLY PLEASE SPECIFY)

1. TYPE OF OPERATION _____

   MONTH AND YEAR HOSPITALIZED _____

   NAME AND LOCATION OF HOSPITAL _____

   _____

2. TYPE OF OPERATION _____

   MONTH AND YEAR HOSPITALIZED _____

   NAME AND LOCATION OF HOSPITAL _____

   _____

3. TYPE OF OPERATION _____

   MONTH AND YEAR HOSPITALIZED _____

   NAME AND LOCATION OF HOSPITAL _____

   _____

SECTION III

PLEASE CIRCLE THE APPROPRIATE RESPONSE.

DURING THE PAST 12 MONTHS...

1. HAS A PHYSICIAN PRESCRIBED ANY FORM OF MEDICATION FOR YOU? YES NO

2. HAS YOUR WEIGHT FLUCTUATED MORE THAN 5 POUNDS? YES NO

3. DID YOU ATTEMPT TO BRING ABOUT THIS WEIGHT CHANGE THROUGH DIET AND/OR EXERCISE? YES NO

4. HAVE YOU EXPERIENCED FAINTNESS, LIGHTHEADEDNESS, OR BLACKOUTS? YES NO

5. HAVE YOU OCCASIONALLY HAD TROUBLE SLEEPING? YES NO

6. HAVE YOU EXPERIENCED BLURRED VISION? YES NO

7. HAVE YOU HAD ANY SEVERE HEADACHES? YES NO

8. HAVE YOU EVER EXPERIENCED CHRONIC MORNING COUGH? YES NO

9. HAVE YOU EVER EXPERIENCED ANY TEMPORARY CHANGE IN YOUR SPEECH PATTERN, SUCH AS SLURRING OR LOSS OF SPEECH? YES NO

10. HAVE YOU EVER FELT UNUSUALLY NERVOUS OR ANXIOUS FOR NO APPARENT REASON? YES NO

11. HAVE YOU EVER EXPERIENCED UNUSUAL HEARTBEATS, SUCH AS SKIPPED BEATS OR PALPITATIONS? YES NO

12. HAVE YOU EVER EXPERIENCED PERIODS IN WHICH YOUR HEART FELT AS THOUGH IT WERE RACING FOR NO APPARENT REASON? YES NO

AT PRESENT...

1. DO YOU EXPERIENCE SHORTNESS OF BREATH OR LOSS OF BREATH WHILE WALKING WITH OTHERS YOUR OWN AGE? YES NO

2. DO YOU EXPERIENCE SUDDEN TINGLING, NUMBNESS, OR LOSS OF FEELING IN YOUR ARMS, HANDS, LEGS, FEET, OR FACE? YES NO

3. HAVE YOU EVER NOTICED THAT YOUR HANDS OR FEET SOMETIMES FEEL COOLER THAN OTHER PARTS OF YOUR BODY? YES NO

4. DO YOU EXPERIENCE SWELLING OF YOUR FEET AND ANKLES? YES NO

5. DO YOU GET PAINS OR CRAMPS IN YOUR LEGS? YES NO

6. DO YOU EXPERIENCE ANY PAIN OR DISCOMFORT IN YOUR CHEST? YES NO

7. DO YOU EXPERIENCE ANY PRESSURE OR HEAVINESS IN YOUR CHEST? YES NO

8. HAVE YOU EVER BEEN TOLD THAT YOUR BLOOD PRESSURE IS ABNORMAL? YES NO

9. HAVE YOU EVER BEEN TOLD THAT YOUR SERUM CHOLESTEROL OR TRIGLYCERIDE LEVEL WAS HIGH? YES NO

10. DO YOU HAVE DIABETES? YES NO
IF YES, HOW IS IT CONTROLLED? (PLEASE CHECK RESPONSES)
DIETARY MEANS _____ INSULIN INJECTION _____
ORAL MEDICATION _____ UNCONTROLLED _____

11. HOW OFTEN WOULD YOU CHARACTERIZE YOUR STRESS LEVEL AS BEING HIGH? (PLEASE CHECK RESPONSE)
RARELY _____ OCCASIONALLY _____
FREQUENTLY _____ CONSTANTLY _____

12. HAVE YOU EVER BEEN TOLD THAT YOU HAVE ANY OF THE FOLLOWING ILLNESSES? (PLEASE CHECK RESPONSES)
MYOCARDIAL INFARCTION _____
CORONARY THROMBOSIS _____
CORONARY OCCLUSION _____
HEART BLOCK _____
ATHEROSCLEROSIS _____
RHEUMATIC HEART _____
ANEURYSM _____
HEART DISEASE _____
HEART ATTACK _____
HEART MURMUR _____
ANGINA _____
STROKE _____

13. HAS ANY MEMBER OF YOUR IMMEDIATE FAMILY BEEN TREATED FOR OR SUSPECTED TO HAVE ANY OF THESE CONDITIONS? (PLEASE SPECIFY RELATIONSHIP TO YOU [I.E. FATHER, MOTHER, BROTHER, SISTER...]) (PLEASE CHECK RESPONSES)
DIABETES _____
HEART DISEASE _____
STROKE _____
HIGH BLOOD PRESSURE _____

SECTION IV

SMOKING HABITS

PLEASE CIRCLE APPROPRIATE RESPONSE OR FILL IN THE BLANK.

1. HAVE YOU EVER SMOKED CIGARETTES, CIGARS, OR A PIPE?
YES NO

2. DO YOU CHEW SMOKELESS TOBACCO? YES NO

3. DO YOU PRESENTLY SMOKE? YES NO
IF YES, PLEASE FILL IN THE NUMBER SMOKED PER DAY.
CIGARETTES: _____ PER DAY
CIGARS: _____ PER DAY
PIPEFULS: _____ PER DAY

4. AT WHAT AGE DID YOU BEGIN SMOKING? _____ YEARS

5. IF YOU HAVE QUIT SMOKING, WHEN DID YOU QUIT (MONTH AND YEAR) _____

DRINKING HABITS

1. DURING THE PAST MONTH, HOW MANY DAYS DID YOU DRINK ALCOHOLIC BEVERAGES? _____ DAYS

2. DURING THE PAST MONTH, HOW MANY TIMES DID YOU HAVE FIVE OR MORE DRINKS PER OCCASION? _____ TIMES

3. ON AVERAGE, HOW MANY GLASSES OF BEER, WINE, OF HIGHBALLS DID YOU CONSUME PER WEEK? (PLEASE FILL IN THE NUMBER)
BEER _____ GLASSES OR CANS
WINE _____ GLASSES
HIGHBALLS _____ GLASSES
OTHER (SPECIFY) _____ GLASSES

EXERCISE HABITS

1. DO YOU EXERCISE VIGOROUSLY ON A REGULAR BASIS?
YES NO

2. WHAT ACTIVITIES DO YOU ENGAGE IN ON A REGULAR BASIS?

_____

3. IF YOU WALK, RUN, OR JOG, WHAT IS THE AVERAGE NUMBER OF MILES YOU COVER PER WEEK? _____ MILES

4. HOW MANY MINUTES, ON AVERAGE, IS EACH OF YOUR WORKOUTS?
_____ MINUTES

5. HOW MANY WORKOUTS PER WEEK DO YOU PARTICIPATE IN ON AVERAGE? _____ WORKOUTS

6. IS YOUR OCCUPATION: (PLEASE CHECK RESPONSE)
INACTIVE (I.E. DESK JOB) _____
LIGHT WORK (I.E. HOUSEWORK, LIGHT CARPENTRY) _____
HEAVY WORK (I.E. LIFTING, HEAVY CARPENTRY) _____
I DO NOT WORK _____

7. CHECK THOSE ACTIVITIES THAT YOU WOULD PREFER ON A REGULAR EXERCISE PROGRAM FOR YOURSELF:

WALKING/RUNNING/JOGGING	_____	TENNIS	_____
HANDBALL/RACQUETBALL/SQUASH	_____	CYCLING	_____
STATIONARY RUNNING	_____	SWIMMING	_____
STATIONARY CYCLING	_____	AEROBICS	_____
JUMPING ROPE	_____	BASKETBALL	_____

OTHERS (SPECIFY) _____

DIETARY HABITS

1. WHAT IS YOUR CURRENT WEIGHT? _____ POUNDS

2. WHAT IS YOUR CURRENT HEIGHT? _____ FEET _____ INCHES

3. WHAT WOULD YOU LIKE TO WEIGH? _____ POUNDS

4. WHAT IS THE MOST YOU HAVE EVER WEIGHED AS AN ADULT? (SINCE AGE 18 YEARS) _____ POUNDS

5. WHAT IS THE LEAST YOU HAVE EVER WEIGHED AS AN ADULT? (SINCE AGE 18 YEARS) _____ POUNDS

6. WHAT WEIGHT LOSS METHODS HAVE YOU TRIED? (PLEASE LIST)

_____

7. WHICH DO YOU EAT REGULARLY: (PLEASE CHECK RESPONSES)

BREAKFAST	_____	MIDMORNING SNACK	_____
LUNCH	_____	MIDAFTERNOON SNACK	_____
DINNER	_____	AFTER-DINNER SNACK	_____

8. HOW OFTEN DO YOU EAT OUT PER WEEK? _____ TIMES

9. WHAT SIZE PORTIONS DO YOU NORMALLY CONSUME? (PLEASE CHECK RESPONSE)
SMALL _____ MODERATE _____
LARGE _____ EXTRA-LARGE _____
UNCERTAIN _____

10. HOW OFTEN DO YOU EAT MORE THAN ONE SERVING? (PLEASE CHECK RESPONSE)
ALWAYS _____ USUALLY _____
SOMETIMES _____ NEVER _____

11. HOW LONG DOES IT TAKE FOR YOU TO EAT A MEAL?
_____ MINUTES

12. DO YOU EAT WHILE DOING OTHER ACTIVITIES (I.E. WATCHING TV, READING, WORKING, STUDYING)? YES NO

13. WHEN YOU SNACK, HOW MANY TIMES PER WEEK DO YOU EAT THE FOLLOWING: (PLEASE CHECK RESPONSES)
COOKIES, CAKE, PIE _____ CANDY _____
SOFT DRINKS _____ DIET SODA _____
DOUGHNUTS _____ FRUIT _____
MILK/MILK BEVERAGE _____ ICE CREAM _____
PRETZELS, POTATO CHIPS... _____ PEANUTS/NUTS _____
CHEESE AND CRACKERS _____
OTHER (SPECIFY) _____

14. HOW OFTEN DO YOU EAT DESSERT?
_____ TIMES PER DAY _____ TIMES PER WEEK

15. WHAT DESSERT DO YOU EAT MOST?
_____

16. HOW OFTEN DO YOU EAT FRIED FOODS? _____ TIMES PER WEEK

17. DO YOU SALT YOUR FOOD AT THE TABLE? YES NO
IF YES, BEFORE TASTING IT _____ AFTER TASTING IT _____

PSYCHOLOGICAL STRESS LEVEL ASSESSMENT AND RELAXATION TECHNIQUES

1. I HAVE AN INTENSE SUSTAINED DRIVE TO GET AHEAD.
YES NO

2. I AM ANXIOUS TO REACH MY GOALS, BUT I AM UNCERTAIN WHAT THOSE GOALS ARE. YES NO

3. I FEEL A NEED TO COMPETE AND WIN. YES NO.

4. I HAVE A PERSISTENT DESIRE FOR RECOGNITION. YES NO

5. I ALWAYS SEEM TO BE INVOLVED IN TOO MANY THINGS AT ONCE. YES NO

6. I AM ALWAYS RACING THE CLOCK, CONSTANTLY ON EDGE, AND HAVE DEADLINES. YES NO

7. I HAVE A NEED TO SPEED THINGS UP, AND TO GET THINGS DONE FASTER. YES NO

8. I AM EXTRAORDINARILY ALERT MENTALLY AND PHYSICALLY. YES NO

SECTION V

CIRCLE THE APPROPRIATE RESPONSE.

1. DO YOU OFTEN EXPERIENCE HEADACHES OR BACKACHES? YES NO

2. WHEN SITTING IN A CHAIR AND TALKING TO SOMEONE, DO YOU CONTINUALLY MOVE IN THE CHAIR TO SEEK A COMFORTABLE POSITION? YES NO

3. WHEN RETIRING FOR THE NIGHT, ARE YOU UNABLE TO FALL ASLEEP IMMEDIATELY? YES NO

4. DO YOU OFTEN GRIND YOUR TEETH WHEN YOU ARE CONFRONTED WITH AN UNPLEASANT EXPERIENCE? YES NO

5. DO YOU EASILY BECOME ANGRY OR FRUSTRATED WHEN YOU ARE FACED WITH A PROBLEM FOR WHICH THERE IS NO IMMEDIATE SOLUTION? YES NO

6. DO YOU OFTEN COMPLAIN OF BEING TIRED? YES NO

7. DOES YOUR FACE OFTEN HOLD EXPRESSIONS OF INTENSE CONCENTRATION? YES NO

8. DO YOU OFTEN DRUM YOUR FINGERS AIMLESSLY OR FORCIBLE TO EXPRESS IRRITATION? YES NO

9. DOES YOUR POSTURE APPEAR STIFF WHEN YOU SIT OR WALK? YES NO

10. ARE YOU UNABLE TO CONCENTRATE ON ONE PROBLEM AT A TIME? YES NO

11. ARE YOU UNABLE TO RELAX VOLUNTARILY? YES NO

12. DO YOU OFTEN EXPERIENCE NERVOUSNESS AND UNEASY FEELINGS? YES NO

13. DO YOU BECOME UPSET WHEN YOUR PLANS ARE INTERRUPTED OR MUST BE CHANGED? YES NO

14. ARE YOU HIGHLY COMPETITIVE IN SPORTS, IN YOUR TEST GRADES, AND IN YOUR DAILY RESPONSIBILITIES? YES NO

15. ARE YOU TIME-CONSCIOUS? YES NO

16. DO YOU EXPERIENCE EXTREME DISSATISFACTION AND ANXIETY WHEN YOU FAIL TO ACHIEVE SUCCESS IN YOUR ENDEAVORS? YES NO

17. ARE YOU AN AGGRESSIVE PERSON? YES NO

18. ARE YOU OFTEN TOO BUSY TO ALLOW TIME FOR PHYSICAL ACTIVITY? YES NO

19. DO YOU PLAN YOUR DAILY ACTIVITIES AND OFTEN BUDGET YOUR TIME? YES NO

20. ARE YOU CRITICAL OF YOURSELF WHEN YOU MAKE A MISTAKE? YES NO

21. DO YOU FEEL "UPTIGHT" AT THE END OF THE DAY? YES NO

22. ARE YOU IMPATIENT WHEN OTHERS ARE LATE FOR AN APPOINTMENT WITH YOU? YES NO

23. DO YOU OFTEN SET HIGH GOALS OR LEVELS OF ACHIEVEMENT FOR YOURSELF? YES NO

24. DO YOU EXPERIENCE BAD MOODS OFTEN? YES NO

25. ARE YOU UNYIELDING WHEN OTHERS DISAGREE WITH YOUR BELIEFS OR CONVICTIONS? YES NO

SECTION VI

1. ARE YOU TAKING ANY MEDICATIONS ON A REGULAR BASIS?
   YES NO
   IF YES, PLEASE LIST ALL MEDICATIONS, BOTH PRESCRIPTION AND NON-PRESCRIPTION DRUGS.

   _____

2. ARE YOU CURRENTLY TAKING A VITAMIN AND/OR MINERAL SUPPLEMENT? YES NO
   IF YES, PLEASE LIST THE FOLLOWING:
   BRAND NAME OF SUPPLEMENT _____

   DOSAGE _____

   CONSUMPTION FREQUENCY _____

3. HAVE YOU EVER SUFFERED A: (PLEASE CHECK RESPONSES)
   BROKEN BONE _____ BONE FRACTURE _____
   SPRAIN _____ TORN LIGAMENT _____
   OTHER (SPECIFY) _____
   IF YES TO ANY, WAS THE INCIDENT EXERCISE RELATED?
   YES NO

4. HAVE YOU EVER SUFFERED A TRAINING RELATED INJURY?
   YES NO
   IF YES, PLEASE ELABORATE _____

   _____

5. GIVE AGE OF MENARCHE. _____ YEARS

6. NUMBER OF MENSES PER YEAR _____

7. DOES YOUR MENSTRUAL PATTERN CHANGE DURING EXERCISE/TRAINING? YES NO
   IF YES, PLEASE ELABORATE. _____

   _____

8. ARE YOU CURRENTLY:

AMENORRHEIC (0 MENSES PER YEAR)	YES	NO
OLIGOMENORRHEIC (0 - 6 MENSES PER YEAR)	YES	NO
EUMENORRHEIC (NORMAL MENSES PATTERN)	YES	NO

FOR ANY CATEGORY YOU RESPONDED YES, PLEASE GIVE LENGTH OF TIME FOR MENSES PATTERN (I.E. FOR PAST FIVE MONTHS, FOR PAST 3 YEARS, ...)

_____

QUESTION SHEET

SUBJECT NAME _____

SUBJECT NUMBER _____ DATE _____

1. Do you presently take oral contraceptives? YES NO

*IF YOU ANSWERED YES TO QUESTION #1 PLEASE COMPLETE THE REST OF THIS QUESTION SHEET. IF YOU ANSWERED NO TO QUESTION #1 YOU MAY STOP.

2. Please indicate which type of oral contraceptives you take.
(LIST THE NAME, i.e. ORTHO NOVUM 7/7/7)

_____

3. Please indicate the length of time you have taken the above oral contraceptive. (i.e. 12 months)

_____

4. If you have taken another type of oral contraceptive, different from the one listed above, please list the name and length of time you have taken it.

_____

APPENDIX D

WRITTEN CONSENT FORMS

CONSENT OF PARTICIPATION IN A NUTRITION STUDY

Department of Human Nutrition and Foods
Virginia Polytechnic Institute and State University

I have received an oral and written explanation of the study, and I understand the following:

I will maintain normal activities and eating patterns throughout the duration of the study.

A boron supplement (3 mg) or placebo (corn starch) will be provided to me, which I will consume on a daily basis. The dosage of boron is consistent with a diet high in fruits and vegetables.

I will keep a three-day food record at the beginning of the study and at six months.

I will keep a menstrual record each month during the study.

I will give a 24-hour urine sample at the beginning of the study and at six months.

I will give a (30 ml) blood sample, which will be drawn by a qualified technician, at the beginning of the study and at six months.

I will have my bone density of the lumbar spine determined by qualified personnel at the beginning of the study and at six months. This procedure will be done at Montgomery Regional Hospital in Blacksburg, VA, by the use of dual photon absorptiometry.

I will have my height, weight, and percent body fat assessed at the beginning of the study and at six months. My body fat will be determined by the use of skinfold measurements.

I will have my aerobic capacity determined at the beginning of the study.

If I or the investigators believe at any time that it is in my best interest to drop from the study, I shall do so.

The following people may be contacted if I have any questions or concerns about my participation in the study:

S. J. Ritchey, Ph.D.
Principle Investigator
Room 229B Wallace Hall
Department of Human Nutrition and Foods
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0430
(703) 231-6393

Beth Thomas, Ph.D.
Principle Investigator
Room 232C Wallace Hall
Department of Human Nutrition and Foods
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0430
(703) 231-8763

Candice Ward, B.S.
Graduate Student
Room 340 Wallace Hall
Department of Human Nutrition and Foods
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0430
(703) 231-7708

I understand the above and agree to participate in the Human Nutrition Study to be at Virginia Polytechnic Institute and State University from October 1993 to April 1994.

Signed _____

Date _____

BLOOD COLLECTION CONSENT FORM

I, the undersigned, consent to donate blood. I have received an explanation of the study and understand the following:

A sample of blood, approximately 30 mls, will be taken.

In case of an "Exposure Incident" a sample of my blood will be tested in order to determine Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV) infectivity. "Exposure Incident" means a specific eye, mouth, other mucous membrane, non-contact skin, or parenteral (puncture or cut) contact with blood or other potentially infectious materials that results from the performance of an employee's duties.

All information obtained in the study will be held strictly confidential.

_____ _____

(Date) (Print Name)

_____

(Signature)

For questions and/or more information contact:

Department of Human Nutrition and Foods
338 Wallace Hall
Blacksburg, VA 24061
(703) 231-5549

LABORATORY FOR HEALTH AND EXERCISE SCIENCES
INFORMED CONSENT FOR EXERCISE EVALUATION

1. Explanation of the Procedure

In order to assess the following, my physical fitness during the course of my participation in the **"Investigation Related to Boron, an Essential Human Nutrient with the Human Nutrition and Foods Department" (IRB approval 92-104),** I hereby consent, voluntarily, to perform an exercise test on a stationary cycle ergometer. The work will begin at a level you can easily accomplish and continually advance in 3-4 minute stages until you reach a work level which you feel is too fatiguing or difficult to continue. Whatever the stopping point, I understand that I may stop the exercise whenever I request to do so. This test will provide a necessary measure of my exercise tolerance for the research project. During the test my heart rate will be monitored using a single ECG lead, which will require 3 electrodes being placed on the skin of my chest area. The technician may stop the test at any time because of my feelings of fatigue or for any other personal reason.

I also understand that I must answer completely a variety of questions related to my personal health history, my physical activity habits and my exercise capabilities. Answers to questions about these matters will be obtained during my test visit to the lab, in some cased obtained by interview and in other cases, by questionnaire. In addition, body weight, height and a number of skinfold fat measurements will be taken to determine my percent body fat.

2. Risks and Discomforts

There exists the possibility of certain changes occurring during the exercise test. They include abnormal blood pressure, fainting, disorders of the heart beat, and very rare instances of heart attack, stroke, or death. Other discomforts which may be more applicable to this population include leg fatigue/soreness.

3. **Benefits to be Expected**

The results obtained from this exercise test and the ones that will be done at future intervals should assist the study investigators in their determination of my physical fitness and allow a tracking of physical fitness changes that may occur over the period of the study.

4. **Inquiries**

I understand that any questions about the procedures used in the exercise test or in the estimation of my physical fitness are welcome. I further understand that if I have any doubts or questions, I may ask questions and receive answers from the laboratory supervisor who will be available in the lab before I take my exercise test (s).

5. **Freedom of Consent**

Permission for you to perform the exercise test is voluntary. You are free to deny consent at any time if you so desire.

6. **Additional Important Points**

I understand that a physician or nurse will not be available during my exercise test, but that the supervising technicians in the lab are trained in CPR and experienced with conducting exercise tests and trained to recognize the appearance of major changes that should lead non-physicians to stop such tests. Emergency medical support is almost never needed as a contingency for exercise tests with healthy subjects, such support is available through response from local rescue squad. I understand that in the event of any harm or injury arising from my participation in this test that no compensation will be available from Virginia Polytechnic Institute and State University and its agents. Finally, I understand that any test results may be used for research purposes only in a manner such that the information is not personally indentifiable with the test subject (me). I have read this form and I understand the test procedures I will perform and freely consent to participate in this test. Questions or concerns about my participation in this exercise test may be directed to the Lab Director (Dr. William Herbert, 231-6565) or the Study Investigator (Dr. Elizabeth Thomas, 231-8763).

7. **Comments**
Questions asked:

Answers given:

Participant's Name _____

Participant's Signature _____

Date _____

Witness' Name _____

Witness' Signature _____

Date _____

APPENDIX E

SUBJECT CONTRACT

CONTRACT

I, _____ , agree to comply with the following rules as part of my participation in the boron study:

A. Comply with height, weight, and skinfold measurement schedules.

B. Keep accurate, well-documented food records and return them on the appropriate dates.

C. Fast for 12-14 hours prior to reporting to scheduled blood draws.

D. Collect 24-hour urine specimens in the provided containers, as instructed; keep the specimens refrigerated; and return the samples and all containers on the appropriate dates.

E. Refrain from smoking any tobacco product for the duration of the study.

F. Inform the investigators when taking medications.

G. Keep accurate, well-documented menstrual records and return them as instructed.

H. Consume one placebo or one 3 mg boron supplement per day as instructed.

I. Consume self-selected diet for the duration of the study.

J. Maintain regular exercise habits for the duration of the study.

K. Report any concerns to the investigators.

In return for my complete cooperation and compliance with the above, I understand that I will receive one hour of undergraduate research credit. I recognize that failure to comply with the above could result in my being dropped from the study, resulting in forfeiture of my undergraduate research credit.

Signed _____

Social Security Number _____ Date _____

Witnesses _____ _____

APPENDIX F

EXERCISE RECORDS

EXERCISE JOURNAL - DAY #1

NAME _____ **DATE** _____

SOCIAL SECURITY NUMBER _____

TYPE OF EXERCISE (AEROBICS, WALKING, RUNNING, SOCCER...)	INTENSITY OF EXERCISE (VIGOROUS, MODERATE, LIGHT...)	DURATION OF EXERCISE (IN MINUTES)

APPENDIX G

MENSTRUAL RECORDS

SUBJECT NUMBER _____

OCTOBER 1993

NAME _____

SUN	MON	TUES	WED	THURS	FRI	SAT
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						

Directions for completing calendar:
Days with heavy flow, mark "H"
Days with medium flow, mark "M"
Days with light flow, mark "L"

NOVEMBER 1993

NAME _____

SUN	MON	TUES	WED	THURS	FRI	SAT
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30				

Directions for completing calendar:
Days with heavy flow, mark "H"
Days with medium flow, mark "M"
Days with light flow, mark "L"

APPENDIX H

FOOD RECORDS AND PROCEDURES

INSTRUCTIONS FOR KEEPING 3-DAY FOOD RECORDS

A. Please print clearly.

B. Include everything you eat for three consecutive days (72 hours), with each day beginning at 6:00 am and ending at 6:00 am the following day. This includes all meals, snacks, beverages, condiments, gravies, butter, medications, supplements, gum, and mints.

C. Write down each food or beverage item immediately after you consume it, this way you will tend to remember the smaller items, such as sugar, ketchup, butter, and salt. If you do not have the food record with you when you eat, please record the food and beverage items on a piece of paper, then copy them into the food record booklet as soon as possible.

D. Describe all foods and beverages in as much detail as possible. For example, cooking method (baked, fried, boiled...), canned/frozen/fresh, brand name, and sizes of food items (large fry/small fry, one thin/thick slice ...).

E. List amounts eaten as precisely as possible. For example, 1/2 cup, 1 cup, 1 tablespoon, 4 ounces (for solid foods, such as meat, 4 ounces is approximately equal to the size of a deck of cards or the palm of your hand, while for liquids, 4 ounces is equal to a 1/2 cup).

F. For mixed food items, such as spaghetti, list out all components of the item. Each item will be listed separately. For example the spaghetti will be listed as follows: 2 cups of thin spaghetti noodles, 3/4 cups of Ragu meat sauce, and 2 tablespoons of Kraft Parmesan cheese.

G. Please do not alter your diet in any way. We are not looking at the amount or type of food you are consuming, but rather are looking to see how much boron you are taking in through the diet.

FOOD RECORD - DAY #1

NAME _____ **DATE** _____

SOCIAL SECURITY NUMBER _____

SUPPLEMENTS TAKEN _____

FOOD\BEVERAGE (DESCRIPTION)	AMOUNT EATEN

NAME _____ **DATE** _____

SOCIAL SECURITY NUMBER _____

DAY #1 CONTINUED

FOOD\BEVERAGE (DESCRIPTION)	AMOUNT EATEN

APPENDIX I

NUTRITIONIST IV™ PROCEDURES AND CODE BOOK

PROCEDURES FOR USING NUTRITIONIST IV™

TO GET INTO THE NUTRITIONIST IV™ PROGRAM FROM DOS:

1. (C:\)
   Type: CD\NUT4 <ENTER>

2. (C:\CD\NUT4\)
   Type: N4 <ENTER>

TO RUN THE NUTRITIONIST IV™ PROGRAM:

1. Select: <1> Start New Diet/Recipe

2. Diet/Recipe Information Screen Appears
   At the "Name" selection, type in subject number <TAB>

   Select RDA (use arrow keys) <ENTER>

3. Enter Food and Servings Screen Appears
   Enter food code or food name itself <ENTER>

   *If code selected: select serving amount <TAB>
   select serving size <ENTER>

   *If food item selected: use arrow keys to scroll down or up the list of food items. To select the item, <ENTER>. Now follow the above procedure as given for "if code selected".

4. Once all foods have been selected, <ESCAPE>, then
   <3> Analyze Diet/Recipe
   Use arrow keys to scroll up or down dietary analysis.

5. Once viewed dietary analysis, save diet to a disk.
   <ESCAPE>, then <6> Save Diet/Recipe
   At the "File Name" selection, enter subject number.
   Hit <TAB>to select drive. Enter in A: for drive A, <ENTER>.

6. To quit the program, <ESCAPE>, then <Q> Quit.
   The following appears on the screen:
   Are you sure you want to quit? Enter <Y> for yes.

NUTRITIONIST IV™ CODE BOOK

The purpose of this code book is to provide a default selection when a subject has not given a detailed description of a food item which specifies it from the other food selections. Both foods and serving sizes of the selected foods have been given so that the coder is consistent with unspecified food choices given by a subject. The code book is divided into the following sections:

- Breads/Cereal/Grains
- Dairy
- Fruit
- Meat/Meat Alternatives
- Vegetables
- Miscellaneous: Beverages/Condiments
  Desserts/Snacks
  Dressings/Miscellaneous Items
  Italian Foods
  Soups/Sauces/Submarine Sandwiches

Breads/Cereals/Grains

Food Item	Code	Default Amount
Bagel	319	1 each
Biscuit	4493	1 each
Bread	352	1 slice
Bread (Banana)	7341	1 miniloaf = 2 slices
Bread (Garlic)	7284	1 slice
Bread (Melba)	4933	1 slice
Bread (Pita)	1409	1 each
Bread (Rye)	336	
Bread (Toast)	353	1 slice
Bread (White)	352	1 slice
Bread (Whole Wheat)	358	1 slice
Cereal	5534	1 oz
Cereal (Corn Bran)	5533	1 cup
Cereal (Corn Flakes)	5534	1 cup
Cereal (Apple Cinnamon Cheerios)	5526	1 cup
Cereal (Cocoa Pebbles)	5529	1 cup
Cereal (Corn Chex)	1210	1 cup
Cereal (Cream of Wheat)	1260	1 cup
Cereal (Frosted Flakes)	5545	1 cup
Cereal (Frosted Mini Wheats)	5571	1 cup
Cereal (Fruity Pebbles)	5553	1 cup
Cereal (Grape Nuts)	5555	1 cup
Cereal (Life)	5567	1 cup
Cereal (Raisin Bran)	5585	1 cup
Cereal (Rice Krispies)	5591	1 cup
Cereal (Shredded Wheat)	3688	1 cup
Cereal (Special K)	5597	1 cup
Crackers	432	4 each
Crackers (Cinnamon Graham)	430	2 each
Crackers (Graham)	430	2 each
Crackers (PB/Cheese)	3729	4 each
Crackers (Ritz)	1869	4 each
Crackers (Saltines)	432	4 each
Crackers (Wheat)	7312	4 each
Crackers (Wheat/Cheddar)	3841	4 each
Crackers (Wheat Thins)	1651	4 each
Croutons	4946	2 TBSP
Grits	364	1 cup
Hushpuppies	3779	1 each
Macaroni	6093	1 cup
Macaroni & Cheese	442	1 cup
Muffin	4999	1 each
Muffin (Blueberry)	4015	1 each
Muffin (English)	4995	1 each
Muffin (Plain)	4999	1 each

Breads/Cereals/Grains (Continued)

Food Item	Code	Default Amount
Noodles	6093	1 cup
Noodles (Chow Mein)	7240	1 cup
Noodles (Egg)	1564	1 cup
Noodles (Fettucine)	6099	1 cup
Oatmeal	366	1 cup
Pancakes	452	2 each
Rice	6086	1 cup
Rice (Brown)	129	1 cup
Rice (Wild)	2871	1 cup
Rice Cake	1723	1 each
Rice Pilaf	3706	1 cup
Roll	4467	1 each
Roll (Hoagie)	491	1 each
Roll (Whole Wheat)	1653	1 each
Sunflower Seeds	5844	2 TBSP
Tortilla	1669	1 each
Tortilla (Corn)	1391	1 each
Tortilla (Flour)	1669	1 each
Waffles	1392	2 each

Dairy

Food Item	Code	Default Amount
Cheese (also Grilled Cheese)	22	2/3 oz = 1 slice
Cheese (Cheddar/Nacho Chips)	3	1 oz 1/2 oz for crackers
Cheese (Colby)	888	1 oz
Cheese (Cottage)	8	1 oz
Cheese (Cream)	3736	1 oz
Cheese (Mozzarella)	13	1 oz = 1/8 cup
Cheese (Muenster)	897	1 oz
Cheese (Natural)	17	1 oz
Cheese (Parmesan)	14	1 oz
Cheese (Processed)	22	2/3 oz
Cheese (Provolone/Steaks)	17	1 oz
Cheese (Swiss)	23	1 oz
Cream (Coffee)	5659	1 TBSP
Cream (Lite-Coffee)	3660	1 TBSP
Cream (Sour)	903	1 TBSP
Cream (Whipped)	5218	1 TBSP
Ice-Cream	76	1 scoop = 1/4 cup
Ice Milk	82	1 scoop = 1/4 cup
Milk	51	1 cup
Milk (2%)	51	1 cup
Milk (Chocolate)	68	1 cup
Milk (Skim)	57	1 cup
Milk (Whole)	50	1 cup
White Sauce	5189	1/4 cup
Yogurt	93	8 oz
Yogurt (Non-Fat)	94	8 oz
Yogurt (With Fruit)	92	8 oz

Fruit

Food Item	Code	Default Amount
Apple	223	1 each
Apple (Juice)	225	6 oz
Applesauce	5614	1/2 cup
Banana	8447	1 each = 1 serving
Cantaloupe	8451	1/2 each
Cranberry (Juice)	242	6 oz
Grapes	5627	10 each = 1/2 cup
Grape (Juice)	258	6 oz
Grapefruit	246	1/4 each
Grapefruit (Juice)	984	6 oz
Honeydew	272	1 cup
Kiwi	8458	1 each
Melon	5628	1/4 each
Mixed Fruit	977	1/2 cup
Nectarine	1005	1 each
Orange	8460	1 each
Orange (Juice)	278	6 oz
Peach	5633	1/2 cup
Peach (Canned)	5633	1/2 cup
Peach (Raw)	283	1 each
Pear	5635	1/2 cup
Pear (Canned)	5635	1/2 cup
Pear (Raw)	291	1 each
Pineapple	5640	1/2 cup
Pineapple (Canned)	5640	1/2 cup
Pineapple (Juice)	1021	1 cup
Pineapple (Raw)	295	1/4 each
Pineapple/Orange (Juice)	6033	6 oz
Plum	301	1 each
Raisins	307	1/2 cup
Strawberries	5649	1/2 cup

Meat/Meat Alternatives

Food Item	Code	Default Amount
Beef	4144	2 oz
Beef (Ground)	5917	2 oz
Beef (Lean)	4123	4 oz
Beef (Roast)	167	4 oz
Beef (Steak)	4144	4 oz
Canadian Bacon	912	2 oz
Chicken	1275	4 oz
Chicken (Fried)	6830	4 oz
Chicken (Grilled)	7404	4 oz
Chicken (No-Skin)	1275	4 oz
Chicken (Roasted)	1273	4 oz
Chicken Tenders	1873	4 oz
Egg	5234	1/2 cup
Egg (Fried)	99	1 each
Egg (Scrambled)	5234	1/2 cup
Fish (Filet)	3767	1 serving
Ham	190	2 oz
Ham (Lunchmeat)	190	2 oz
Meatloaf	7751	4 oz
Nuts	6084	1/4 cup
Nuts (Peanuts)	6084	1/4 cup
Nuts (Walnuts)	530	1/4 cup
Omelet	1407	1 serving
Ribs	4240	2 oz
Ribs (Baby)	4240	2 oz
Salmon	6058	2 oz
Salmon (Poached)	6058	2 oz
Sausage	7108	2 oz
Shrimp	7701	4 oz
Tuna	355	2 oz
Turkey	7154	4 oz
Turkey (Lunchmeat)	7154	4 oz
Turkey (Roasted)	222	4 oz

Vegetables

Food Item	Code	Default Amount
Beans	573	1/2 cup
Beans (Baked)	1804	1/2 cup
Beans (Garbanzo)	1678	1/2 cup
Beans (Green)	573	1/2 cup
Beans (Kidney)	6081	1/2 cup
Beans (Pinto)	2784	1/2 cup
Beans (Refried)	1810	1/2 cup
Beans (Sprouts)	5764	1 TBSP
Broccoli	590	1 spear = 30 g 1 cup = 184 g
Broccoli (Boiled From Frozen)	590	1 spear = 30 g 1 cup = 184 g
Broccoli (Boiled From Raw)	588	1 cup = 156 g
Broccoli (Raw)		1 spear = 30 g
Brussel Sprouts	5764	1/4 cup
Brussel Sprouts (Boiled)	591	1/4 cup
Carrots	5768	1 each = 1/2 cup
Carrots (Cooked)	5767	1/2 cup
Carrots (Raw)	5768	1 each = 1/2 cup
Cauliflour	5770	1/2 cup
Celery	5773	1 each = 1/2 cup
Celery (Raw)	5773	1 each = 1/2 cup
Cole Slaw	6834	1 ounce
Corn	617	1/2 cup
Corn (Canned)	617	1/2 cup
Cucumber	619	4 slices
Lettuce	627	1 cup
Mixed Vegetables	1132	1/2 cup
Mushrooms	5781	1/2 cup
Mushrooms (Canned)	5781	1/2 cup
Olives	5026	1/4 cup
Olives (Green)	5026	1/4 cup
Onion	8465	1/4 cup
Peas	5785	1/2 cup
Peas (Blackeye)	516	1/2 cup
Peppers	5787	1/2 cup
Peppers (Green)	5787	1/2 cup
Pickle	703	1 each
Pickle (Dill)	703	1 each
Pickle (Sweet)		1 each
Potato	645	1 each
Potato (Baked)	645	1 each
Potato (Boiled)		1 each

Vegetables (Continued)

Food Item	Code	Default Amount
Potato (French-Fried-Home)	649	1 cup = 15 each
		small = 15 each
		medium = 25 each
		large - 40 each
Potato (Fried)	2761	1/2 cup
Potato (Hash Browns)	650	1 cup
Potato (Mashed)	652	1/2 cup
Potato (Scalloped)	1093	1/2 cup
Radish	657	1 each
Salad	1826	1 each
Spinach	659	1 cup
Spinach (Cooked)	661	1/2 cup
Spinach (Raw)	659	1 cup
Squash	664	1/2 cup
Tomato	6332	1 each = 123 g
		1 each = 8 slices
		4 slices = 1/2 cup
		4 slices = 61.5 g

Miscellaneous: Beverages/Condiments

Food Item	Code	Default Amount
Beer	686	12 fl. ounces
Coffee	731	1 cup
Fruit Punch	1718	1 cup
Juice	See Fruit Section	
Kool-Aid	6016	1 cup
Lemonade	6023	1 cup
Soft Drink	693	12 oz
Soft Drink (Cola)	693	12 oz
Soft Drink (Diet Cola)	1415	12 oz
Soft Drink (Slice/7-Up)	1876	12 oz
Tea	733	1 cup
Vegetable Juice	1434	1 cup
Bacon Bits	1408	1 TBSP
Butter	5195	1 TBSP
Gravy	842	1 TBSP
Jam/Jelly	551	1 TBSP
Ketchup	674	1 TBSP
Margarine	924	1 TBSP
Mayonnaise	138	1 TBSP
Mustard	5001	1 TBSP
Peanut Butter	6082	2 TBSP
Salt	822	1 TSP
Sugar	561	1 TBSP
Syrup	4795	2 TBSP

Miscellaneous: Desserts/Snacks

Food Item	Code	Default Amount
Apple Turnover	4459	1 each
Brownie	3561	1 each
Brownie (Fudge)	3555	1 each
Cake	4530	1 serving
Cake (Angel-Food)	386	1 serving
Cake (Carrot)	4543	1 serving
Cake (Chocolate)	4538	1 serving
Cake (White)	4530	1 serving
Candy Bar	4665	1 each
Candy Bar (5th Avenue)	4665	1 each
Candy Bar (Bar None)	4654	1 each
Chewing Gum	1666	1 each
Chocolate	538	2 oz
Cobbler	1607	1 slice
Cookie	4456	1 each
Cookie (Chocolate Chip)	4457	1 each
Cookie (Fat Free)	6952	1 each
Cookie (Peanut Butter)	7249	1 each
Cookie (Sugar)	4456	1 each
Corn Chips	1389	4 oz = 25 chips
Croissant	1889	1 each
Doughnut	437	1 each
Ice Cream Cone	4972	1 each
Jello	2620	1/2 cup
Licorice	4934	1 oz
Nachos & Cheese	1899	1 cup
Pie (Apple)	455	1 slice
Pie (Cream)	1667	1 slice
Pie (Lemon)	464	1 slice
Popcorn	477	1 cup
Popsicle	207	1 each
Poptart	499	1 each
Potato Chips	5790	4 oz = 25 chips
Pretzels	481	1 cup = 125 thin sticks
Pretzels (Hard)	481	1 hard = 50 thin sticks
Pretzels (Soft)	3955	1 each
Pudding	4749	1/2 cup
Pudding (Vanilla)	4749	1/2 cup
Tootsie Rolls	3956	1/2 ounce

Miscellaneous: Dressings/Miscellaneous Items

Food Item	Code	Default Amount
Dressing	5445	1 oz = 2 TBSP
Dressing (Fat-Free)	5444	1 oz = 2 TBSP
Dressing (Italian)	136	1 oz = 2 TBSP
Dressing (Oil & Vinegar)	120, 5176	1 oz = 2 TBSP
Dressing (Ranch)	1765	1 oz = 2 TBSP
Dressing (Thousand Island)	142	1 oz = 2 TBSP
Vegetable Dip	4810	1 serving
Bean Burrito (+ Cheese)	1897	1 each
Chicken Salad	1778	1 cup
Chicken Alfredo	3655	1 serving
Chili	1809	1 cup
Egg Roll	7254	1 each
Egg Roll & Vegetable	7254	1 each
Hog Dog With Bun	1411	1 each
Stir Fry	7267	1 cup

Miscellaneous: Italian Foods

Food Item	Code	Default Item
Lasagna	471	1 serving
Manicotti	1914	1 serving
Pasta (Salad)	3698	1 serving
Pasta (Spinach)	2883	1 serving
Pizza	4072	1 serving
Pizza (Cheese)	4072	1 serving
Pizza (Dough)	3579	1 serving
Pizza (Mushroom)	2318	1 serving
Pizza (Pepperoni)	4073	1 serving
Pizza (Sausage)	4071	1 serving
Ravioli	7441	1 serving
Spaghettio's	7655	1 can = 17 oz
Stromboli	4073	1 serving
Stuffed Shells	1411	1 serving
Tortolini (Cheese)	2383	1 serving

Miscellaneous: Soups/Sauces/Submarine Sandwiches

Food Item	Code	Default Amount
Soup	3078	1 cup
Soup (Chicken Noodle)	3078	1 cup
Soup (Clam Chowder)	828	1 cup
Soup (Cream of Broccoli)	7500	1 cup
Soup (Cream of Vegetable)	824	1 cup
Soup (Minestrone)	717	1 cup
Soup (Tomato)	1355	1 cup
Soup (Vegetable)	3103	1 cup
Salsa	7222	1/2 cup
Sauce	5813	1/2 cup
Sauce (Alfredo)	3723	1/2 cup
Sauce (BBQ)	685	1/2 cup
Sauce (Marinaria)	1122	1/2 cup
Sauce (Meat)	5150	1/2 cup
Sauce (Picante)	348	1/2 cup
Sauce (Soy)	841	1 TBSP
Sauce (Spaghetti)	5813	1/2 cup
Sauce (Sweet & Sour)	7249	1/2 cup
Sauce (Tomato)	7249	1/2 cup
Sub	3885	1 each
Sub (Ham & Cheese)	3885	1 each
Sub (Turkey & Cheese)	3675	1 each

APPENDIX J

PWC_{170} TEST SUBJECT INFORMATION

PWC_{170} TEST SUBJECT INFORMATION

1. Name _____

2. Your evaluation is scheduled at _____ on _____
   time day/date

3. The test will be conducted in the VPI & SU Laboratory for Health and Exercise Scineces in War Memorial Hall. Park in front of the building and go to Room 230 on the second floor.

4. Your laboratory evaluation will involve the following:
   - •Health and Physical Activity Histories, Risk Factor Evaluation
   - •Your written informed consent to participate
   - •Anthropometric (fat) Measurements
   - •Resting blood pressure and heart rate
   - •Spirometry (lung measurement) - forced expired volume
   - •Electrocardiogram (single lead using 3 electrodes on chest)
     - Supine (lying on back)
     - Exercise - electrocardiographically monitored Graded Exercise Test using a stationary cycle ergometer with 1 channel ECG recorder, constant monitoring of heart rate and periodic blood pressure determinations
     - Recovery

5. Apparel for GXT
   Women - comfortable walking clothes and appropriate walking shoes, as well as a snug fitting halter top (i.e., as with a two-piece bathing suit) with no clothing that covers the upper arm; light rubber soled walking shoes must be worn.

6. Do not eat, drink or smoke within 4 hours of reporting to this lab, do not exercise at any time during the day you take this exercise test.

7. If you have questions about prescription medications, consult your physician or our office (231-7277) at least 2 days before your scheduled evaluation. Please bring with you a bottle of each medication you may be taking; the bottle may be an empty one in that we simply need to look at the prescription.

8. The supervisor of our exercise staff in attendance for your evaluation will be either Jeff Ocel, Laura Craft, or Dr. Herbert.

9. Upon your arrival, please report to the staff supervisor.

10. If it looks as if you will be late or if you are ill on the scheduled day, notify us at 231-5006 or 231-7277 immediately. If you have to cancel your appointment, let us know in order that consideration can be given for testing another subject.

APPENDIX K

BORG'S RATING OF PERCEIVED EXERTION SCALE

BORG'S RATING OF PERCEIVED EXERTION SCALE*

6

7 = VERY, VERY LIGHT

8

9 = VERY LIGHT

10

11 = FAIRLY LIGHT

12

13 = SOMEWHAT HARD

14

15 = HARD

16

17 = VERY HARD

18

19 = VERY, VERY HARD

20

*Adapted from Pollock et al., 1984

APPENDIX L

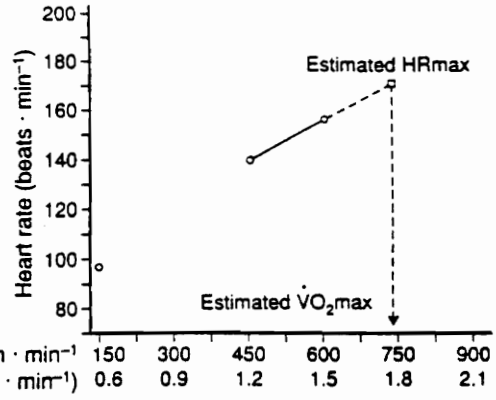
PREDICTING VO_{2MAX} FROM HEART RATE AND WORK LOAD

Name: _____ Estimated HRmax: 170 Ht: _____ in. Wt: _____ lb

Sex: Female Age: 50 85% HRmax: 145 _____ cm _____ kg

1. Plot 3rd min HR for each work rate.
2. Draw line though points starting at HR > 110.
3. Extrapolate line to subject's estimated HRmax.
4. Drop vertical line from HRmax to baseline.
5. Record estimated $\dot{V}O_2$ max in L · min^{-1}.

Work rate kgm · min^{-1}	Heart rate 2nd min	3rd min
150	95	96
300		
450	138	140
600	153	156



Prediction of Maximum Oxygen Uptake

WOMEN

Heart Rate	Maximal Oxygen Uptake (liters/min.)					Heart Rate	Maximal Oxygen Uptake (liters/min.)				
	300 (kpm/ min.)	450 (kpm/ min.)	600 (kpm/ min.)	750 (kpm/ min.)	900 (kpm/ min.)		300 (kpm/ min.)	450 (kpm/ min.)	600 (kpm/ min.)	750 (kpm/ min.)	900 (kpm/ min.)
120	2.6	3.4	4.1	4.8		148	1.6	2.1	2.6	3.1	3.6
121	2.5	3.3	4.0	4.8		149		2.1	2.6	3.0	3.5
122	2.5	3.2	3.9	4.7		150		2.0	2.5	3.0	3.5
123	2.4	3.1	3.9	4.6		151		2.0	2.5	3.0	3.4
124	2.4	3.1	3.8	4.5		152		2.0	2.5	2.9	3.4
125	2.3	3.0	3.7	4.4		153		2.0	2.4	2.9	3.3
126	2.3	3.0	3.6	4.3		154		2.0	2.4	2.8	3.3
127	2.2	2.9	3.5	4.2		155		1.9	2.4	2.8	3.2
128	2.2	2.8	3.5	4.2	4.8	156		1.9	2.3	2.8	3.2
129	2.2	2.8	3.4	4.1	4.8	157		1.9	2.3	2.7	3.2
130	2.1	2.7	3.4	4.0	4.7	158		1.8	2.3	2.7	3.1
131	2.1	2.7	3.4	4.0	4.6	159		1.8	2.2	2.7	3.1
132	2.0	2.7	3.3	3.9	4.5	160		1.8	2.2	2.6	3.0
133	2.0	2.6	3.2	3.8	4.4	161		1.8	2.2	2.6	3.0
134	2.0	2.6	3.2	3.8	4.4	162		1.8	2.2	2.6	3.0
135	2.0	2.6	3.1	3.7	4.3	163		1.7	2.2	2.6	2.9
136	1.9	2.5	3.1	3.6	4.2	164		1.7	2.1	2.5	2.9
137	1.9	2.5	3.0	3.6	4.2	165		1.7	2.1	2.5	2.9
138	1.8	2.4	3.0	3.5	4.1	166		1.7	2.1	2.5	2.8
139	1.8	2.4	2.9	3.5	4.0	167		1.6	2.1	2.4	2.8
140	1.8	2.4	2.8	3.4	4.0	168		1.6	2.0	2.4	2.8
141	1.8	2.3	2.8	3.4	3.9	169		1.6	2.0	2.4	2.8
142	1.7	2.3	2.8	3.3	3.9	170		1.6	2.0	2.4	2.7
143	1.7	2.2	2.7	3.3	3.8						
144	1.7	2.2	2.7	3.2	3.8						
145	1.6	2.2	2.7	3.2	3.7						
146	1.6	2.2	2.6	3.2	3.7						
147	1.6	2.1	2.6	3.1	3.6						

APPENDIX M

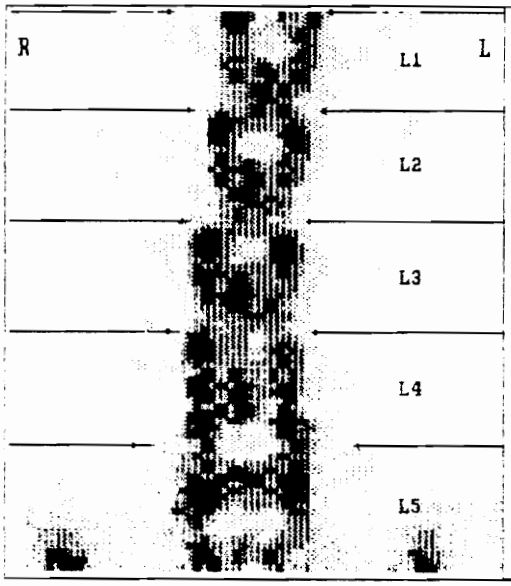
SAMPLE DATA SHEET FROM DUAL PHOTON ABSORPTIOMETER

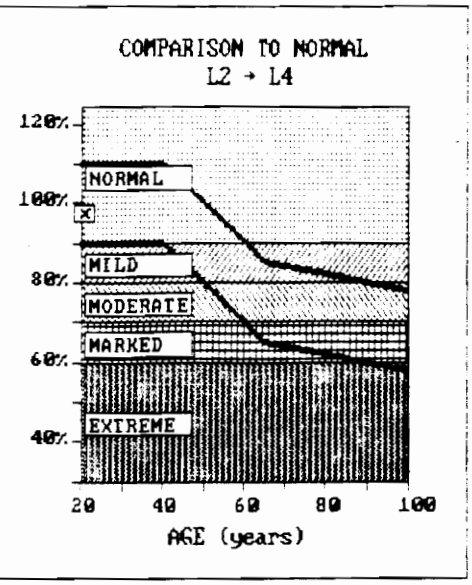
MONTGOMERY REGIONAL HOSPITAL
BONE MINERAL DENSITOMETRY

ROUTE 460 SOUTH, BLACKSBURG, VA, [illegible]

ID: 07-79-54
NAME:

SCAN: [illegible]
ANALYSIS: [illegible]





Age (years).....	21	Large Standard.....	13.57	Scan Speed (mm/s)...	5.0
Sex.....	Female	Medium Standard.....	10.98	Step Distance (mm)..	4.5
Weight (lb).....	[illegible]2.0	Small Standard.....	9.94	Collimation (mm)....	13
Height (in).....	68	44 keV Air Value....	26952	Corrected R value...	1.42
Ethnic.....	White	100 keV Air Value...	[illegible]5218		

REGION	BMD g/cm²	% Young Normal	% Age* Matched	Fracture Risk
L1	1.177	[illegible]	[illegible]	NORMAL
L2	1.253	[illegible]	[illegible]	NORMAL
L3	1.252	[illegible]	[illegible]	NORMAL
L4	1.196	[illegible]	[illegible]	NORMAL
L1 -> L2	1.223	[illegible]	[illegible]	NORMAL
L1 -> L3	1.227	[illegible]	[illegible]	NORMAL
L1 -> L4	1.214	[illegible]	[illegible]	NORMAL
L2 -> L3	1.252	[illegible]	[illegible]	NORMAL
L2 -> L4	1.224	97.3	97.3	NORMAL
L3 -> L4	1.209	[illegible]	[illegible]	NORMAL

* Age matched adjusted for sex, age, ethnic.

LUNAR

APPENDIX N

HUMAN WASTE COLLECTION PROCEDURES

HUMAN WASTE COLLECTION PROCEDURES

PROTOCOL FOR WASTE DISPOSAL

I. CATEGORIES OF WASTE INCLUDE:

A. Biological/"Infectious" Waste.

B. Glass Waste and "Sharps" (Includes Biological/"Infectious" Sharps).

C. Chemical Waste and Toxic Waste.

D. General Lab Waste, i.e., trash.

E. Radioactive Waste.

II. DEFINITION OF EACH CATEGORY OF WASTE:

A. Biological/"Infectious" Waste:

1. **INFECTIOUS** - biomedical waste which is infectious to humans (not necessarily infectious but is treated as though capable of producing disease in humans or is perceived as "infectious").

2. Infectious waste:

a. Cultures and biologicals.
b. Blood and blood products, including anything that comes in contact with blood, i.e., gloves, paper towels, Kimwipes, pipet tips, plastic tubes.
c. Pathological waste.
d. Sharps.
e. Animal blood, carcasses, tissues, etc. (presently human and animal waste treated the same).

B. Glass Waste and "Sharps":

1. "Sharps":

a. Needles
b. Syringes
c. Vacutainers

d. Glass slides
e. Pipet tips
f. Razor blades, scalpels
g. Pasteur pipets
h. All other pipets
i. All blood or serum tubes for disposal
j. All other glass tubes (broken)
k. Broken plastic
l. Small disposable filter units
m. Disposable loops
n. Swabs
o. Capillary tubes
p. Droppers
q. Glass vials
r. Any glass, plastic, or metal which can puncture a biohazard bag

C. Chemical Waste and Toxic Waste:

1. Reagents and solvents used in lab procedures.

D. General Lab Waste:

1. Regular trach includes everything from candy to flowers.

E. Radioactive Waste:

1. Never handle unless authorized.

III. DISPOSAL PROCEDURE

A. Biological/"Infectious" Waste:

1. All "infectious" waste **MUST BE PLACED IN AN INFECTIOUS WASTE BOX.** Therefore, dispose of all non-sharp materials in Orange Biohazard Bags. Place all Orange Biohazard Bags into an Infectious Waste Box. These boxes are located in Laboratory 328, Wallace Hall. Once Infectious Waste Boxes are filled they are taken to Laboratory Animal Resources (LAR) for commercial disposal. Health and safety should be notified when boxes have been taken to LAR for pick-up. A log is kept for record keeping in Laboratory 328, Wallace Hall. This log lists the dates when boxes are taken to LAR.

B. Glass Waste and "Sharps":

1. Dispose of "sharps" in a sharp container and place the container in an Orange Biohazard Bag. These bags should be kept in a puncture-resistant, leakproof container until they can be placed in an infectious waste box.

2. Follow procedure as listed above under section A.

3. Non-infectious broken glass may be placed in a puncture resistant box, labeled properly as broken glass and placed in the regular trash.

C. Chemical Waste and Toxic Waste:

1. Follow MSDS (material safety data sheet) supplied from the company from which the reagent or solvent was purchased.

2. Fill out waste label.

3. TA's and students involved with research check with Carolyn Harris, Kathy Reynolds, or Janet Rinehart, who call Health and Safety, the Hazardous Waste Department for pick-up.

D. General Lab Waste:

1. Keep regular trash segregated from "infectious" waste.

E. Radioactive Waste:

1. Special guidelines.

2. All procedures using radioactivity are conducted in Laboratory 328, Wallace Hall. Procedures are conducted and waste handled only by licensed and responsible persons for radioisotopes in the Department of Human Nutrition and Foods.

GENERAL SAFETY GUIDELINES

1. Follow Universal Precautions!

2. Assume that no waste goes down the sink!

3. When in doubt, ask!

ENVIRONMENTAL HEALTH AND SAFETY SERVICES

1. Medical and Training Program:

    a. Laboratory Safety: Deborah Young 231-8751
    b. Hazardous Waste and Radiation Safety: Doug Smiley 231-5364

THE UNIVERSITY MUST COMPLY WITH ESTABLISHED DISPOSAL PROCEDURES. FAILURE TO COMPLY WILL RESULT IN CENSURE AND FINES TO THE DEPARTMENT AND LOSS OF YOUR LABORATORY PRIVILEGES.

APPENDIX O

24 HOUR URINE COLLECTION PROCEDURES

INSTRUCTIONS FOR COLLECTION A 24-HOUR URINE SAMPLE

It is very important to make a total collection of all your urine for 24 hours. If a void is lost within the 24 hour period, please record the details on a sheet of paper with your name and include this when you turn in your urine sample.

You will be given 3 urine containers (2 large and 1 small) for each collection period. These bottles will contain ascorbate which is used as a preservative.

A. On the first day, void into the toilet before you start the collection. This is the last time that you will void into the toilet until after the 24 hour collection period is done. In other words, the collection begins with an empty bladder. Remember the time you began your collection and continue until for 24 hours. Be sure to urinate directly into the collection containers.

B. On the second day, save your urine up to the end of the 24 hour collection time. For most of you this will mean including the first void of the morning.

C. Please make every attempt to fill one of the large containers before starting with the next container.

D. The small container may be used one time within the 24 hour period, regardless of whether the large containers are filled. Do not reuse this container again within the same 24 hour period.

E. Keep the collected urine specimens cool at all times. If you do not have a refrigerator available for this purpose during the day, please use a styrofoam cooler with an ice pack.

F. Since we are studying boron, care must be taken to avoid contamination of the urine specimen, as much as possible. For this reason it is important that you avoid touching the inside of the urine bottle with your fingers.

G. Please try to maintain a normal fluid intake during each collection period. This is important in order to avoid producing overly concentrated urine.

Thank you for your cooperation.

APPENDIX P

WET-ASHING PROCEDURES

WET-ASHING PROCEDURES

In an attempt to minimize trace mineral contaminaion, powder-free gloves and deionized water were used during sample preparation and analysis. No glassware came in contact with the samples since glass itself may contain traces of boron. All equipment, including Teflon tubes and caps, Sardstedt tubes and caps, urine collection bottles, and pipet tips were acid washed in 2 N hydrochloric acid (HCl) (Fisher Scientific, Pittsburgh, PA 15219) prior to use. After soaking in the acid bath for at least 24 hours prior to use, the equipment was rinsed three times with deionized water and left to air dry on kimwipes™. Each Teflon tube and cap, and Sardstedt tube and cap were labeled and recorded.

The following procedures of Hunt and Shuler (1989) were used for sample preparation.

Plasma:

1. Plasma samples were thawed at room temperature.
2. Pipet 2ml plasma into Teflon tubes
3. Add 2ml 16.1N HNO_3 (Fisher Scientific Trace Metal Grade, Pittsburgh, PA 15219) to tube, mix throughly by swirling, recap, and let stand overnight.

   Release gas intermittently.

4. Remove caps (place down in container lined with kimwipes™) and let stand (in hood). Preheat heating block (in hood) for ten minutes on setting seven.

5. Place uncapped tubes into heating block. Gently raise temperature to approximately 100°C. Heat tubes until almost dry (until liquid just pulls away from sides). Intermittently mix by swirling to assure full digestion.

6. Remove tubes and let cool for approximately five minutes.

7. Add 2ml 16.1N HNO_3 to each tube. Mix well.

8. Repeat steps 4 and 5. Turn off heating block while performing steps 8 and 9.

9. Add 0.5ml 16.1N HNO_3 (use pipette-man and acid washed tip).

10. Add 2ml 30% H_2O_2 (GFS Chemicals, Inc., 30% Reagent [ACS] Columbus, OH 43223) drop-wise to each sample.

    CAUTION: HAVE ICE BATH READY

11. Preheat heating block to approximately 85°C on setting six.

12. Place samples on heating block. Watch carefully for rapid boiling and formation of foam on top.

    NOTE: Steps 11 and 12 do five to ten samples at a time.

13. When sample foams, remove from heating block and place on ice. Once foaming stops, replace on heating block and five to ten new samples.

    NOTE: Repeat steps 11 and 12 until all samples are done.

14. Heat tubes until almost dry. Remove and let cool.

15. Label and weigh out acid-washed Sardstedt tubes with cap and record weight.

16. Add 0.5ml 6N HCl (GFS Chemicals, Inc., Redistilled 6M, Columbus, OH 43223) to tubes with sample and heat on heating block on low for five minutes.

17. Remove samples and transfer to corresponding Sardstedt tubes.

18. Rinse digestion tubes with 0.5ml 0.1N HCl (rinse sides) and transfer to Sardstedt tube. Rinse two more times and bring volume up to 2ml.

19. Re-weigh each Sardstedt tube (cap and sample). Record weight. Centrifuge ten minutes at 4000 rpm.

20. Refrigerate until analysis is run.

Urine:

1. Urines samples were thawed at room temperature and vortexed.

2. Pipet 5ml urine from the top of the container into Teflon tube.

3. Add 1ml 16.1N HNO_3 (Fisher Scientific Trace Metal Grade, Pittsburgh, PA 15219) to tube, mix throughly by gently swirling, recap, and let stand overnight. Release gas intermittently.

4. Remove caps (place down in container lined with kimwipes™) and let stand (in hood). Preheat heating block (in hood) for ten minutes on setting seven.

5. Place uncapped tubes into heating block. Gently raise temperature to approximately 100°C. Heat tubes until almost dry (unitl liquid just pulls away from sides to form a ball). Intermittently mix by swirling to assure full digestion.

6. Remove tubes and let cool for approximately five minutes.

7. Add 1ml 16.1N HNO_3 (use pipette-man and acid washed tip).

8. Add 3ml 30% H_2O_2 (GFS Chemicals, Inc., 30% Reagent [ACS] Columbus, OH 43223) drop-wise to each sample.

CAUTION: HAVE ICE BATH READY

9. Preheat heating block to approximately 85°C at setting six.

10. Place samples on heating block. Watch carefully for rapid boiling and formation of foam on top.

NOTE: Steps 10 and 11 do 5-10 samples at a time

11. When sample foams, remove from heating block and place on ice. Once foaming stops, replace on heating block and add 5-10 new samples.

NOTE: Repeat steps 10-11 until all samples are done

12. Heat tubes until almost dry. Remove and let cool.

13. Label and weigh out acid-washed Sardstedt tubes with cap and record weight.

14. Add 1ml 6N HCl (GFS Chemicals, Inc., Redistilled 6M, Columbus, OH 43233) to tubes with sample and heat on heating block on low for five minutes.

15. Remove samples and transfer to corresponding Sardstedt tubes.

16. Rinse digestion tubes with 1ml 0.1N HCl (rinse sides) and transfer to Sardstedt tube. Rinse two more times and bring volume up to 5ml.

17. Re-weigh each Sardstedt tube (cap and sample). Record weight. Centrifuge 10 minutes at 4000 rpm.

18. Refrigerate until analysis is run.

APPENDIX Q

SAMPLE PREPARATION FOR ATOMIC ABSORPTION SPECTROSCOPY

SAMPLE PREPARATION FOR ATOMIC ABSORPTION SPECTROSCOPY

The following procedures of Perkin-Elmer (1982) were used for sample preparation.

Plasma Calcium and Magnesium:

1. Dilute plasma sample 1:50 with a 0.1% (w/v) lanthanum chloride diluent.

Urine Calcium:

1. Dilute urine sample 1:100 with 0.5% (w/v) lanthanum chloride diluent.

Urine Magnesium:

1. Dilute urine sample 1:200 with deionized water.

A Perkin-Elmer 2100 Atomic Absorption Spectrophotometer was used to analyze the samples.

APPENDIX R

PROCEDURES FOR PHOSPHORUS DETERMINATION

PROCEDURES FOR PHOSPHORUS DETERMINATION

The following procedures of Sigma Diagnostics (1985) were used for phosphorus sample preparation and determination.

Calibration:

1. Pipet into test tube the following:

Tube #	Phosphorus Standard (ml)	Water (ml)	Serum Inorganic Phosphorus mg/dl)
1	0	5.00	0
2	0.25	4.75	2.5
3	0.50	4.50	5.0
4	0.75	4.25	7.5
5	1.00	4.00	10.0
6	1.25	3.75	12.5

2. To each tube, add 1.0 ml Acid Molybdate Solution. Mix by swirling.

3. to each tube add 0.25 ml fiske and SubbaRow Solution. Mix by inversion, and allow to stand 10 minutes for color development.

4. Transfer to cuvet, read and record absorbance at 660 ± 40 nm using Tube 1 as reference (Spectronic 501 Spectrophotometer, Milton Roy Co., Rochester, NY 14625).

   NOTE: Complete absorbance readings within 10 minutes after color development.

5. Prepare a calibration curve by plotting the absorbance values vs. the corresponding inorganic phosphorus concentration, in mg/dl.

NOTE: Calibration curve should be verified periodically.

Plasma and Urine:

1. Into a test tube, pipet:
   0.5 ml serum or a 10-fold dilution of a 24-hour urine collection.
   2.5 ml water
   2.0 ml Trichloroacetic Acid (TCA) 20% (w/v)

   Cap tube, mix thoroughly by shaking, and allow to stand 5-10 minutes.

2. Centrifuge until clear, or filter through ashless filter paper.

3. Label 2 or more test tubes or cuvets BLANK, TEST 1, TEST 2, etc. Pipet the following:

Reagents	Blank	Test
TCA, 20% (w/v)	2.0 ml	-
Filtrate from Step #2	-	2.0 ml
Water	3.0 ml	3.0 ml
Acid Molybdate Solution	1.0 ml	1.0 ml

   Mix contents by gently shaking.

4. Pipet into each tube 0.25 ml Fiske and SubbaRow Solution. Mix by inversion and allow to stand 10 minutes for color development.

5. Transfer solutions to cuvets and read and record absorbance [A] of TEST using the BLANK as reference at 660 ± 40 nm. Use the same wavelength, cuvet size and instrument as used in the preparation of the calibration curve.

   NOTE: Complete absorbance readings within 10 minutes after color development.

6. Determine inorganic concentration from the calibration curve.

APPENDIX S

RADIOIMMUMOASSAY PROCEDURES

RADIOIMMUMOASSAY PROCEDURES

Estradiol (Diagnostic Products Corporation, 1991)

1. Thaw serum at room temperature and then vortex.

2. **Plain Tubes:** Label four plain (uncoated) 12x75mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.

   Coated Tubes: Label 14 Estradiol Ab-Coated tubes A (maximum binding) and B through G in duplicate. Label additional antibody-coated tubes, also in duplicate, for controls and patient samples.

3. Pipet **100µL** of the zero calibrator A into the NSB and A tubes, and **100µL** of each remaining calibrator, control and patient sample into the tubes prepared. **Pipet directly to the bottom.**

4. Add **1.0mL** of [^{125}I] Estradiol to every tube. Vortex.

5. Incubate for **three hours at room temperature.**

6. Decant throughly.

7. Count for **one minute** in a gamma counter (Beckmam Gamma 5500, Beckmam Instruments, Inc., Arlington Heights, IL 60004).

Progesterone (Diagnostic Products Corporation, 1993)

1. Thaw serum at room temperature and then vortex.

2. **Plain Tubes:** Label four plain (uncoated) 12x75mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.

   Coated Tubes: Label 14 Progesterone Ab-Coated Tubes A (maximum binding) and B through G in duplicate. Label additional antibody-coated tubes, also in duplicate, for controls and patient samples.

3. Pipet **100µL** of the zero calibrator A into the NSB and A tubes, and **100µL** of each of the calibrators B through G into correspondingly labeled tubes. Pipet **100µL** of each control and patient sample into the tubes prepared.

4. Add **1.0mL** of [^{125}I] Progesterone (Yellow) to every tube. Vortex.

5. Incubate for **three hours at room temperature.**

6. Decant throughly.

7. Count for **one minute** in a gamma counter.

Testosterone (Diagnostic Products Corporation, 1992)

1. Thaw serum at room temperature and then vortex.

2. **Plain Tubes:** Label four plain (uncoated) 12x75mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.

   Coated Tubes: Label 12 Total Testosterone Ab-Coated tubes A (maximum binding) and B through F in duplicate. Label additional antibody-coated tubes, also in duplicate, for controls and patient samples.

3. Pipet **50µL** of the zero calibrator A into the NSB and A tubes, and **50µL** of each remaining calibrator, control and patient sample (serum or plasma) into the tubes prepared. Pipet **50 µL** of each urinary **hydrolyzate** into appropiately labeled tubes. **Pipet directly to the bottom.**

4. Add **1.0mL** of [^{125}I] Total Testosterone to every tube. Vortex.

5. Incubate for **three hours at room temperature.**

6. Decant throughly.

7. Count for **one minute** in a gamma counter.

Calcitonin (Diagnostic Products Corporation, 1992)

1. Thaw serum at room temperature and then vortex.

2. Label 16 tubes in duplicate: T (total counts), NSB (nonspecific binding) and B through F. Label additional tubes, also in duplicate, for serum samples and controls.

3. Pipet **200µL** of the zero calibrator A into the NSB and A tubes, and **200µL** of each of the remaining calibrators B through F into correspondingly labeled tubes. Pipet **200µL** of each patient serum sample and control into the tubes prepared.

4. Add **100µL** of Calcitonin Antiserum (Red) to all tubes except the NSB and T tubes. Vortex.

5. Incubate for **three hours at room temperature.**

6. Add **100µL** of [^{125}I] Calcitonin (Yellow) to all tubes. Vortex.

7. Incubate for **18 hours at 0-4°C.**

8. Add **1.0mL** of **cold** Precipitating Solution (Blue) to all tubes. Vortex.

9. Incubate for **30 minutes at room temperature.**

10. Centrifuge for **15 minutes at 3000 x g.**

11. Using a foam decanting rack, decant (or aspirate) the supernatant, retaining the precipitate for counting.

12. Count each tube for **one minute** in a gamma counter.

Parathyroid Hormone (PTH-M) (Diagnostic Products Corporation, 1993)

1. Thaw serum at room temperature and then vortex.

2. Label 18 polyporpylene tubes in duplicate: T (total counts), NSB (nonspecific binding), A (maximum binding), and B thorugh G. Label additional tubes, also in duplicate, for patient samples and controls.

3. Pipet **50µL** of the zero calibrator A into the NSB and A tubes, and **50µL** of each of the remaining calibrators B through G into correspondly labeled tubes. Pipet **50µL** of each patient sample and control into the tubes prepared.

4. Add **100µL** of [^{125}I] PTH-M (Green) to all tubes. Shake the rack.

5. Add **100µL** of PTH-M Antiserum (Red) to all tubes expect the NSB (and T) tubes. Vortex.

6. Incubate **overnight (16-20 hours) at 4°C.**

7. Add **1.0mL** of **cold** Precipitating Solution (Blue) to all tubes. Vortex.

8. Centrifuge for **15 minues at 3000 x g** in a refrigerated centrifuge.

9. Using a foam decanting rack, decant (or aspirate) the supernatant, retaining the precipitate for counting.

10. Count each tube for **one minute** in a gamma counter.

APPENDIX T

RAW DATA

SUBJECT CLASSIFICATION INTO ACTIVITY, SUPPLEMENT, AND ORAL CONTRACEPTIVE GROUPS, PATTERN OF MENSTRUAL CYCLE, AND WEEK OF CYCLE AT TIME OF BLOOD COLLECTIONS

Subject Number	Activity Group*	Supplement Group*	OC Use*#	Pattern of Menstrual Cycle*	Week of Cycle at Blood Collection
1	A	B	OC(11)	E	3
2	A	B	OC(4)	E	2
3	A	B	OC(22)	E	3
4	NA	B	NOC	E	1
5	A	B	NOC	E	3
6	NA	B	OC(3)	E	3
7	A	B	NOC	E	4
8	NA	B	NOC	E	1
9	NA	B	NOC	E	4
10	A	B	OC(12)	E	3
12	A	B	OC(1)	E	3
14	A	B	OC(12)	E	3
15	NA	B	NOC	E	3
16	NA	P	NOC	E	1
19	NA	P	NOC	E	2
20	NA	P	NOC	O	2
21	A	P	OC(1 WEEK)	E	1
22	NA	P	NOC	E	1
23	A	P	NOC	E	4
27	A	B	OC(33)	E	4
30	A	B	NOC	E	4
32	NA	B	OC(13)	E	1
33	NA	B	NOC	E	2
34	NA	B	OC(14)	E	3
35	A	B	NOC	E	3
36	A	B	NOC	E	4
37	A	B	NOC	E	1
38	A	B	NOC	E	3
40	A	B	OC(7)	E	1

*A = ATHLETE, NA = NON-ATHELTE, B = BORON, P = PLACEBO, OC = ORAL CONTRACEPTIVES, NOC = NO ORAL CONTRACEPTIVES, E = EUMENORRHEIC, O = OLIGOMENORRHEIC

#NUMBER IN PARENTHESIS REPRESENTS LENGTH OF TIME (IN MONTHS) ORAL CONTRACEPTIVES TAKEN (AT BASELINE)

SUBJECT ANTHROPOMETRIC DATA

Subject	Height (cm)	Baseline Weight (kg)	Six Months Weight (kg)	Age (years)
1	165	54	55	19
2	160	51	53	18
3	170	58	59	20
4	168	67	66	19
5	170	66	68	19
6	165	59	56	18
7	165	53	53	18
8	175	66	69	18
9	165	68	65	19
10	163	54	56	20
12	170	42	43	19
14	155	57	57	19
15	152	44	43	18
16	155	50	51	18
19	168	56	57	18
20	170	64	66	19
21	173	57	56	19
22	165	72	74	19
23	163	53	51	19
27	170	58	59	19
30	165	56	56	20
32	170	68	70	18
33	163	65	64	19
34	170	53	57	19
35	160	61	63	19
36	152	54	54	20
37	160	62	64	19
38	147	48	47	29
40	165	53	54	19

SUBJECT ANTHROPOMETRIC/FITNESS DATA CONTINUED

Subject	VO_{2MAX} (ml/kg/min)	% Body Fat	Body Frame*	Fitness Rating*
1	49	25	S	E
2	57	20	M	E
3	46	34	S	E
4	38	30	M	A
5	43	22	S	E
6	37	28	S	A
7	51	24	S	E
8	33	29	M	F
9	40	34	M	A
10	59	23	S	E
12	62	10	S	E
14	45	33	M	E
15	25	34	M	P
16	40	30	S	A
19	34	19	S	A
20	35	26	S	A
21	48	24	S	E
22	28	36	L	P
23	46	28	M	E
27	44	27	S	E
30	46	29	S	E
32	38	28	M	A
33	36	36	S	A
34	30	25	S	P
35	42	33	S	E
36	49	38	L	E
37	45	31	S	E
38	46	37	M	E
40	37	29	S	A

*FOR BODY FRAME: S = SMALL, M = MEDIUM, L = LARGE
FOR FITNESS RATING: E = EXCELLENT, A = AVERAGE, F = FAIR, P = POOR

BASELINE THREE-DAY DIETARY RECORD AVERAGES

Subject	Total Energy (kcal)	Protein (%)	Carbohydrate (%)	Fat (%)
1	1043	35	48	17
2	1778	11	81	7
3	2054	12	56	32
4	3160	14	40	46
5	1200	21	66	14
6	1573	12	60	29
7	2224	15	47	38
8	1889	12	57	31
9	1621	17	54	28
10	3037	14	64	22
12	1000	17	66	17
14	1818	16	57	28
15	4098	16	50	34
16	2091	12	64	23
19	3431	12	53	35
20	2040	10	61	30
21	2762	9	59	32
22	1264	15	68	17
23	1190	10	70	20
27	2200	17	66	16
30	2135	16	66	17
32	3494	11	45	43
33	2382	12	78	10
34	2715	13	55	32
35	3634	20	31	48
36	1155	10	75	15
37	1506	15	57	27
38	1319	17	67	16
40	1155	14	68	19

BAESLINE THREE-DAY DIETARY RECORD AVERAGES CONTINUED

Subject	Calcium (mg)	Magnesium (mg)	Phosphorus (mg)	Dietary Fiber (g)
1	1030	232	1470	6
2	689	441	1569	41
3	939	271	1413	13
4	851	312	1359	19
5	955	296	1526	28
6	1132	212	1250	14
7	958	344	1902	17
8	905	289	1551	12
9	954	331	1328	13
10	1162	402	1561	37
12	765	246	1103	18
14	657	219	926	19
15	2951	456	3556	19
16	1274	347	1556	16
19	948	255	1369	15
20	986	183	1300	9
21	965	196	1238	9
22	662	178	924	17
23	530	165	1049	12
27	1016	418	1534	27
30	1984	303	2069	23
32	1145	310	1884	20
33	858	357	1424	28
34	566	198	956	11
35	1435	336	2522	14
36	311	220	638	18
37	989	305	1446	25
38	257	221	749	12
40	479	152	603	22

SIX MONTH THREE-DAY DIETARY RECORD AVERAGES

Subject	Total Energy (kcal)	Protein (%)	Carbohydrate (%)	Fat (%)
1	1369	21	58	21
2	1824	13	76	11
3	2479	11	62	27
4	2895	12	55	19
5	1228	19	59	22
6	1102	17	60	17
7	1663	14	52	33
8	1540	16	55	29
9	1231	17	55	29
10	2444	18	59	23
12	1099	16	70	14
14	2817	26	52	22
15	1993	17	58	25
16	1657	23	44	23
19	1549	12	54	16
20	1990	16	52	31
21	1127	12	57	30
22	1313	14	54	31
23	1890	14	50	36
27	2801	14	66	20
30	2587	20	50	31
32	2924	15	56	29
33	1764	12	76	12
34	1232	13	71	17
35	2909	13	56	31
36	1689	9	75	10
37	1622	12	59	30
38	942	13	76	11
40	1695	13	51	24

SIX MONTH THREE-DAY DIETARY RECORD AVERAGES CONTINUED

Subject	Calcium (mg)	Magnesium (mg)	Phosphorus (mg)	Dietary Fiber (g)
1	817	219	1103	13
2	804	247	1079	23
3	659	214	1094	18
4	1099	439	1587	28
5	1027	223	1178	21
6	922	226	1091	17
7	499	171	889	22
8	811	188	9241	11
9	568	173	957	13
10	1381	367	1775	27
12	604	898	781	17
14	1052	408	2553	25
15	1378	279	2116	14
16	880	277	1836	7
19	608	213	860	10
20	1107	274	1707	14
21	492	480	6548	13
22	1019	195	1010	14
23	157	165	631	17
27	1897	368	2114	29
30	1444	365	2035	21
32	4645	316	1239	29
33	662	252	1007	24
34	678	115	786	7
35	605	282	1957	10
36	729	253	809	23
37	707	290	1124	26
38	445	224	677	22
40	487	257	1055	26

SUBJECT BONE MINERAL DENSITY MEASUREMENTS

Region of the Spine

Subject	L2	L3	L4
1	1.29	1.33	1.27
2	1.44	1.43	1.28
3	1.23	1.20	1.25
4	1.29	1.28	1.29
5	1.38	1.32	1.29
6	1.29	1.22	1.24
7	1.01	1.13	0.98
8	1.34	1.13	1.28
9		NO DATA	
10	1.17	1.20	1.17
12	1.02	1.02	0.99
14	1.10	1.12	1.27
15	1.11	1.11	1.05
16	1.35	1.28	1.21
19	1.34	1.46	1.40
20	1.37	1.38	1.32
21	1.20	1.18	1.23
22	1.13	1.28	1.13
23	1.17	1.14	1.07
27	1.04	1.11	1.17
30	1.18	1.27	1.31
32	1.40	1.44	1.38
33	1.32	1.38	1.25
34	1.30	1.38	1.17
35	1.35	1.43	1.39
36	1.37	1.38	1.27
37	1.09	1.18	1.02
38	1.13	1.24	1.13
40		NO DATA	

BASELINE PLASMA MINERAL CONCENTRATIONS

Subject	Calcium (mg/dl)	Magnesium (mg/dl)	Phosphorus (mg/dl)	Boron (µg/ml)*
1	8.5	1.7	3.3	0.11
2	9.1	1.8	3.2	0.05
3	9.4	1.8	3.2	0.04
4	9.1	1.8	4.1	0.04
5	9.4	1.9	4.1	0.10
6	9.1	1.7	3.0	ND
7	9.0	1.7	3.9	0.08
8	9.5	1.9	3.6	0.02
9	5.2	1.1	3.9	0.04
10	9.5	1.9	3.5	ND
12	9.7	1.9	2.7	0.09
14	8.8	1.8	3.9	0.004
15	8.8	1.8	3.6	0.02
16	8.1	1.5	4.1	ND
19	9.0	1.8	3.6	ND
20	9.4	1.9	3.1	ND
21	7.3	1.5	2.9	ND
22	9.4	1.9	3.5	ND
23	8.7	1.7	4.5	ND
27	8.6	1.7	3.0	ND
30	9.0	1.8	3.3	0.01
32	7.0	1.3	3.4	0.02
33	8.9	1.8	3.6	ND
34	8.2	1.7	3.4	0.11
35	8.5	1.8	3.8	ND
36	8.8	1.9	3.5	ND
37	8.5	1.7	3.3	ND
38	8.3	1.6	3.1	ND
40	7.0	1.4	2.7	ND

*ND = NON-DETECTABLE

SIX MONTH PLASMA MINERAL CONCENTRATIONS

Subject	Calcium (mg/dl)	Magnesium (mg/dl)	Phosphorus (mg/dl)	Boron (µg/ml)*
1	9.6	2.0	3.8	0.06
2	10.0	1.9	3.0	0.01
3	9.3	1.7	2.9	0.01
4	9.5	1.9	4.2	0.33
5	8.8	1.9	4.2	0.06
6	9.2	1.8	3.8	0.03
7	9.9	1.9	3.6	ND
8	9.5	1.9	3.6	0.01
9	9.4	2.0	3.0	0.12
10	9.0	1.7	3.7	ND
12	9.7	2.0	3.3	0.06
14	9.3	1.9	3.4	0.04
15	9.3	1.9	4.0	0.003
16	9.1	1.8	4.0	ND
19	9.5	1.8	3.0	ND
20	8.9	2.0	2.8	ND
21	9.6	1.8	3.5	ND
22	9.1	2.0	4.5	ND
23	9.3	1.7	3.6	ND
27	8.8	1.8	3.1	ND
30	9.5	2.0	3.6	ND
32	9.0	2.0	4.4	ND
33	9.8	2.0	3.4	ND
34	9.7	2.0	3.7	ND
35	9.3	2.0	3.7	0.08
36	9.7	1.9	3.6	ND
37	9.0	1.7	3.6	ND
38	9.5	1.9	3.4	ND
40	9.7	2.0	3.7	ND

*ND = NON-DETECTABLE

BASELINE URINE MINERAL CONCENTRATIONS

Subject	Calcium (mg/dl)	Magnesium (mg/dl)	Phosphorus (g/24 hours)	Boron (µg/ml)
1	22.8	4.8	0.55	5.3
2	2.2	2.0	0.75	0.8
3	8.0	5.8	0.46	2.9
4	11.4	3.2	2.04	3.0
5	20.7	14.2	1.28	5.3
6	5.7	5.7	0.47	3.1
7	14.7	7.7	0.21	3.9
8	28.1	3.5	0.26	4.6
9	6.3	3.7	0.58	1.6
10	15.3	7.8	0.67	3.1
12	22.0	2.7	0.28	11.5
14	7.2	8.0	0.52	3.2
15	12.6	7.5	0.53	3.1
16	18.2	9.3	0.68	1.0
19	8.9	6.6	1.00	0.8
20	12.0	5.4	1.96	0.8
21	19.3	3.3	0.85	1.3
22	12.4	5.8	0.89	0.8
23	18.6	9.5	0.48	1.0
27	14.3	8.2	0.51	5.0
30	10.4	4.5	2.03	2.2
32	7.7	3.2	0.86	7.8
33	2.1	2.9	1.00	1.7
34	21.2	13.9	0.79	3.2
35	20.9	8.3	0.35	4.2
36	6.4	3.5	0.74	2.4
37	15.8	5.2	0.18	3.1
38	5.5	5.8	0.59	5.0
40	16.9	10.8	0.09	1.5

SIX MONTH URINE MINERAL CONCENTRATIONS

Subject	Calcium (mg/dl)	Magnesium (mg/dl)	Phosphorus (g/24 hours)	Boron (µg/ml)
1	14.3	7.2	0.88	1.1
2	3.9	4.1	0.58	1.7
3	9.2	6.3	0.84	2.9
4	26.8	11.6	0.21	7.6
5	8.5	6.4	0.59	3.4
6	6.2	10.1	0.25	0.6
7	16.1	6.2	0.93	0.7
8	18.1	11.1	0.60	2.3
9	4.6	2.6	0.81	0.8
10	7.8	4.6	1.09	0.6
12	22.3	3.7	0.35	11.1
14	4.4	7.1	0.81	3.5
15	24.9	8.0	0.41	6.3
16	17.8	8.2	0.52	2.3
19	4.0	4.2	0.29	1.0
20	12.7	6.3	0.56	0.9
21	27.5	3.6	0.33	1.8
22	14.4	6.8	0.78	1.3
23	21.0	11.9	0.26	1.0
27	13.6	6.1	0.71	2.7
30	12.9	9.2	0.66	2.4
32	11.6	4.2	0.53	7.1
33	3.3	4.2	0.49	1.4
34	8.8	8.3	0.47	0.7
35	6.6	2.7	1.71	2.1
36	6.7	3.1	0.39	5.3
37	6.2	4.1	0.79	0.9
38	9.1	6.1	0.44	3.2
40	14.5	8.4	0.38	0.9

BASELINE SERUM HORMONE CONCENTRATIONS*

Subject	Estradiol (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
1	20	0.39	0.20
2	22	0.74	0.42
3	20	0.50	0.20
4	44	0.34	0.44
5	52	0.76	0.63
6	20	0.32	0.20
7	166	24.22	0.32
8	88	2.33	0.28
9	140	15.1	0.20
10	20	0.57	0.22
12	20	0.27	0.94
14	64	0.85	0.38
15	246	1.03	0.72
16	36	0.80	0.30
19	73	0.23	0.75
20	88	0.72	0.60
21	206	14.38	0.57
22	31	0.61	0.48
23	279	21.90	0.24
27	20	0.71	0.38
30	129	16.44	0.43
32	20	0.66	0.43
33	46	0.56	0.39
34	20	0.39	0.20
35	56	1.20	0.70
36	102	6.28	0.86
37	38	0.45	0.54
38	82	2.92	0.67
40	20	0.31	0.20

*CALCITONIN AND PARATHYROID HORMONE LEVELS WERE NON-DETECTABLE

SIX MONTH SERUM HORMONE CONCENTRATIONS*

Subject	Estradiol (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
1	20	0.46	0.20
2	24	0.85	0.27
3	20	0.68	0.20
4	110	2.62	0.48
5	27	0.85	0.39
6	20	0.36	0.20
7	110	6.95	0.35
8	122	3.64	0.42
9	310	0.79	0.30
10	160	0.74	0.26
12	20	0.43	0.23
14	80	0.84	0.98
15	244	2.02	0.59
16	52	1.20	0.76
19	226	0.80	0.40
20	116	0.45	0.47
21	40	1.31	0.43
22	32	0.40	0.41
23	211	12.76	0.43
27	20	0.38	0.20
30	162	16.34	0.38
32	35	0.60	0.42
33	44	0.63	0.72
34	43	0.58	0.24
35	143	12.14	0.44
36	94	1.64	0.55
37	227	6.71	0.69
38	86	1.12	0.62
40	20	0.38	0.25

*CALCITONIN AND PARATHYROID HORMONE LEVELS WERE NOT MEASURED

APPENDIX U

STATISTICAL ANALYSES

BASELINE ANALYSIS OF VARIANCE (ANOVA) TABLES FOR ACTIVITY, ORAL CONTRACEPTIVE, AND TREATMENT GROUPS			
VARIABLES	F VALUES		
	ACTIVITY (DF = 1)	ORAL CONTRACEPTIVE (DF = 1)	TREATMENT (DF = 1)
SERUM			
TESTOSTERONE	1.08	4.63	5.08
PROGESTERONE	11.50	2.36	0.91
ESTRADIOL	14.22	5.95	3.11
VO_{2MAX}	49.03	3.85	1.45
AGE	2.13	0.67	0.14
HEIGHT	0.95	2.36	0.14
WEIGHT	3.73	1.78	0.20
BODY FAT	0.90	5.18	0.27
TOTAL CALORIES	0.23	0.05	0.01
DIETARY			
PROTEIN	0.74	0.00	5.85
CARBOHYDRATE	3.31	1.22	0.12
FAT	4.23	1.16	0.23
CALCIUM	0.53	0.77	0.20
MAGNESIUM	0.17	0.17	4.69
PHOSPHORUS	0.04	1.34	0.90
FIBER	1.62	0.75	3.95
URINARY			
CALCIUM	0.31	0.01	0.38
MAGNESIUM	0.00	0.00	0.09
BORON	2.04	2.84	9.00
PHOSPHORUS	1.32	2.46	1.94
PLASMA			
CALCIUM	1.69	0.29	0.02
MAGNESIUM	2.00	0.09	0.00
BORON	0.18	3.70	0.93
PHOSPHORUS	0.10	12.74	0.60
BONE DENSITY			
L2	4.15	0.18	0.24
L3	2.51	0.50	0.37
L4	1.30	0.05	0.06

ANALYSIS OF VARIANCE (ANOVA) TABLES FOR CHANGE SCORE VALUES BETWEEN ACTIVITY, ORAL CONTRACEPTIVE, AND TREATMENT GROUPS			
VARIABLES	F VALUES		
	ACTIVITY (DF = 1)	ORAL CONTRACEPTIVE (DF = 1)	TREATMENT (DF = 1)
SERUM			
TESTOSTERONE	4.96	0.02	1.72
PROGESTERONE	6.18	1.31	1.31
ESTRADIOL	9.06	7.29	1.19
WEIGHT	0.87	1.04	0.01
TOTAL CALORIES	1.47	0.60	1.78
DIETARY			
PROTEIN	0.35	0.36	4.69
CARBOHYDRATE	0.27	0.04	7.38
FAT	1.10	0.28	1.13
CALCIUM	0.28	1.73	0.00
MAGNESIUM	0.41	1.62	0.67
PHOSPHORUS	0.06	2.61	0.31
FIBER	0.12	0.00	0.23
URINARY			
CALCIUM	0.89	0.11	0.87
MAGNESIUM	1.05	0.28	0.01
BORON	1.92	2.17	1.90
PHOSPHORUS	3.26	1.37	2.74
PLASMA			
CALCIUM	1.47	0.66	0.21
MAGNESIUM	3.81	0.09	0.12
BORON	0.48	1.99	1.45
PHOSPHORUS	0.48	0.55	0.57

BASELINE ANALYSIS OF VARIANCE (ANOVA) TABLES FOR INTERACTION GROUPS			
VARIABLES	F VALUES		
	ACTIVITY * TREATMENT (DF = 1)	ACTIVITY * ORAL CONTRACEPTIVE (O) (DF = 1)	TREATMENT * ORAL CONTRACEPTIVE (DF = 1)
SERUM			
TESTOSTERONE	1.07	0.00	0.71
PROGESTERONE	7.55	0.97	3.80
ESTRADIOL	13.18	0.01	6.80*
VO_{2MAX}	0.04	1.00	0.48
AGE	0.45	0.41	0.12
HEIGHT	1.05	0.20	0.37
WEIGHT	0.00	0.00	0.05
BODY FAT	0.03	0.43	0.09
TOTAL CALORIES	0.13	0.35	0.28
DIETARY			
PROTEIN	3.84	0.00	0.05
CARBOHYDRATE	0.59	0.12	1.78
FAT	1.93	0.28	1.72
CALCIUM	0.02	0.54	0.29
MAGNESIUM	0.88	2.96	0.00
PHOSPHORUS	0.01	0.38	0.30
FIBER	1.17	0.19	0.57
URINARY			
CALCIUM	0.62	0.02	0.39
MAGNESIUM	0.04	2.47	1.58
BORON	0.06	0.62	1.97
PHOSPHORUS	0.66	0.49	0.05
PLASMA			
CALCIUM	4.35	1.66	0.03
MAGNESIUM	4.07	2.19	0.88
BORON	0.00	1.83	0.50
PHOSPHORUS	0.16	0.03	0.95
BONE DENSITY			
L2	0.03	0.45	0.16
L3	1.69	1.45	0.56
L4	0.41	0.00	0.01

*SAMPLE SIZE TOO SMALL FOR SIGNIFICANCE (ORAL CONTRACEPTIVES/PLACEBO N=1)

ANALYSIS OF VARIANCE (ANOVA) TABLES FOR CHANGE SCORE VALUES BETWEEN INTERACTION GROUPS			
VARIABLES	F VALUES		
	ACTIVITY * TREATMENT (DF = 1)	ACTIVITY * ORAL CONTRACEPTIVE (O) (DF = 1)	TREATMENT * ORAL CONTRACEPTIVE (DF = 1)
SERUM			
TESTOSTERONE	0.66	1.12	0.22
PROGESTERONE	5.51	0.02	4.10
ESTRADIOL	6.74	0.15	4.93*
WEIGHT	3.50	0.32	1.28
TOTAL CALORIES	0.79	0.04	2.89
DIETARY			
PROTEIN	0.03	1.04	1.09
CARBOHYDRATE	0.62	0.31	2.86
FAT	0.07	0.65	0.54
CALCIUM	1.28	0.95	1.37
MAGNESIUM	0.38	0.07	1.06
PHOSPHORUS	1.10	0.88	2.59
FIBER	1.77	2.38	0.67
URINARY			
CALCIUM	2.21	1.39	1.39
MAGNESIUM	1.25	1.96	0.05
BORON	0.11	2.64	0.24
PHOSPHORUS	0.07	0.15	0.09
PLASMA			
CALCIUM	4.68	1.45	2.10
MAGNESIUM	1.78	1.30	0.32
BORON	0.37	2.07	1.22
PHOSPHORUS	0.11	0.72	0.05

*SAMPLE SIZE TOO SMALL FOR SIGNIFICANCE (ORAL CONTRACEPTIVE/PLACEBO, N=1)

APPENDIX V

INDIVIDUAL DATA SHEETS

INDIVIDUAL DATA SHEETS FOR BORON STUDY

SUBJECT NAME: _____

SUBJECT NUMBER: _____

SUPPLEMENT: _____ BORON _____ PLACEBO

DATE TAKEN: _____ BASELINE _____ 6-MONTHS

WEIGHT: _____ lbs HEIGHT: _____ inches

AEROBIC CAPACITY: VO_2MAX: _____ ml/kg/min

ANTHROPOMETRIC VALUES:

% BODY FAT: _____ % FUTREX

_____ % CALIPERS

DUAL PHOTON ABSORPTIOMETRY:

BONE DENSITY:

_____ g/cm^2 L2

_____ g/cm^2 L3

_____ g/cm^2 L4

INDUCTIVELY COUPLED PLASMA SPECTROSCOPY ANALYSIS:

PLASMA BORON: _____ µg/g URINE BORON: _____ µg/g

ATOMIC ABSORPTION SPECTROSCOPY:

PLASMA CALCIUM: _____ mg/dl MAGNESIUM: _____ mg/dl

URINE CALCIUM: _____ mg/dl MAGNESIUM: _____ mg/dl

COLORIMETRIC PROCEDURES:

PLASMA PHOSPHORUS: _____ mg/dl

URINE PHOSPHORUS: _____ mg/dl

RADIOIMMUNOASSAY ANALYSIS:

HORMONES:

ESTRADIOL: _____ pg/ml

PROGESTERONE: _____ ng/ml

TESTOSTERONE: _____ ng/ml

CALCITONIN: _____ ng/ml

PARATHYROID HORMONE: _____ ng/ml

DIETARY ANALYSIS: (three day average)

CALORIES:_____ %

CARBOHYDRATES: _____ %

FAT: _____ %

PROTEIN: _____ %

DIETARY FIBER: _____ g/day

MAGNESIUM: _____ mg/day

PHOSPHORUS: _____ mg/day

CALCIUM: _____ mg/day

VITA

Candice Lyn Ward was born on December 2, 1969 in Warren, Ohio. She was raised in McDonald, a suburb of Youngstown, Ohio. Candice attended Youngstown State University for her Bachelor of Science degree from September 1988 to March 1992, and graduated Magna Cum Laude with a major in Nutrition and minors in Chemistry, Biology, Social Science, and Business. Candice married Gregory F. Ward, Jr. in April 1992.

Candice attended Virginia Polytechnic Institute and State University for her Master of Science degree in Human Nutrition from August 1992 to May 1994. Candice intends to continue her education to the doctoral level. Upon completion of a Ph.D., she intends to become a college professor. Candice enjoys sports, traveling, and spending time with her husband, family, and puppy.

Candice Lyn Ward