REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	18 JUL 97	

4. TITLE AND SUBTITLE
THE EFFECTS OF CALCIUM INTAKE AND PHYSICAL ACTIVITY ON THE BONE MINERAL CONTENT AND BONE MINERAL DENSITY OF UNITED STATES NAVAL ACADEMY MIDSHIPMEN

5. FUNDING NUMBERS

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
UNIVERSITY OF MARYLAND AT COLLEGE PARK

8. PERFORMING ORGANIZATION REPORT NUMBER
97-095

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
DEPARTMENT OF THE AIR FORCE
AFIT/CI
2950 P STREET
WRIGHT-PATTESON AFB OH 45433

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS	15. NUMBER OF PAGES
	108
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

THE EFFECTS OF CALCIUM INTAKE AND PHYSICAL ACTIVITY ON THE BONE MINERAL CONTENT AND BONE MINERAL DENSITY OF UNITED STATES NAVAL ACADEMY MIDSHIPMEN

by

Angela Marie Ogawa

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland at College Park in partial fulfillment
of the requirements for the degree of
Master of Science
1997

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ABSTRACT

Title of Thesis: THE EFFECTS OF CALCIUM INTAKE AND PHYSICAL ACTIVITY ON THE BONE MINERAL CONTENT AND BONE MINERAL DENSITY OF UNITED STATES NAVAL ACADEMY MIDSHIPMEN

Degree candidate: Angela Marie Ogawa

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Prevention of osteoporosis depends upon the identification of lifestyle factors that increase peak bone mass. We investigated the effects of calcium intake, weight bearing exercise (WBE), and strength training (ST) on total bone mineral content (TBMC) and bone mineral density (BMD) in 22 female and 13 male United States Naval Academy Midshipmen (MIDN). Each received 4 dual energy x-ray absorptiometry scans to measure TBMC and spine and hip BMD during the 2.8 year study period. Calcium intake was estimated at baseline and follow-up using a food frequency questionnaire. Frequency of WBE was determined at each visit using a questionnaire.

The female's TBMC was predicted by calcium intake at baseline ($p<.01$) and increased during the study ($p<.001$) as did spine BMD. At follow-up calcium was a significant variable in a model predicting TBMC ($p<.001$), but was weaker than at baseline and not independent of body weight. In the male subjects, TBMC and spine BMD increased significantly ($p<.005$), ($p<.05$). Hip BMD did not increase in males or

females. WBE was predictive of TBMC ($p<.01$), in female subjects weighing greater than 60 kg. This suggests that a threshold level of weight bearing (from body weight and exercise) is necessary to stimulate osteogenesis. We conclude that bone accretion occurs early in the third decade in MIDN, and is highly influenced by body weight. Calcium intake and WBE in female MIDN may positively modify peak bone mass, although these effects were not independent of body weight.

DEDICATION

I dedicate this thesis to my husband, Lester S. Ogawa. I thank you not only for your love and support during this experience, but also for being my sounding board, encouraging me to think more analytically and challenging me to grow in every aspect of life.

Special thanks is granted to my mentors, Dr. Alma Blake for chairing my committee and guiding me along the research process. Dr. David W. Armstrong III, thank you for allowing me the opportunity to work within your monumental research project, and for endless cooperation, and advice. Thank you Dr. Phylis Moser-Veillon, for it was your expert teaching that sparked my interest in this subject.

I also wish to thank Jan Yoshimoto for assisting me with the dietary assessments for this study and Dr. Larry Douglass for invaluable consultation during the statistical analysis. Finally, Mrs. Sue Douglass and Ms. Pat Ritale deserve special thanks for helping me present my results magnificently.

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
CSFII	Continuing Surveys of Food Intake by Individuals
DEXA	Dual Energy X-Ray Absorptiometry
DPA	Dual Photon Absorptiometry
EDI	Eating Disorders Inventory
FFQ	Food Frequency Questionnaire
FN	Femoral neck
HHHQ	Health, Habits and History Questionnaire
MIDN	Midshipmen
NHANES III	Third National Health and Nutrition Examination Survey
NNMC	National Naval Medical Center
RDA	Recommended Dietary Allowance
ST	Strength Training
TBMC	Total Body Bone Mineral Content
USDA	United States Department of Agriculture
USNA	United States Naval Academy
WBE	Weight Bearing Exercise

Chapter I.

INTRODUCTION

Twenty-six million American women are at risk for fracture due to osteopenia and osteoporosis (1). Minor trauma in osteoporotic individuals results in 1.5 million fractures annually (2). Although men have lower incidence of osteoporosis, they are not exempt from the morbidity associated with low bone mass as hip fracture rates for men have risen steadily in numerous communities studied (3,4). Hip fracture alone results in over 250, 000 admissions to United States hospitals each year (5) at a total cost of 13.8 billion dollars annually (2). With the aging U.S. population, the number of hip fractures will likely increase (6). Fractures in the elderly are especially debilitating and are associated with co-morbidity and mortality. Care of these patients contributes to rising health care costs and decreased quality of life.

Involutional osteoporosis is an age related disease characterized by progressive loss of bone mass and subsequent increased susceptibility to fracture (7). Riggs and Melton (8) have defined two types of involutional osteoporosis: Type I occurs in women approximately twenty years post menopausal, causing trabecular bone loss, vertebral and radial fractures. Type II occurs in elderly men and women due to gradual loss of both cortical and trabecular bone and is responsible for hip, humeral, pelvic and vertebral fractures. The World Health Organization defines osteopenia as bone mineral density (BMD) more than one standard deviation below the young normal mean, while 2.5 standard deviations below the mean is diagnostic of osteoporosis (9).

Bone remodeling occurs constantly in adults with osteoclast activity resorbing bone and osteoblast activity depositing bone. Activity of these cells is usually balanced until middle age when osteoblast activity slows, resulting in net bone loss (10). The mechanism for the age related reduction in osteoblastic activity is not fully understood, however, as bone loss ensues, it may eventually exceed the fracture threshold. Minor trauma occurring after a person has reached the fracture threshold may result in an elevated risk of fracture leading to increased morbidity.

Bone accretion was generally thought to conclude with cessation of linear growth and some recent studies (11,12) maintain this theory. In one prospective study BMD was measured by dual energy x-ray absorbtiometry (DEXA) twice with a one year interval period in male and female adolescents ranging in age from 7 to 20. The most dramatic changes were observed in the increased bone mineral content (BMC) and BMD in the spine and femoral neck of both sexes. In these young girls, the increase in BMD and BMC occurred during a two year period surrounding menarche, while the males had rapid bone accretion over a four year period between ages 13-17. By age 20, bone accretion had diminished markedly in both sexes (11). The longer period of rapid, linear, growth observed in males, results in larger bone volume and cortical thickness, while actual trabecular density is similar to that of females. These factors appeared to account for most of the difference in total bone mineral content (TBMC) between genders (11).

A second study measured BMD at baseline and one year later in 20-35 year old males and females to examine the relationship of age to peak bone mass. There were no

significant changes in BMC or BMD despite precise measurement by DEXA, and the authors concluded that no bone accretion occurs during the 3rd decade (12).

Other cross sectional (13,14) and longitudinal studies (15,16) report that bone accretion extends into the third decade of life. Men ranging in age from 15-85 showed an increase in TBMC and BMD up to age 29. Bone mass was correlated with body weight and muscle mass (13). These results agree with those from a smaller study of similar aged men in whom spine BMD measured by dual photon absorptiometry increased until age 29 and began to decline at age 40 at a rate of 0.23% annually (14). Differences in peak bone mass between the youngest and oldest groups in the study showed that men older than 70 years experienced a 6.5% loss of bone mass compared to younger men. Male and female subjects from a previous study by the same authors, had similar spine and femoral neck BMD until age 50. As the women underwent menopause, their rate of trabecular bone loss surpassed the men's resulting in a 31.6% loss by age 70 compared to the men (14). Women also lose more cortical bone, and these losses do not reach a plateau, even in very elderly women (17).

Increases in peak bone mass early in life, may prolong the age of the fracture threshold (18). Intervention therapies once fractures have occurred, have limited success (19), so the present emphasis in research and public health is to slow the progression of osteopenia by maximizing bone mass in the early decades of life. Peak bone mass is largely determined by genetic factors including race, sex, and body mass (20,21,22). However, lifestyle factors such as dietary intake, physical activity, estrogen exposure in

women, alcohol consumption and cigarette smoking may modify peak bone mass, although the extent of their role remains unclear (23).

Study Rationale

Studying factors that affect peak bone mass, specifically calcium intake and physical activity, may define strategies to increase peak bone mass early in life. Optimizing peak bone mass ensures that the inevitable bone loss during the elderly years starts from a higher baseline, thereby decreasing the risk of fractures.

Improving calcium status and bone health of active duty service women is a priority of the Defense Women's Health Research Program. As young men and women enlist in the armed services, the potential health effects of physically demanding training and duties, need to be examined. Basic training programs such as those encountered by male and female Midshipmen (MIDN) at the United States Naval Academy (USNA) change the lifestyles of these personnel. The long term impact of these training programs on peak bone mass is unknown at the present time.

Study Goals

The goal of this study was to evaluate whether bone accretion occurs in 17-24 year old MIDN. We also hoped to measure the effects of calcium intake and exercise on the TBMC and BMD of the spine and hip of MIDN. Results from this study may demonstrate whether calcium intake and physical activity in this population contribute to bone accretion. Significant findings will aid in the development of strategies for increasing peak bone mass thereby lowering risk of osteoporotic fractures later in life.

Chapter II.

LITERATURE REVIEW

Dietary Intake

Positive relationships between dietary calcium and BMD have been observed in some populations but not others (24). Calcium intake exerts its greatest impact on BMD during the rapid growth of infancy and adolescence. During the adolescent growth spurt (pubertal stages 2-5), 37% of the TBMC is accumulated (25). Five to ten percent of the difference in TBMC achieved during childhood and early adolescence may result from variations in calcium intake (25). Continued high calcium intake during the post-menopausal years contributes a preventive effect by minimizing bone loss. In the classic study by Matkovic et al, (26) significant differences in metacarpal bone mass in two populations living in different areas of Yugoslavia were due to different patterns of dairy product consumption and the subsequent differences in calcium intake. Both men and women with higher calcium intakes had higher TBMC than their counterparts with lower calcium intakes.

The National Academy of Science states in the 10th edition Recommended Dietary Allowances (RDA) that males and females should consume 1200 mg of calcium per day between the ages of 11 and 24, after which the calcium RDA decreases to 800 mg per day (27). Unfortunately, U.S. adolescents decrease milk consumption at a point in the life cycle when its high calcium content could be most beneficial. Calcium was one of 5 nutrients most frequently consumed below the RDA in a recent study of 933 males and females ages 11-18 (28). Apparent changes in food preferences, high consumption

of soda and desire to be slim are among the reasons for decreased dairy product consumption and subsequent inadequate calcium intake.

Cross sectional studies have shown that the RDAs for calcium are below the threshold at which no additional bone accretion occurs (29). The calcium intake threshold applies to the level below which skeletal accumulation is a function of calcium intake and above which skeletal accumulation is constant, regardless of continued increases in calcium intake. Threshold behavior of calcium was first demonstrated by Forbes et al in a rat model (30), and has recently been shown in humans in a meta analysis by Matkovic and Heany (31). Adequate calcium intake occurs at or above this threshold where bone deposition is not dependent on intake, but rather on the genetically determined growth process. Matkovic and Heany analyzed 517 calcium balance studies from male and female subjects at various stages of the life cycle. Threshold calcium intakes per day for infants, children, adolescents, and young adults were 1090, 1390, 1480, and 957 mg calcium per day respectively. Calcium intake at these levels resulted in calcium retention of 50% for infants, 18% for children, 27% for teenagers and 12% for young adults (31). These threshold levels at which balances no longer increased with intake, were higher than the RDA for calcium in all age groups, especially in adolescents. During periods of growth, calcium balance must be positive to insure attainment of the genetic potential for peak bone mass, whereas adults need only achieve zero balance to maintain their skeletal structure (29).

Matkovic's and Heany's meta analysis compiled balance studies from numerous sources. We do not know the methodology for conducting and analyzing the studies

especially the length of time the subjects consumed the test diets before their calcium balance was measured. According to Mertz this is problematic because a subject's calcium balance does not determine their requirement for the nutrient, but rather the intake required to maintain the subject's body pool of calcium (32). Calcium balance is a function of the body's pool size, so intakes greater than one's habitual intake result in increased pool size. Eventually, however, losses of calcium occur such that the intake and output are balanced again. Now, if the calcium intake decreases, losses will exceed intake for some time, until equilibrium is established once again. The authors of the meta-analysis report that the large sample permits evaluating calcium balance behavior and nutrient requirements in healthy, growing subjects (31).

Despite the lack of longitudinal intervention studies in which calcium is supplemented above the RDA, some experts have already concluded that these RDA's are too low. The National Institutes of Health consensus statement has recommended at least 1000 mg of calcium per day for adults ages 24-65 and as much as 1500 mg per day for post menopausal women, men greater than age 65 and all persons ages 11-24 (33). This recommendation comes at a time when many Americans are not consuming the RDA for calcium according to data from the Third National Health and Nutrition Examination Survey (NHANES III) which reported a mean calcium intake in adults of 857 mg per day (34). Continuing Surveys of Food Intakes by Individuals (CSFII) by the USDA between 1988-1991, showed median calcium intakes for college aged females (19-24) of 572 mg per day (35). NHANES III data show increased, but still inadequate

calcium intake in this female age group, with mean intakes of 778 mg per day compared to college aged men with means ranging from 1075-1274 mg calcium per day (34).

Research on dietary intake shows that many female athletes restrict calories to maintain a desirable weight and enhance their performance. In doing so, calcium intake is often inadequate (36). Male athletes usually consume the RDA for calcium, although this was not true in male wrestlers. Female Army soldiers had inadequate calcium, energy, protein and iron intakes when they were deployed and when they were not eating in an institutional setting (37). Dietary data show that female subjects in previous studies at the USNA (Armstrong-unpublished) had mean baseline calcium intakes of 550 mg per day upon admission. It's likely that they are facing similar calcium nutrition issues as the military and college aged women described above.

Cross sectional studies have found a positive association between calcium intake and BMD. Higher phosphorus and calcium intakes during the teen years were associated with a 6% higher hip BMD when measured at ages 30-39 (38). The implications of increased calcium intake are important to bone health since increases of just 5-10% in peak bone mass are associated with a 50% reduction in hip fracture rate in the elderly years (29). Another study of 38 women ages 24-28 showed that their usual calcium intake of 1000 mg per day was positively associated with radial BMC and BMD (39).

A longitudinal study in pre-pubertal identical twins where one twin received a calcium supplement and the other received a placebo showed a positive association with increases in TBMC (40). The supplemented twins averaged 1600 mg of calcium per day and had a greater rate of increase in BMD compared to the control group twins who

averaged 900 milligrams of calcium per day (40). Similar, but less dramatic results were found in slightly older adolescent females who took calcium supplements to meet 110% of the RDA. Compared to the control group, whose dietary intake met 80% of the RDA, the supplemented group gained an additional 24 grams of bone mineral which translates to 1.3% greater accretion of bone mineral annually. Such accretion during adolescence may lower future fracture risk (41).

Calcium intake may benefit bone accretion after adolescence as well. Several years after cessation of linear growth, calcium intake (adjusted for protein intake) continued to exert a positive effect on BMD of the lumbar spine in college aged women (16). In this study of 156 college women, bone mineral accretion slowed by age 29, similar to that reported by studies of young men (12,13). No dietary or exercise intervention was included during the five year study. Self selected calcium intake of the women was evaluated by multiple food records, and the mean intake was 781 mg per day. Physical activity was evaluated using accelerometers and also exerted a significant, positive effect on lumbar spine bone accretion in this population. This study is noteworthy because moderate calcium intake and physical activity were associated with a positive effect on bone accretion during the third decade.

A recent multi-center study followed 153 females and 111 males between the ages of 9-18 for a period of 11 years. At the end of the study, calcium intake appeared to have a weak relationship to increased hip BMD in the females, but there was no suggestion of this relationship in the males. In the multiple regression analyses, physical activity, smoking, age and body weight explained 46% of the variance in femoral neck

bone density in the men and 38% of the variance in the women (42). These prospective, longitudinal studies and others reviewed by meta-analysis suggest the same relationship of women's calcium intake to bone mineral content seen in cross sectional studies. Much less research has been done with male subjects however, and a similar relationship of their calcium intake to bone mineral content remains unconfirmed (43).

High calcium intake, commonly observed in male subjects, may exceed the threshold where the effects of calcium on bone accretion can be shown in a linear model. Experts point out, however, that lack of a linear relationship should not be interpreted as calcium being unimportant to bone health of males or females. A 15 year longitudinal study of Dutch males and females from age 13-28 evaluated the effect of body weight, calcium intake and weight bearing exercise on bone density. Calcium intake was not a significant predictor of lumbar spine BMD in the final regression analyses, however body weight and physical activity were. The researchers concluded that adequate body weight, and activity level were more important factors affecting peak bone mass than calcium (44). Heany countered this conclusion (45) explaining that calcium is known to be a threshold nutrient. Strong relationships of calcium intake to TBMC are seen at or near this threshold, the exact value of which is unknown. In the Dutch population studied (44), calcium intake at age 27 was greater than the Dutch RDA (700-900 mg per day) even in the groups consuming the least calcium. Heany explains the lack of a relationship between calcium intake and TBMC by suggesting that the subject's calcium intakes were above the threshold where the effect of calcium can be shown in a linear model.

Physical Activity

Kanders et al found calcium intake and physical activity to be equal, independent determinants of bone mass in women aged 24-35 (46). Those with calcium intakes less than 755 mg per day and lower physical activity had 16.6% less spine BMC than those with higher calcium intakes and physical activity. This case is noteworthy in that calcium and physical activity were both independently, positively associated with BMD.

Physical activity is often suspected of confounding the relationship between calcium intake and TBMC and BMD in cross sectional studies because calcium intake is reported to be related to bone indices regardless of energy expenditure. In an individual, as physical activity increases, caloric intake will likely increase to maintain energy balance. As overall food intake increases, it is likely that calcium intake will increase, especially in individuals who consume dairy products. Analysis may reveal that the calcium intake is high, as well as some bone indices and the researcher concludes that calcium intake is related to the increased bone indices. In reality, the high bone indices could be due to the individual's high level of physical activity, and the calcium intake be merely a consequence of higher food intake.

Epidemiological studies showing positive relationships between calcium intake and bone mass raise questions about the potential confounding effect of physical activity (47). In the study by Matkovic et al of two Yugoslavian populations, the group with higher calcium intake also had higher energy intake (26). Mean body weight was identical between the groups suggesting greater energy expenditure due to higher physical activity among the group with higher calcium and energy intake. Researchers

question whether the higher bone mass and lower fracture rate is due to the higher calcium intake or increased physical activity.

Kanis (47) advised researchers to prospectively evaluate calcium intake as a ratio to energy intake to evaluate the independence of any relationship of calcium intake to bone indices. If calcium intake is positively correlated to energy intake, it may be difficult to evaluate if bone density is high due to calcium intake, or because the individual has a high level of physical activity (as evidenced by their high energy intake).

To date only two prospective studies have demonstrated a positive association between calcium intake and bone mineral content independent of energy intake (46,48). Male and female subjects in the Rancho Bernardo cohort had positive relationships between calcium intake and hip BMD measured 18 years later. As measured by one 24 hour recall, calcium intake was positively related to hip BMD, but was not associated with caloric intake (48).

Weight bearing, physical activity and muscular contraction present an osteogenic stress which stimulates osteoblast activity and deposition of mineral raising TBMC. During inactivity and absence of this osteogenic stress, osteoclasts dominate in the bone remodeling process and result in net bone loss (49). The osteogenic stimulus of physical activity is dramatically demonstrated in its absence. Hospitalized patients as well as healthy, young men and women who were immobilized for 7-20 days had losses of spine BMD (50,51). Astronauts subjected to the zero gravity environment of space, also experience loss of bone mineral (52). Sedentary individuals would not likely experience the same degree of bone loss as astronauts, or immobilized subjects, however these

examples demonstrate that bone loss occurs with inactivity even in young individuals. There is no evidence to suggest that a high calcium intake alone will prevent mineral loss during periods of inactivity. Likewise, physical activity in those with inadequate calcium intake may compromise BMD. Even athletes may be at risk for low BMD despite the effects of their physical activity on bone accretion (53).

BMD, particularly of the spine, seems to decrease if calcium intake is inadequate in highly active populations. This may have been the case with male, Swiss military recruits in whom tibia BMD increased during heavy physical training while spine BMD decreased possibly due to an inadequate intake of dietary calcium (54). The effect of concomitant decreases in spine BMD and increases in BMD at lower extremity sites has been observed in female runners and gymnasts as well (55). Other studies have shown that physical activity was positively related to spine BMD, specifically that walking increased lumbar spine BMD by 0.5% compared with a 7% decrease in sedentary controls (36). Male athletes suffering lower extremity stress fractures had significantly lower femoral and spine BMD than uninjured, athletic controls matched for height, weight and training regimens. Calcium intake during the late teen years and early twenties was significantly less in the males with fractures (56). This raises the question as to whether physically active individuals with inadequate calcium intakes will show increased BMD at sites specifically related to their activity and suffer bone loss at others.

When collegiate gymnasts, runners and non-athletic college aged women were compared, the runners had lower lumbar spine BMD. Femoral neck BMD was significantly different among all groups, being highest in gymnasts and lowest in the

runners. Despite similar calcium intakes and incidence of oligo and amenorrhea in the gymnasts and runners, the gymnasts had greater femoral neck BMD. Conversely, in the runners, physical activity did not maintain BMD of the spine compared with non-athletic women. Apparently, the effects of muscular contraction from gymnastics training are an effective osteogenic stimulus, and appear to even compensate for the increased bone resorption that occurs in oligo and amenorrhea (57).

Type of physical activity appears to affect the BMD of men as well. Healthy male runners had significantly less upper limb BMD than those who lifted weights. This difference remained after adjusting for significantly different body weights between the runners and weight lifters (58). Similar to differences observed between female gymnasts and runners, the distinct mechanical loading characteristic of weight lifting, appears to preserve upper extremity BMD.

Female athletes may also be at risk for low TBMC and BMD if the combination of low caloric intake, low body fat and intense physical activity induce amenorrhea. Studies show that intense physical training may cause menstrual dysfunction in 50% of elite runners and 44% of ballet dancers but only 12% of swimmers and cyclists (59). Amenorrhea is not a benign condition as it increases an athlete's risk of musculoskeletal injury, reduces circulating estradiol and progesterone levels, and reduces bone density. Unlike gradual bone loss due to inadequate calcium intake, estrogen deficiency is more immediate and severe in its negative effect on BMC (60). This effect has been observed in amenorrheic runners who had lower spine BMD than eumenorrheic runners of similar fitness levels and with similar training regimens (61).

Dual Energy X-Ray Absorptiometry

Dual energy x-ray absorptiometry (DEXA) uses x-rays to measure bone mineral content. A scanner emits x-rays beneath the supine subject while a scintillation counter above tries to detect the transmitted x-rays. Bone attenuates passage of the x-rays in proportion to its mineral content. The DEXA converts the degree of attenuation into units of bone mineral (62). Bone mineral content (BMC) is measured in g/cm and bone mineral density (BMD) g/cm^2. Note that bone area, but not volume can be measured using DEXA, so the term BMD refers to area density g/cm^2 and not g/cm^3 (62). DEXA is more precise and faster than its predecessor dual photon absorptiometry, and is the gold standard for assessing BMC (63). Its precision is exceptional with a coefficient of variation of about 1.5%. This method is very safe with the radiation dose for a whole body scan being less than 5 mrem (less than a chest X-ray). Manufactures of DEXA machines have produced user friendly software that allows researchers with minimal training to consistently conduct safe, accurate reliable scans (64).

Dietary Assessment

Food frequency questionnaires (FFQ) are designed to obtain usual intakes of foods, or groups of foods over a specified time period. They are particularly useful for grouping subjects into broad categories of nutrient intakes (65). When combined with reported portion sizes, FFQ can be semi-quantitative for nutrient intake. FFQ tend to be more valid predictors of nutrients provided they are concentrated in a relatively small

number of foods or food groups. Examples include fresh fruits and juices as predictors of vitamin C and dairy products as predictors of calcium (66).

Respondent burden is low because the FFQ is quick to complete and has few open ended questions. For this reason, FFQ are very useful in epidemiological studies and are also useful in combination with more precise quantitative methods to provide confirmation. Numerous studies have shown that FFQ were a valid measurement method for nutrient intakes particularly for energy, protein, calcium and vitamin C (67,68,69). To the contrary, Krall and Dwyer found that the FFQ underestimated calcium intake by 12% and performed worse for other micronutrients (70).

The Health Habits and History Questionnaire (HHHQ) is a semi-quantitative FFQ developed by Block et al for epidemiological and clinical use (71). It can be self administered and has been validated for a wide age range of adults. It lists foods with portion sizes determined to be commonly consumed by the Second National Health and Nutrition Examination Survey (NHANES II). Foods included in the HHHQ were those that contributed at least 90% or more to the United States diet as determined by NHANES II. A software package has been developed to quantify nutrient intake from the questionnaires. HHHQ lists foods that represent 96.5% of the US population's calcium intake based on the NHANES II data (71). Extensive modifications to the software and lists of foods included on the questionnaire are expected after the detailed results from the NHANES III study are available (72).

The HHHQ has been adapted for use with IBM compatible personal computers (73). Subjects can be interviewed and have their responses immediately entered into the

program eliminating possible coding errors. The paper form of the HHHQ was designed to be self administered. Repeated administration of a FFQ has shown that respondents complete it more quickly and possibly less accurately. Being able to interview subjects and enter answers into a personal computer allows the researcher to reduce misclassification error and associated sample size requirements (72). Recent revisions of the dietary analysis software have not changed the validity. The portion size designation on the HHHQ as small, medium and large allows respondents to rate their portion size as smaller or larger than the medium, reference portion. This method has produced higher correlations to 7 day food records than methods which ask respondents to rate their portion in common household measurements (74).

The HHHQ is intended to estimate the usual intake of an individual's self selected diet over an extended time period such as one year. It is primarily useful for ranking individuals intakes, although it is also useful for estimating nutrient intakes and thereby can estimate a group's mean nutrient intake. The HHHQ was validated against four sets of four day food records in white and black men and woman ranging in ages from 20-80 years of age as a self administered FFQ. The HHHQ group means related well to sets of food records for most nutrients with correlations ranging from .42-.68 (75). Correlations for calcium and energy were .56 and .62 respectively (75).

Twenty-four hour dietary recalls are frequently used to estimate usual nutrient intake of large groups. Subjects are asked to report food intake of the previous twenty four hours from memory. Food models and household measuring utensils are used to assist the subject in recalling portion sizes. Their use in this setting is advantageous due

to low cost and low respondent burden. In smaller groups, the 24 hour recall can be repeatedly administered to estimate actual or usual nutrient intakes for individuals. The number of 24 hour recalls required depends on the nutrient(s) to be studied, the degree of precision required and the population under consideration (76). Seasonal variation of intake and days of the week need to be considered especially if intake varies considerably with respect to these factors.

The disadvantage of using repeated 24 hour dietary recalls lies in their high intra-subject variation. Beaton et al studied the sources of error in 24 hour recalls extensively (77). Their findings indicate that different interviewers did not significantly affect the variance of repeated recalls. Day of the week was contributory to variance for females for all nutrients except calcium, vitamin A, vitamin C, riboflavin and fiber. This was due to increased energy intake on the weekends for females. Clearly, the largest partition of variance was the intra-individual variance. This creates difficulty in grouping subjects by level of nutrient intake. The presence of high intra-individual variance may mask correlations or bias regressions toward zero (78). Fortunately, this error can be evaluated in repeated 24 hour recalls prior to using them in an analysis. Intra-individual variation for a given population can be estimated and then the appropriateness of the 24 hour dietary recall method can be assessed, and the number of repeated 24 hour recalls required can be determined. Due to the variable nature of dietary intake data, some form of cross checking between methods or with an objective variable should always be performed (79). This provides the investigators with a rough measure of the validity of a dietary assessment method in a given situation.

Validation studies for FFQ have used repeated 24 hour recalls for reference data. Block et al found that 4-24 hour recalls correlated well with calcium intake(.88) and energy (.84) as measured by 4-3 day food records (75). A study comparing reported food intake to measured energy expenditure found no significant differences in energy and macronutrient intake between 2-24 hour recalls and 14 days of food records (79).

With respect to intra-individual variation, a one day food record should perform similarly to a 24 hour recall. A comparison of dietary methods for estimating nutrient intake was conducted using 1, 3, and 7 day food records and a FFQ. One 24 hour diet record was not significantly different than the mean of a seven day record for calcium or energy intake in a group of 40 lactating women (80). Actual differences between the group means for the two methods were 34 mg of calcium and 29 Kcal for energy. Calcium intake in Japanese women as measured by seven-24 hour diet records had a .59 correlation to that by a FFQ (81). These significant correlations are due in part to the fact that calcium is likely to be more accurately estimated by all dietary methodologies due to being relatively concentrated into one group of foods-dairy products. Regardless of this fact, nutrient estimates from repeated 24 hour recalls should only be used given full recognition of their limitations and after intra-individual variance has been analyzed.

Chapter III.

HYPOTHESES

1. Bone accretion occurs early in the third decade of life in male and female MIDN.

2. TBMC and BMD of the spine and hip positively correlate with calcium intake of male and female MIDN.

3. BMD of the lumbar spine and hip positively correlate with weight bearing exercise and strength training in male and female MIDN.

Questions To Be Answered During Hypotheses Testing

1. What is the mean calcium intake of male and female MIDN, does it change over the 3 year study period, and is it correlated to energy intake?

2. Does estimated calcium and energy intake, as measured by a single 24 hour dietary recall, correlate with that measured by a FFQ in male and female MIDN?

3. Are TBMC and BMD of the spine and hip related to usual calcium intake as measured by a FFQ at baseline and study endpoint?

4. Is change in TBMC and BMD of the spine and hip related to change in calcium intake between the 2nd and 3rd study year in male and female MIDN?

5. Is there a difference in TBMC and BMD of the spine and hip between two groups of female subjects--those consuming greater than the median calcium intake per day and less than the median calcium intake per day?

6. Is BMD of the spine and hip related to number of hours spent in weight bearing exercise and in strength training in male and female MIDN?

7. Is there a difference in BMD of the spine and hip between two groups of female MIDN--those participating in WBE or strength training greater than 2 hours per week compared to those participating in the activities less than 2 hours per week?

Chapter IV.

METHODS

Principle Research Protocol

The Department of Endocrinology at the National Naval Medical Center at Bethesda (NNMC) is conducting a prospective study of 468 subjects at the USNA approved by the Institutional Human Subject Review Board at NNMC. A description follows of the 4 year study from which we obtained data collected between June 1993 and Spring 1995 and for which we collected year 3 ancillary dietary data. The purpose of this study is to evaluate change in TBMC and BMD of the lumbar spine, femoral neck, wards triangle, trochanter and the distal tibia of male and female MIDN. Changes in these bone indices are evaluated for relationship to menstrual status (female subjects), stress fractures and other orthopedic injuries, physical activity and nutrient intake.

Upon entry to the USNA, dramatic lifestyle changes occur, notably increased physical activity, mental and emotional stress. Many female MIDN experience menstrual dysfunction and weight loss during the plebe summer followed by weight gain during the first academic year. Additionally, there is a high incidence of stress fractures among male and female MIDN which significantly interrupts their training. This study is evaluating whether the stressors unique to the USNA affect menstrual status (in the female subjects) and bone accretion in males and females thereby increasing the risk of stress fracture. Data collected from June 1993 to Spring 1995 is summarized in Table 1.

Table 1: Data collected in conjunction with the principal research protocol

Date	Type of Data Collected	Method Used
June 1993 Pre-USNA	3-Day Food Record HHHQ[1] Exercise, Medical, and Menstrual History	Mailed food record Self administered questionnaire Self administered questionnaire
July 1993	Bone Densitometry	DEXA-Norland XR-26
Aug 1993	24 Hour Dietary Recall	University of Minnesota protocol
Spring 1994	Bone Densitometry Exercise, Medical, and Menstrual History	DEXA-Norland XR-26 Interview
Spring 1995	24 Hour Dietary Recall	University of Minnesota protocol
	Bone Densitometry	DEXA-Norland XR-26
	Exercise, Medical, and Menstrual History	Interview

[1]Health, Habits and History Questionnaire (FFQ)

Ancillary Study

The ancillary study reported here was approved by the Human Subjects Review Committee of the Department of Nutrition and Food Science at the University of Maryland at College Park, College Park, MD.

Subjects

Thirty-five MIDN (22 females and 13 males) from the class of 1997, provided informed consent (Appendix A) and completed the data described in Table 2 in conjunction with the principal, research protocol. The data collected in 1993, 1994 and 1995 including an exercise history (Appendix B) and medical history (Appendix C), were used to provide baseline and interval data for this study. Additional data collected from these subjects for use in this study are summarized in Table 3.

Table 2 Retrospective data used in this ancillary study

Date	Type of Data Collected	Method Used
June 1993 Pre-USNA	FFQ Exercise History Medical History	Self administered questionnaire Self administered questionnaire Self administered questionnaire
July 1993	Bone Densitometry	DEXA-Norland XR-26
Spring 1994	Bone Densitometry Exercise Questionnaire Medical Questionnaire	DEXA-Norland XR-26 Interview Interview
Spring 1995	24 Hour Dietary Recall	University of Minnesota protocol
	Bone Densitometry	DEXA-Norland XR-26
	Exercise Questionnaire Medical Questionnaire	Interview Interview

Table 3: Data collected in April 96 for use in the proposed study

	Method Used and Specific Data to be collected
APPOINTMENT 1 24 Hour Recall FFQ	 University of Minnesota research protocol Interview
APPOINTMENT 2 Bone Densitometry Exercise Questionnaire Medical Questionnaire	 DEXA Interview Interview

Dietary Intake

The investigator conducted a 24-hour dietary recall interview during the Spring of 1996 using the University of Minnesota research protocol for data collection (82). The recall took place during the first scheduled appointment, and the subjects were notified in advance that the recall would occur. During the interview, subjects were presented with the previous day's King Hall dining menu to assist with their food consumption recollection. Subjects reported foods consumed during the recall period and were not interrupted. After the subjects completed their recall, the investigator

probed for details such as portion size, quantity, and ingredients. Finally, subjects were prompted to recall snacks and/or beverages consumed between meals. All questions were posed in a non-judgmental manner to provide the subject a neutral forum for responding honestly and accurately.

At the end of the 24-hour recall, subjects were asked to review the recalled intake for accuracy and were asked if it accurately depicted a typical day's intake. Three subjects reported that their recalled intake was atypical due to their schedule, a special occasion or illness. These recalls were not analyzed, and the subject provided another 24-hour recall at a later date. Recipes and food product information of the ingredients of foods and brand names were available to the investigator.

High intra-subject variability in dietary intake is a limitation of the 24-hour recall method, however, several factors in this study made this dietary assessment method worthwhile. First and foremost, the subjects eat the majority of their meals in a single institutional setting 10 out of 12 months per year. MIDN are required to attend breakfast and lunch Monday through Saturday. After the first year, dinner may be eaten off campus, however, MIDN are responsible for the cost of this food, limiting the number of meals eaten away from campus. The USNA MIDN foodservice provides family style dining 3 meals per day, 7 days per week. The cycle menu pattern likely decreases intra-subject variability in dietary intake especially with respect to calcium, which is concentrated in a relatively small number of foods. The dining hall does not serve any non-dairy, calcium fortified foods.

The FFQ (Appendix D) was administered to all subjects at the first appointment, during Spring 1996 following the 24-hour dietary recall. The questionnaire was administered in an interview format.

Weight Bearing Exercise and Strength Training

The Interval Exercise Questionnaire (Appendix E) was administered during an interview at the second appointment while the subject received their bone densitometry scan. The subjects were queried as to what physical activities he or she had engaged in on a regular basis over the past year. Time spent in exercise and other physical activity was recorded as number of hours per week spent in weight bearing exercises (WBE) such as running, aerobics and stair climbing machines, and number of hours spent each week in strength training activities. Standards for the low and high exercise classifications were derived from a recent consensus statement by the American College of Sports Medicine and the Centers for Disease Control and Prevention (83,84).

The Interval Medical Questionnaire (Appendix E) was also administered during an interview at the second appointment. This was the 3rd administration of this questionnaire allowing the investigators to update the subject's records as to any injury, fracture, or serious illness that had occurred in the past year. Consumption, dosage and duration of medications, or vitamins and mineral supplements consumed were recorded.

Bone Densitometry Scan Protocol

The Norland XR-26 DEXA at the USNA has been in continuous use since August 1992 and maintains excellent precision with a coefficient of variation of 0.76% for the spinal, hydroxyappetite phantom and 1.5% for in vivo measures. These figures are consistent with published reports regarding DEXA precision (63,64). All bone densitometry measurements were conducted by the principle investigators of the larger study according to the procedure recommended by Norland (85). The DEXA was calibrated daily using the protocol recommended by Norland. The Norland calibration standard was placed in the brackets supplied. The Anterior/Posterior (AP) spine phantom was positioned on the table, and the automatic calibration procedure was initiated by the operator and allowed to run to completion. An AP spine phantom coefficient of variation of less than or equal to 1.5% is acceptable.

Subjects reported for DEXA bone scans dressed in a t-shirt, gym shorts and socks. They removed all jewelry, eyeglasses and watches prior to scanning. Undergarments containing metal were also removed. Subjects were measured for height using a calibrated stadiometer and weighed on a calibrated electronic scale. These data were entered into the Norland subject data file at the time of the bone scan.

Anterior/Posterior (AP) spine

Subjects were positioned supine on the table, centered and parallel to the sides. Using the laser guide, the boom arm was positioned over the xiphoid process and marked. The boom was then moved to a position below the iliac crests and this position

was marked after which, the scan sequence was initiated. When lumbar spine segments 1-5 had been scanned, the sequence was terminated and lumbar segments 2-4 were analyzed by Norland software.

Hip (trochanter, femoral neck, Ward's triangle)

The subject remained supine, and the Norland hip positioning device was positioned over the center of the non-dominant hip. A pilot scan sequence was initiated to precisely locate the center of the femoral neck. Following the pilot scan, the positioning cursor was located in the center of the femoral neck. The hip scan sequence was initiated and allowed to run to completion. Following the scan, the image was analyzed by Norland software and the results stored.

Total Body

The hands and feet of the supine subject were held in place by a sheet. The boom was positioned one cm above the top, center of the subject's head and marked. The boom was moved into position and the scan sequence initiated and run to completion. The scan was analyzed by the Norland software.

Data Analysis

Subject's responses on the FFQ were entered into a personal computer by the interviewer, checked for errors and analyzed using the Dietsys software package version 3.4. This software, developed by the National Cancer Institute is used in conjunction

with the FFQ (73). Responses to the 24 hour recalls were shipped to University of Minnesota for analysis as was done for dietary recalls collected in previous study years.

Individual measurements of the dependent variables were quantified:

(1) Usual calcium and energy intake from the FFQ at baseline and study follow-up 2.8 years later.

(2) Change in calcium intake determined from the difference between single 24 hour recalls obtained in the 2nd and 3rd study year.

(3) Mean hours WBE per week during the study period.

(4) Mean hours of strength training per week.

Comparisons of the outcome variables were made between gender groups. The female subjects were divided into two sub-classes based on the following dependent variables: calcium intake, hours per week spent in WBE and hours per week spent in strength training. The smaller sample of male subjects did not allow such classifications.

Means were computed for each gender group for energy and calcium intakes from the baseline and follow-up FFQ. They were also used to classify the female subjects into two groups based on whether their usual calcium intake was greater or less than the median intake.

Calcium and energy intake from the follow-up FFQ and the Spring 96 twenty-four hour dietary recall were compared. The correlation between these two methods was obtained out of interest as to how closely they estimated the subject's calcium and energy intake. The Spring 1995 and Spring 1996 twenty-four hour recalls were used to determine change in calcium intake during that period and to evaluate whether change in

calcium intake was related to change in TBMC and BMD. Mean hours spent in WBE and ST per week over the three year study period were evaluated for relationship to TBMC, lumbar spine and hip BMD. Differences in exercise patterns between genders and subsequent effects on BMD were also evaluated.

Two classifications for physical activity were made to avoid confounding differences between subgroups of females by misclassification. The female subjects were classified into two sub-groups based on duration of WBE and ST to determine whether any association between physical activity and the bone indices were different between individuals with different exercise patterns.

Running or brisk walking at 6 miles per hour is classified as moderate physical activity (83). Women running at this speed, for 30 minutes, 3 times per week would run 9 miles and spend 1.5 hours per week in WBE. Benefits of WBE on BMD would not be expected in individuals who exercise below the moderate level. Therefore, females spending less than 2 hours per week were assigned to the low WBE group. Females spending at least 2 hours per week in WBE were classified to the high WBE group. Hours spent in other WBE such as stair climbing, and aerobics were considered comparable to running provided the subject's body weight was not supported as in the case of an exercise bike. All hours spent in WBE were pooled together and averaged over the study period to quantify a WBE score for each female subject.

Regular strength training was defined as spending at least 2 hours per week in this activity. Females who strength train at this level comprised the strength training

group and were compared to subjects who did not strength train regularly for differences in BMD of the spine and hip.

The levels at which subjects were divided for the exercise group classifications were not intended to show relationship between a specific duration of an activity and BMD. Rather, the levels were used to classify a subject's physical activity relative to the other female MIDN.

Statistical analysis

This study was an observational, split unit design with repeated measures. For the 22 female subjects there was 90% power to detect a 4% change in TBMC and BMD at the 5% level of significance. For the 13 male subjects there was 80% power to detect a similar change in bone indices at the 5% level of significance (86).

Calcium and energy intake data as estimated by the endpoint FFQ and the Spring 96 twenty-hour hour recall were analyzed for correlation. Strength of this correlation indicated how well the 24 hour recalls represented the subject's self reported usual calcium and energy intake as estimated by the FFQ. Linear regression analysis was performed to determine if percentage change in TBMC and BMD between the 2nd and 3rd study year was related to change in calcium intake during that time.

Linear regression analysis was performed for each gender group to determine if significant relationships existed for each of the independent variables and TBMC and BMD at selected sites. Multiple linear regression analysis was performed for all the independent variables and TBMC and BMD of the spine and hip. These models were

subsequently reanalyzed to verify that none of the subjects were outliers that influenced the model. Analysis of co-variance (ANCOVA) with body weight as a covariate, was also conducted on each of the independent variables and TBMC and BMD of the spine and hip.

Analysis of variance (ANOVA) was conducted to determine if there were significant differences in percentage change in TBMC, spine BMD, and hip BMD in the males versus the females. Likewise, ANOVA was conducted to determine if there were differences in TBMC between female subjects with calcium intakes above versus below the median both at baseline and study endpoint. Comparisons within the male subjects based on levels of the independent variables were not done due to the small sample size.

In addition to linear regression analysis, ANOVA was used to determine whether differences in TBMC, lumbar spine BMD and hip BMD existed between the sub-groups of female subjects who participated in WBE greater than versus less than 2 hours per week. This analysis was repeated on the female subjects for the ST data. As mentioned previously, no comparisons between sub-groups of the male subjects were done.

The female subjects were divided into two groups--those with body weight above and below the median weight of 60 kg. ANOVA was used to determine if these two groups had significant differences in their exercise patterns and bone indices.

Finally, the female subject's menstrual cycle data was evaluated using ANOVA to determine if the number of cycles per year was different in the three years of the study.

Chapter V.

RESULTS

Subjects

Physical characteristics of the study subjects are summarized in Table 4. Twenty-two females and 13 male MIDN aged 18.5 and 18.6 years respectively, completed the 2.8 year study period. Height of the subjects did not increase significantly over the study period, however body weight increased significantly for both males and females. Weight gain in the females was 2.6 kg or 4.6% ($p<.001$) while the male subjects gained an average of 5.3 kg or 7.4% ($p<.001$).

Table 4: Description of study population

	Females n=22			Males n=13		
	Baseline	Follow-up[1]	Change	Baseline	Follow-up	Change
Age (yr)	18.5 (0.3)[2]	21.3 (0.3)	2.8	18.6 (1.1)	21.4 (1.1)	2.8
Ht. (m)	1.63 (.18)	1.64 (.15)	.01 NS	1.76 (.02)	1.77 (.04)	.01 NS
Wt. (kg)	57.1 (7.6)	59.7 (8.1)	2.6*	71.8 (8.1)	77.1 (9.2)	5.3*
BMI (kg/m^2)	21.2 (2.3)	22.2 (1.9)	1.0	23.2 (1.7)	24.6 (0.9)	1.4

*$p<.001$

[1]Refers to the measurements taken at the end of the 2.8 year study period

[2]Mean(SD)

Dietary Intake

During the study period, dietary assessments were taken to evaluate calcium and energy intake. Results for the baseline and follow-up FFQ and the two 24 hour dietary recalls are summarized in Tables 5 and 6 respectively. Calcium intake values from the FFQ include calcium from supplementation, although calcium was primarily derived from

dietary sources. Nine female and 4 male MIDN had supplemental calcium intakes during the study period, in all but two cases from a multiple vitamin and mineral supplement. The 24 hour recall data for calcium intake also includes calcium from supplementation.

Table 5: Subject's calcium and energy intake at baseline and follow-up

	Females n=22			Males n=13		
FFQ	Baseline[1]	Follow-up[2]	Diff.	Baseline	Follow-up	Diff.
Calcium (mg)	1213 (649)[3]	1190 (614)	23 NS	1541 (995)	1304 (697)	237 *
Energy (kcal)	2196 (898)	1772 (473)	424 NS	3176 (1447)	2753 (1129)	423 *

*p<.05
[1]Two female subjects entered the study 6 months later than the original 20 subjects and therefore were not included.
[2]Refers to the measurements taken at the end of the 2.8 year study period
[3]Mean (SD)

Table 6: Subject's calcium and energy intakes during the Spring of 1995 and 1996

	Females 24 Hour Recall n=22			Males 24 Hour Recall n=13		
	Spring 95	Spring 96	Change	Spring 95	Spring 96	Change
Calcium (mg)	808 (388)[1]	916 (489)	108 NS	1541 (743)	1530 (679)	-11 NS
Energy (kcals)	1812 (601)	1710 (430)	-102 *	3823 (967)	3684 (1499)	-139 NS

*p< .01
[1]Mean (SD)

As measured by the FFQ the mean calcium intake for female subjects at baseline was 1213 mg calcium per day. This is slightly above the RDA of 1200 mg, and slightly higher than the female's mean calcium intake at follow-up of 1190 mg calcium per day. Calcium and energy intake were correlated at baseline ($r=.45$ $r^2=.20$ $p<.05$) and at follow-up ($r=.67$, $r^2=.46$ $p<.001$). Despite the insignificant difference of 23 mg of

calcium per day between mean intakes at baseline and follow-up, half of the subjects had calcium intakes that were different relative to the median at baseline and follow-up. For many subjects, the difference in calcium intake was rather dramatic such that the mean individual difference in calcium intake between baseline and follow-up for individuals was 725 mg calcium per day ($p<.001$). At the study endpoint, 11 female subjects were noted to have calcium intakes similar to their baseline intakes (relative to the median). These 11 subjects were analyzed separately in some cases to determine if there were stronger relationships between calcium intake and TBMC in this group with possibly more consistent calcium intake patterns.

Usual calcium intake of male subjects, measured by the FFQ, was 1541 mg calcium per day at baseline and 1304 mg calcium per day at follow-up $p<.05$ (Table 5). Both amounts represent calcium intakes above the RDA of 1200 mg for young men. The male subject's calcium intake (FFQ) was highly correlated to their energy intake at baseline ($r=.84$, $r^2=.71$ $p<.001$) and follow-up ($r=.94$, $r^2=.88$ $p<.001$).

Twenty four hour dietary recalls were taken during Spring 95 and Spring 96 for all subjects to evaluate changes in their calcium and energy intake. Results are shown in Table 6. The female's mean calcium intake from the Spring 95 and Spring 96 twenty-four hour recalls was not significantly different at 808 mg and 916 mg of calcium per day respectively. However, the calcium intake from the Spring 96 twenty-four hour recall was significantly different than the calcium intake from the 1996 follow-up FFQ ($p<.001$), and there was no correlation between these two methods in the female subjects. Among the male subjects, mean calcium intake from the Spring 95 and Spring

96 twenty-four hour recalls was not significantly different at 1541 and 1530 mg of calcium per day respectively--nearly twice that of the female subjects. Like the FFQ, the male's calcium intake from the Spring 96 twenty-four hour recall was highly correlated to their energy intake ($r=.82$, $r^2=.67$ $p<.001$). Additionally, the calcium intake measured by the follow-up FFQ correlated to that measured by the Spring 1996 twenty-four hour recall ($r=.75$ $r^2=.55$ $p<.01$). It is interesting to note that the two methods of estimating calcium intake were correlated for the male subjects, but not in the females, whose Spring 96 twenty-four hour recall showed no correlation to their FFQ for calcium intake.

Weight Bearing Exercise and Strength Training

Exercise data collected during the study--the number of hours per week spent in weight bearing exercise (WBE) and strength training (ST), is summarized in Table 7. As shown, the female subjects participated in WBE 2.4 hours per week, and in ST activities 1.2 hours per week for a total of 3.6 hours per week of exercise. Similarly, the males performed WBE 2.5 hours per week, but spent more time ST than the females.

Table 7: Exercise Data[1]

Subjects	n	Wt Bearing Exercise (WBE) (hrs/wk)	Strength Training (ST) (hrs/wk)
Females	22	2.4 (.85)[2]	1.2 (.75)
Males	13	2.5 (1.5)	2.1 (.95)
Difference		0.1 (NS)	0.9 (NS)

[1] The mean number of hours spent exercising during the 2.8 year study period
[2]Mean (SD)

Bone Densitometry

The baseline and follow-up DEXA measurements for TBMC, spine BMD and hip BMD as well as the percent change for each of these indices are summarized in Table 8. The female subjects had a mean increase in TBMC of 87 gm, indicating a 3.37% change in TBMC ($p<.001$) over the 2.8 year study period. Spine BMD increased 3.32% ($p<.001$) however, hip BMD did not increase significantly over study period.

Also shown in Table 8, the male subjects exhibited an increase in TBMC of 122 gm for a 4.1% gain ($p<.01$). Similar to the female subjects, the males mean spine BMD increased by 3.28% ($p<.05$). Hip BMD in the male subjects increased 2%, but like the females, this change failed to reach significance. The changes in the bone indices were not significantly different between the male and female subjects.

Table 8: Female & male subject's bone indices

FEMALES	n	TBMC gm	Spine BMD mg/cm^2	Hip BMD mg/cm^2
Baseline	20[1]	2584 (341)[2]	1.08 (.10)	1.06 (.12)
Follow-up[3]	22	2671 (331)	1.12 (.92)	1.08 (.12)
Difference	22	87	0.04 (.1)	0.02
% Change	20	3.37%***	3.32%***	2.32% (NS)
MALES				
Baseline	13	2976 (311)	1.10 (.14)	1.09 (.11)
Follow-up	13	3098 (399)	1.14 (.12)	1.11 (.10)
Difference	13	122	0.04	0.02
% Change	13	4.1**	3.28*	2.02 (NS)

* $p<.05$
** $p<.005$
***$p<.001$
(NS) not significant
[1]Two female subjects entered the study 6 months later than the original 20 subjects and therefore were not included in the analysis of change in the bone indices.
[2]Mean (SD)
[3] Refers to measurements taken at the end of the 2.8 year study period

Details of the male and female subject's hip BMD changes for each study year are shown in Table 9. Although mean changes in hip BMD over the study period were not significant, negative changes occurred in individual male and female subject's hip BMD throughout the study period--especially during the first year. Thirteen females lost hip BMD during the first study year, and all but 3 females had some negative changes in their hip BMD during the study. Seven female subjects had net loss of hip BMD during the 2.8 year study period with decreases ranging from 2.3-11.3% with an average of 6%.

Six males lost hip BMD during the first study year, and all but one subject had some negative change in their hip BMD during the 2.8 year study period. Four males had net losses of hip BMD at the study follow-up ranging from 3.1-4.6%. Changes in hip BMD were not correlated to changes in body weight in the male or female subjects.

Table 9: Female and male subject's hip BMD changes by year

FEMALES	n	Hip BMD mg/cm^2	% Change
Baseline	20[1]	1.06 (.12)	
End of year 1	20	1.05 (.12)	-1.0 NS
End of year 2	20	1.05 (.11)	0.0 NS
End of year 3	20	1.08 (.13)	3.0 NS
Total change[3]	20		2.3 NS
MALES	n	Hip BMD mg/cm^2	% Change
Baseline	13	1.09 (.12)	
End of year 1	13	1.08 (11)	-1.0 NS
End of year 2	13	1.10 (.11)	2.0 NS
End of year 3	13	1.11 (.11)	1.1 NS
Total change	13		2.0 NS

(NS) not significant

[1]Two female subjects entered the study 6 months later than the original 20 subjects and therefore were not included in the analysis of change in the hip bone indices.

[2]Mean (SD)

[3]Refers to the change that occurred between baseline and follow-up

TBMC was significantly correlated to body weight for the females at baseline ($r=.79$ $r^2=.63$ $p<.001$) (Figure 1) and follow-up ($r=.80$ $r^2=.64$ $p<.001$) (Figure 2). For the male subjects, body weight was predictive of TBMC at follow-up ($r=.78$ $r^2=.61$ $p<.01$), and this relationship is also shown in Figure 2. The male and female subject's bone indices were better predicted by body weight than height or body mass index (BMI). In the female subjects, correlations to TBMC ranged from .70 for height ($p<.001$) to .48 for BMI ($p<.05$). Similar correlations were found for the male subjects as the correlation between their height and TBMC was .66 ($p<.001$), while the correlation between their BMI and TBMC was .43 ($p<.05$).

Figure 3 shows the male's percentage change in TBMC, which was correlated to change in body weight ($r=.67$ $r^2=.45$ $p<.01$). This relationship was very similar to that seen in the female subjects also shown in Figure 3 where the percentage change in TBMC was correlated to change in body weight ($r=.66$ $r^2=.44$ $p<.001$).

Using linear regression, the female's baseline hip BMD was positively predicted by body weight, ($p<.05$) although this relationship was not seen in the follow-up data. Among the male subjects, there was no association between hip BMD and body weight. Change in body weight was not related to change in hip or spine BMD in the females or the males.

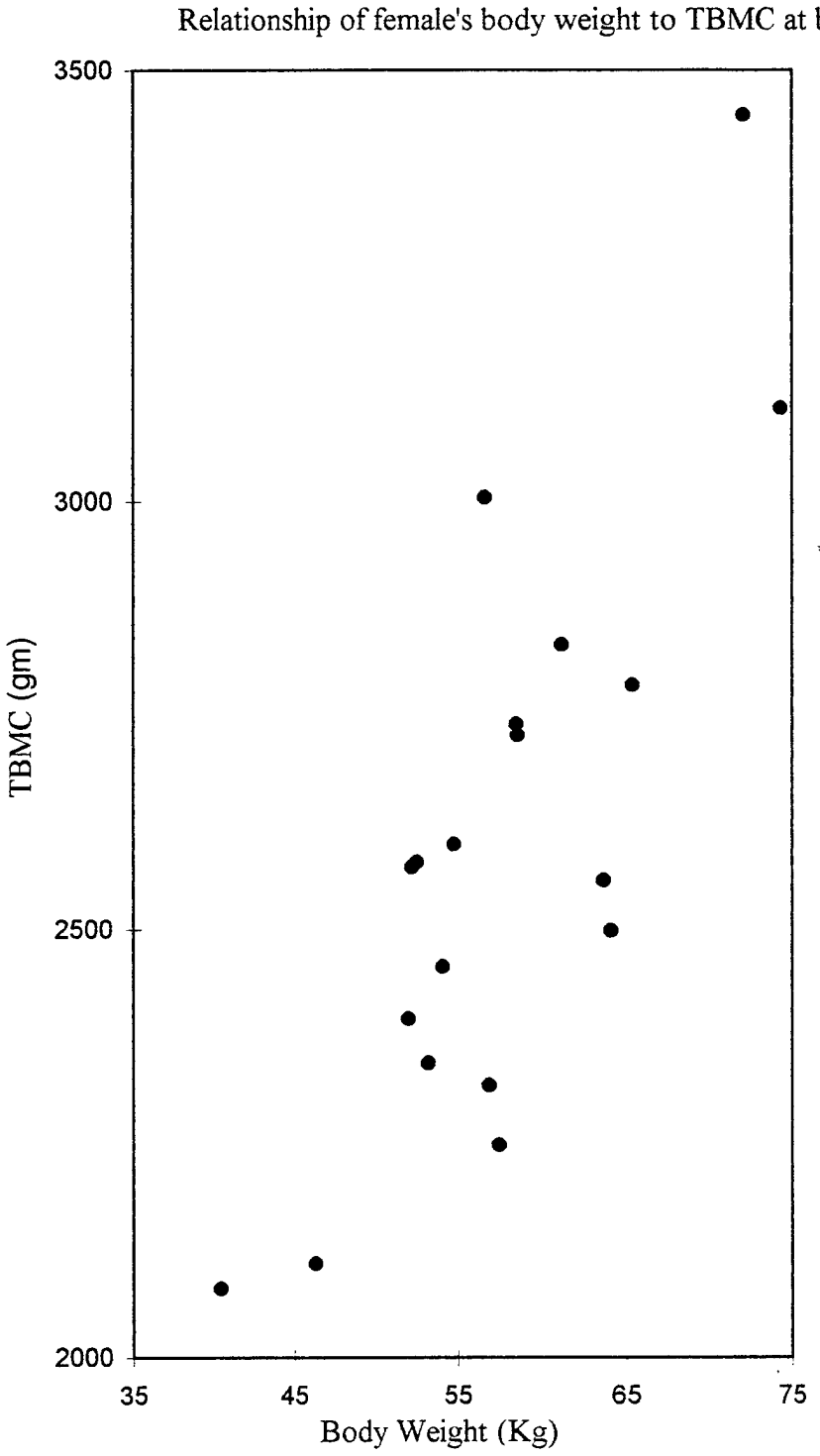

FIGURE 1: Females, n=20 r=.79 p<.0001.

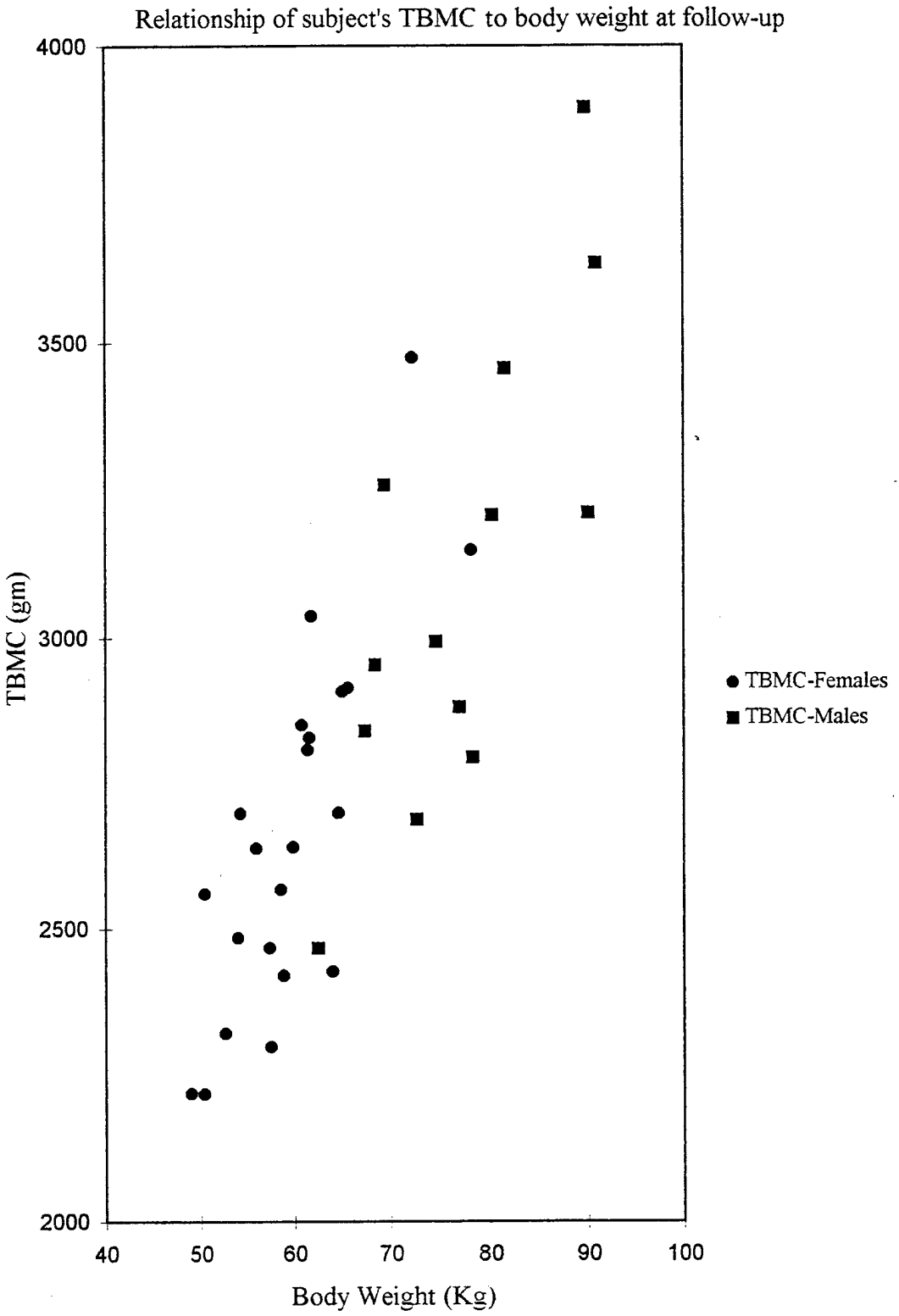

Figure 2: Females: n=22, r=.80 p<.0001 Males: n=13, r=.78 p<.005

Percentage Change in TBMC Versus Percentage Change in Weight

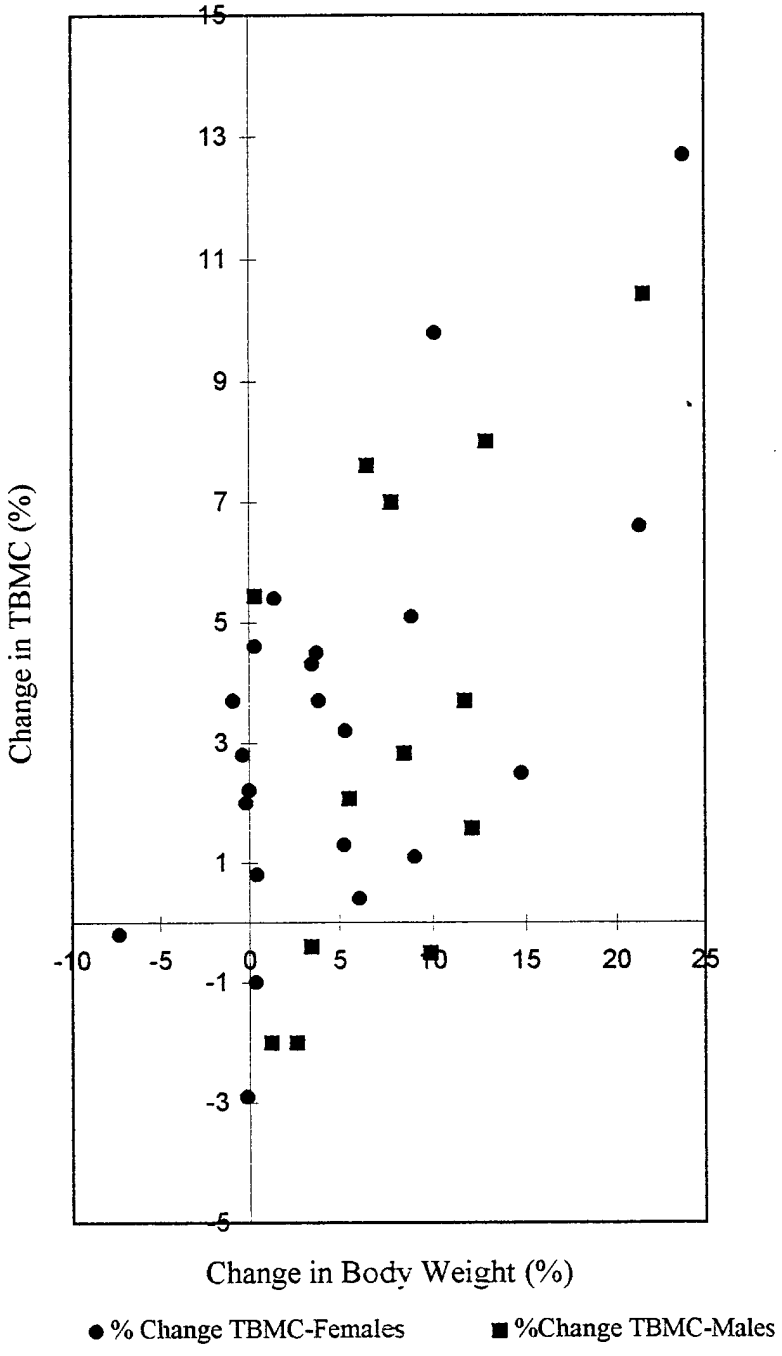

Figure 3: Females, n=20 r=.66 p<.001. Males, n=13 r=.67 p<.01

Calcium and Bone Accretion

In the female subjects, baseline TBMC was positively and significantly correlated to baseline calcium intake as measured by the FFQ ($r=.58$, $r^2=.33$ $p<.01$). This relationship, shown in Figure 4, was not seen in the follow up data. A different model including calcium intake from the follow-up FFQ and the ratio of this calcium intake to body weight, however, significantly predicted TBMC ($p<.05$). Thus, when the ratio of calcium intake to body weight (mg calcium/kg body weight) is held constant, TBMC increases with increasing calcium intake. Although the two variables (calcium mg and calcium mg/kg) in this model were highly correlated, they predict 35% of the variation in the female's TBMC ($r=.60$ $p<.005$).

As mentioned previously, 11 of the female subjects' FFQ indicated that their calcium intakes were similar at baseline and follow-up relative to the median, and these subjects were analyzed separately. Despite similar calcium intake at baseline and follow-up, there was not an independent relationship between TBMC and calcium intake at study follow-up in this subgroup of females. however, the model using calcium (mg/day) and calcium (mg/kg) was more predictive of the variation in TBMC ($r=.90$ $r^2=.81$ $p<.001$) in this subgroup (Figure 5).

As shown in Table 10 and Figure 6, the female subjects were divided into two groups around the median baseline calcium intake of 1059 mg per day. ANOVA showed that the group with the calcium intakes above the median had greater TBMC then those with calcium intakes below the median. ($p<.05$). Closer observation revealed

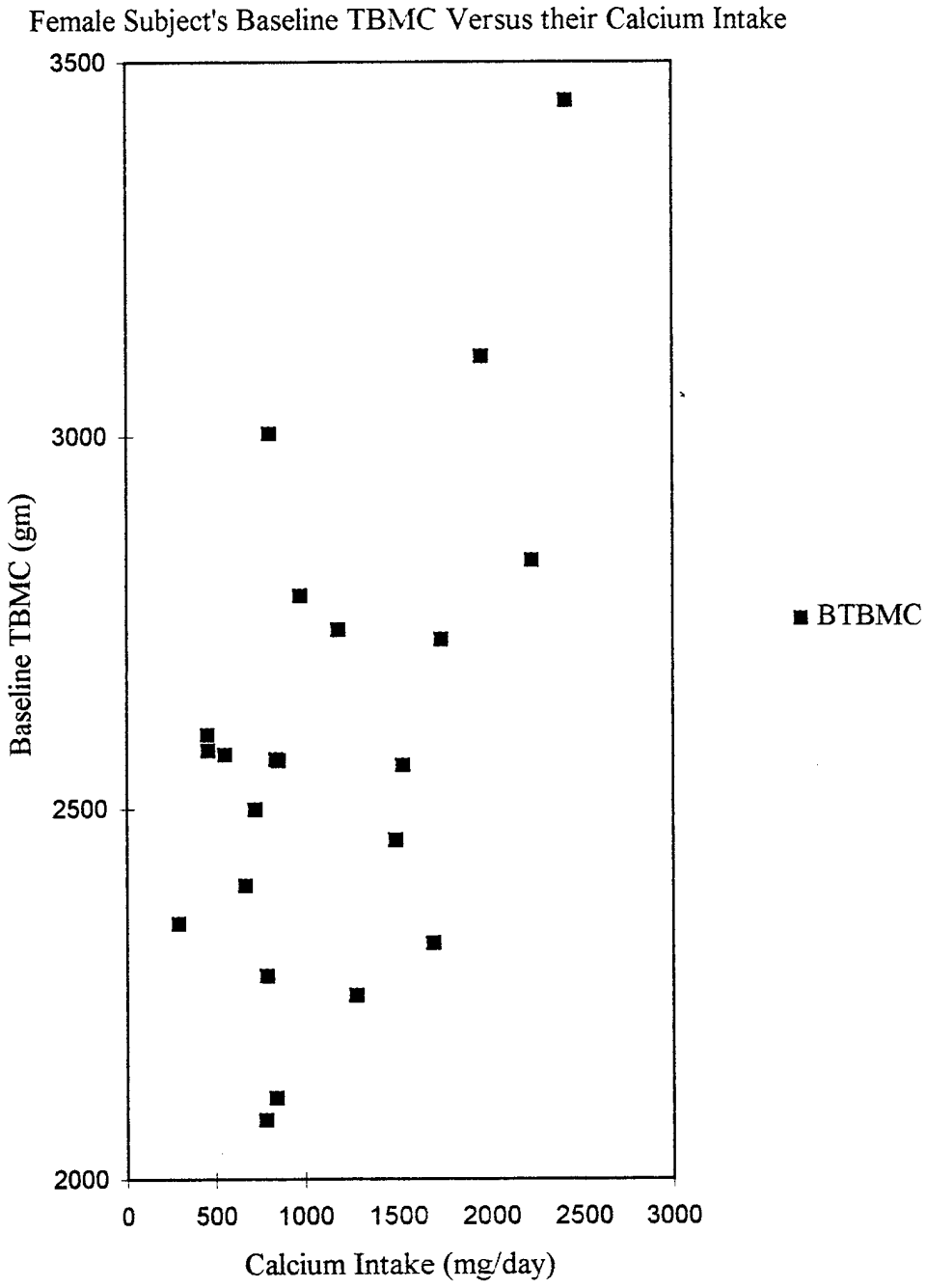

FIGURE 4: n=20, r=.55 p<.01

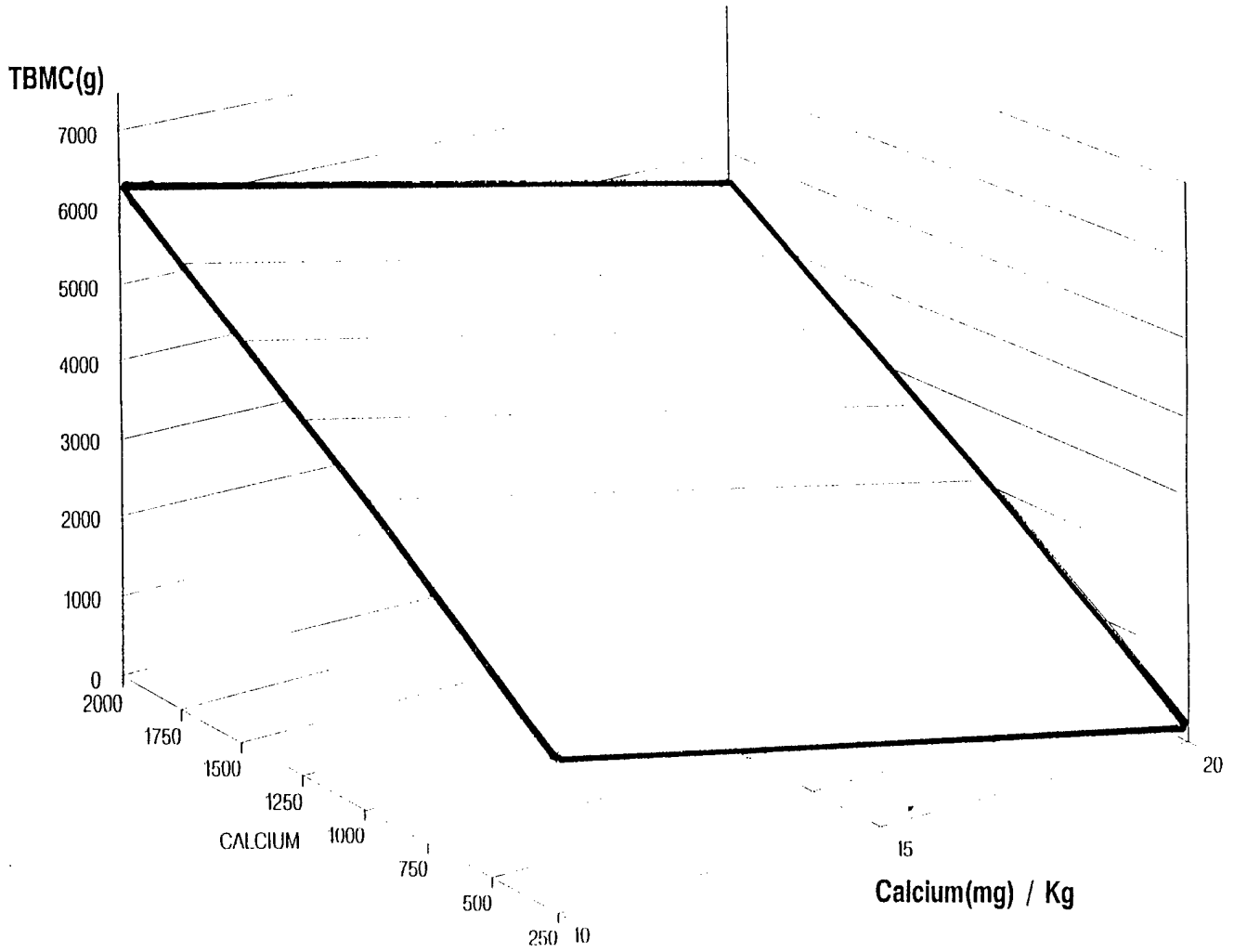

Figure 5: Relationship of TBMC to dietary calcium in females with consistant calcium intake.

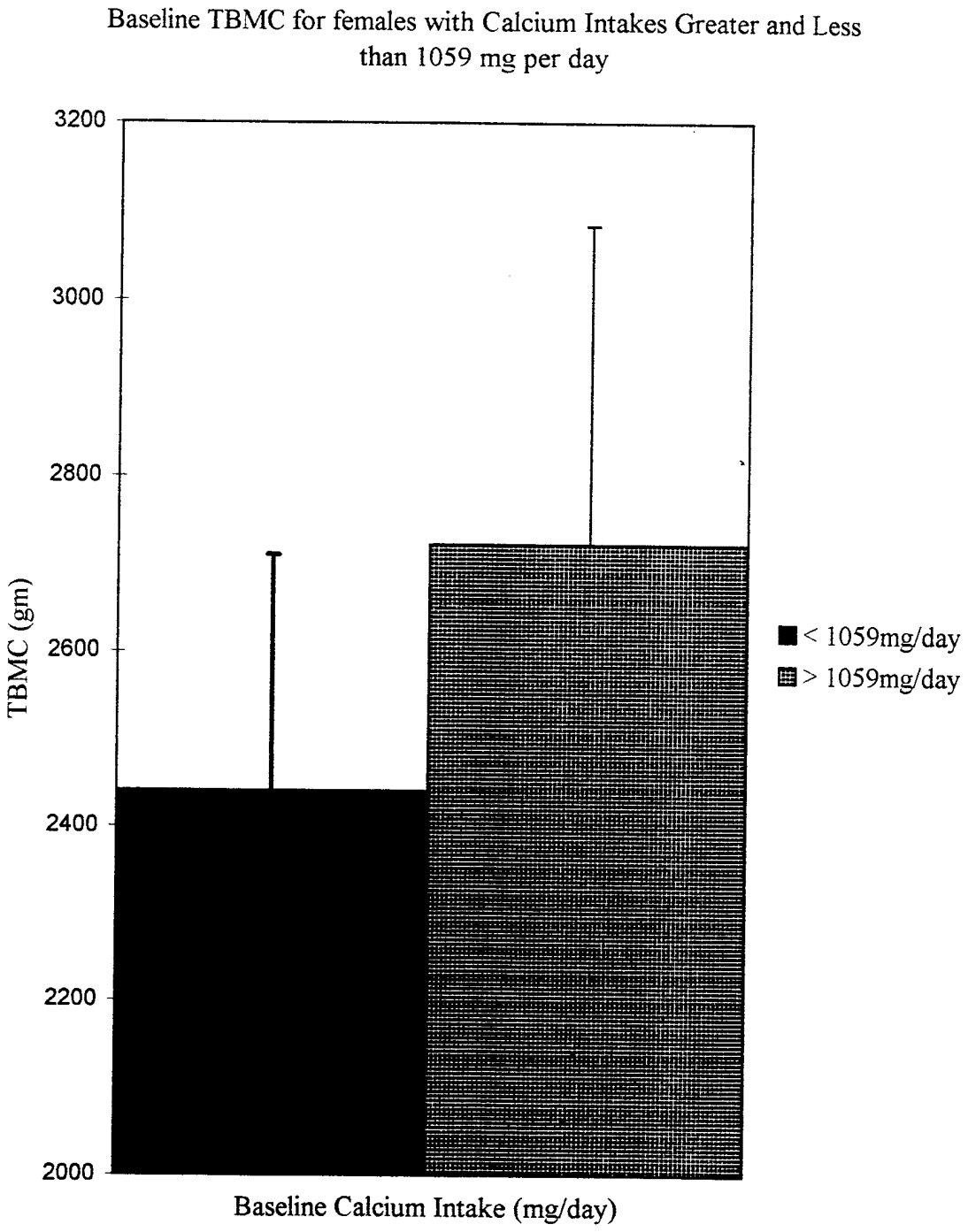

FIGURE 6: Bars indicate standard deviation. $p<.05$

that the group consuming more calcium weighed 16% more than the group with calcium intakes below the median ($p<.01$) (Table 10). Still, when calcium intake was evaluated in terms of milligrams of intake per kilogram of body weight, the group with higher TBMC was shown to consume 58% more calcium per kilogram ($p<.001$). At the study endpoint, there was no difference in TBMC between females with calcium intakes above and below the median of 1078 mg calcium per day (Table 11).

Table10: TBMC for females with baseline calcium intake greater & less than 1059 mg

Calcium Intake[1] (mg/day)	TBMC (gm)	Calcium Intake (mg/day)	Weight (kg)	Calcium (mg/kg)
Above Median >1059 (n=10)	2723 (361)[2]	1779 (383)	62.2 (6.6)	28.6
Below Median <1059 (n=10)	2441 (270)	647 (177)	52.5 (6.1)	12
Difference	282*	1132***	9.7**	16.6***

[1]Denotes data from the FFQ
[2]Mean (SD)
*p<.05
**p<.01
***p<.001

Table 11: TBMC for females with follow-up calcium intake greater & less than 1078mg

Calcium Intake[1] (mg/day)	TBMC (gm)	Calcium Intake (mg/day)	Weight (kg)	Calcium (mg/kg)
Above Median >1078 (n=11)	2582 (245)	1617 (596)	59 (5.6)	27.4
Below Median <1078 (n=11)	2746 (367)	762 (184)	60.7 (8.5)	12.6
Difference	164 (NS)	909***	1.7 (NS)	14.8***

1 Denotes data from the FFQ
2 Mean (SD)
***p<.001

In the female subjects, there was a trend towards calcium intake and body weight positively predicting baseline spine BMD ($p=.07$), but this trend was not present at

follow up. There was also a trend towards a correlation between change in calcium intake between years 2 and 3 (measured by the difference between the Spring 1995 and 1996 twenty-four hour recalls) and change in hip BMD ($r=.38$, $r^2=.15$ $p=.08$), but this association was not true for spine BMD.

In the male subjects, at baseline and follow-up, TBMC and BMD of the hip were not significantly related to usual calcium intake. At baseline, there was a significant negative correlation of calcium intake to BMD of the lumbar spine ($r= -.58$ $r^2=.34$ $p<.05$), but this relationship was not found in the follow up data. Change in the male's calcium intake between the 2nd and 3rd third study years as measured by the difference between the Spring 1995 and 1996 twenty-four hour recalls, showed a trend toward a correlation to change in BMD of the hip ($r=.52$ $r^2=.26$ $p=.09$). The mean hip BMD increased by 1.3% during this period. This was similar to the trend in the female subjects' change in hip BMD

Weight Bearing Exercise, Strength Training and Bone Accretion

Time spent in WBE or ST was not significantly related to BMD of the spine or hip in the male or female MIDN. Even when WBE and ST were combined as total time spent exercising, there was no relationship. Furthermore, when the females were divided into two groups-those exercising at least 2 hours per week and those exercising less than two hours per week, there was no difference between them for any of the bone indices.

A model including WBE and body weight was predictive of the female's follow-up TBMC, ($p=.01$) however the effect of WBE was only positive in subjects weighing

more than the mean body weight of 60 kg (Figure 7). There was no correlation between hours spent in WBE and body weight. Table 12 shows the female subjects divided into two groups-those weighing more and less than 60 kg. The heavier subjects performed WBE exercises 30% longer ($p<.05$) than the lighter subjects and had 5.7% greater hip BMD, although this figure did not reach significance. There were no significant relationships among the male subject's WBE and ST to their BMD or TBMC.

Table 12: WBE data and Hip BMD for female subjects divided at the median body weight

Female Subject's Weight	WBE (hours/week)	Hip BMD (gm/cm2)
< 60 Kg (n=12)	2.08 (.30)[1]	1.05 (.11)
>60 Kg (n=10)	2.76 (.94)	1.11 (.11)
Difference	0.68*	0.05 NS

$p<.05$

[1] Mean (SD) of time spent in exercise during the 2.8 year study period

Other Findings

Female Subject's Menstrual Cycle Data

Table 13 shows the mean number of menstrual cycles that the female subjects had during each year of the study. This data was collected in conjunction with the principle research protocol. During the first year, the female subjects had significantly fewer menstrual cycles than in the following two years ($p<.01$).

Table 13: Female's Menstrual Cycle History

Menstrual Cycles	n	1st Year	2nd Year	3rd Year
Cycles per year	22	9. 40 (3.23)[1]*a	11.36 (2.4)b	11.41 (1.1)b

[1]Mean (SD)

*$p<.01$

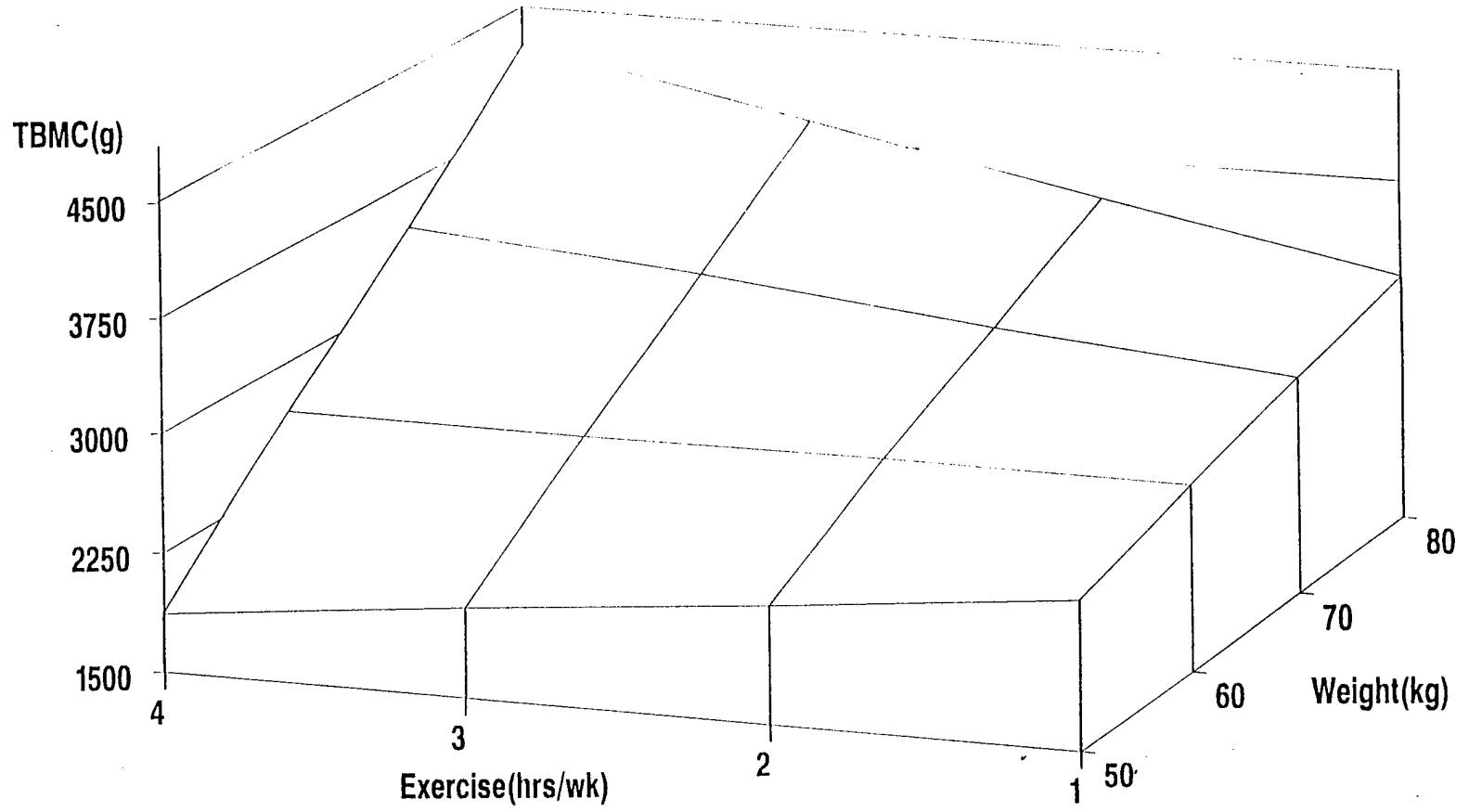

Figure 7: Relationship of females TBMC to weight bearing exercise at various body weights

Chapter VI.

DISCUSSION

Bone accretion occurs early in the third decade in male and female MIDN. Specifically, TBMC and spine BMD increased, but hip BMD did not. Body weight was highly influential to TBMC throughout the study in males and females. At baseline, the female's calcium intake was predictive of TBMC as well, however, this relationship was considerably weaker by the follow-up point and was no longer independent of body weight. The high calcium intakes of the male subjects may have been above the threshold where its positive effects can be shown in a linear model.

Hip BMD decreased during the first study year in males and females. This is possibly due to menstrual disturbances in the females. The female subjects had significantly fewer menstrual cycles during the first study year, a phenomenon that has been observed in other MIDN (87). It is possible that this menstrual disturbance caused an estrogen deficit that was sufficient to cause hip BMD loss (88,89). Another explanation of the hip BMD loss in both genders is that the intense physical activity characteristic of the first year at the USNA, causes adaptive changes in the bone which may appear to lower BMD (90,91,92). Hip bone accretion resumed in the last two years of the study, however, did not reach significance. WBE was only predictive of TBMC in female subjects weighing more than 60 kg suggesting that a threshold level of weight bearing from body weight and exercise is necessary to stimulate osteogenesis. Our results are consistent with much of the literature that reports a positive influence of calcium intake and WBE exercise. Our data are somewhat unique, however, because the

effect of body weight on TBMC modified the effects of calcium and WBE. In contrast to the literature (39,42,46), we found no relationship between ST and any of the bone indices.

Dietary Calcium

In general the female MIDN reported consuming more calcium than other college aged females. NHANES III data show that females in this age group had mean intakes of 778 mg calcium per day (39). The female MIDN, had mean calcium intakes at or near the RDA at the beginning and end of the study period as measured by the FFQ. The higher than average calcium intake may be attributed to the fact that the subjects ate the majority of their meals in a family style dining facility, where calcium rich foods are served regularly. The male MIDN had calcium intake very similar to other men in their age group according to NHANES III data.

Calcium intake showed a positive and significant relationship to TBMC at baseline in the female subjects, and this is consistent with the findings of a meta analysis of the effects of calcium on bone mass in young females (43). At follow-up there was no relationship between calcium intake and TBMC, however, a different model including calcium (mg/day) and calcium (mg/kg/day) positively, significantly predicted TBMC. Although the effect of calcium in this follow-up model was considerably weaker than in the baseline model and dependent on body weight, it predicted 35% of the variation in TBMC.

When the female subjects were divided into two groups based on their calcium intake being above or below the median at follow-up, only 5 of the 10 subjects initially in the lower calcium intake group remained there at the study follow-up point. Similarly, of the 10 subjects in the higher calcium group at baseline, only 6 of them remained in the higher calcium group at the 3 year follow-up point. The FFQ was only administered at baseline and follow-up, so it's impossible to precisely assess when the changes in calcium intake occurred during the study. We suspected that since half of the female subjects changed their calcium intake relative to the median during the study period, that a clear relationship between calcium intake and the bone indices may have been confounded.

At follow-up, eleven subjects' FFQ suggested that their calcium intakes were not different relative to the median at baseline and follow-up. When the model using calcium intake (mg/day) and calcium intake (mg/kg/day) was applied to this subgroup however, it predicted 81% of the variation in the TBMC compared to 63% predicted by body weight in a different model. These findings suggest that the positive influence of calcium intake is dependent on body weight in our female subjects. This principle has not been previously reported to our knowledge, however, Recker et al reported that the influence of calcium on TBMC in 24 year old females was dependent on adjustment for protein intake (16). This study, similar to ours in design and population, also demonstrated that white, college aged females gain BMC and BMD. Subjects followed up to five years had bone mineral content gains of 4.8-12.5% (per decade) for the forearm, lumbar spine and total body. These robust gains in BMC were attributed to physical activity, calcium intake (after adjustment for protein intake) and oral contraceptive use.

Lack of an independent relationship between calcium intake and TBMC at follow-up in our 21 year old female MIDN may be due to the subjects approaching the age of peak bone mass. In a study of 49 women whose mean age was 26.3 years, there was no relationship whatsoever of calcium intake to any of the measured bone indices (93). Bone accretion slows near its peak, making it more difficult to evaluate relationships to single predictive factors such as calcium intake. In our subjects for instance the relationship between calcium intake and TBMC was strong and significant at age 18.5, but weaker and dependent on body weight by age 21.3.

Other prospective studies (94,95,96,97) have suggested that calcium from supplements or dairy products may promote bone accretion and bone mineral maintenance in young women, and prevent or delay bone loss in perimenopausal women. Researchers intervened by supplementing dairy products and calcium supplements in premenopausal women ages 21-55 and noted significant increases in TBMC and BMC of regional sites (94), trends towards increased BMD of the lumbar spine, (95) or retarded losses of BMD of the lumbar spine compared to placebo controls (96). Even in women as much as 65 years old, Smith et al (97) found that calcium supplementation of 1500 mg per day significantly retarded the loss of BMC and BMD of the humerus.

These 5 prospective studies like ours, suggest that young women can increase their peak bone mass after cessation of linear growth and emphasize that calcium plays a role in optimizing peak bone mass. None of these researchers reported the relationship between calcium and bone accretion to be dependent on body weight. Having larger sample sizes in their studies (16,94,95,96,97) and intervening with the subject's calcium

intake (in the later 4 studies) likely enabled them to draw stronger conclusions regarding the positive effects of calcium on bone accretion.

In contrast, Riggs et al (98) found no significant association between calcium intake and rate of change in BMD. In their 2.5 year study of women ages 25-55, dietary calcium intake averaged 991mg per day implying that most women satisfied their requirement for this nutrient and may have exceeded the threshold where the effects of calcium can be shown in a linear model (31). This study, like ours, had a small sample size (45 subjects) and may have lacked the statistical power to show an association to calcium intake.

Among the male subjects, calcium intake was not related to TBMC. Another longitudinal study of young men failed to show significant relationships between calcium intake and BMD (44). Heany cautions however, that the lack of an observed relationship between dietary calcium and bone indices should not be interpreted as calcium being unimportant to bone health in males or females as calcium is known to exhibit threshold behavior (45). Identifying this threshold level of calcium intake for humans--suspected to be higher for females than the current U.S. recommended dietary allowance, would be extremely valuable for determining the optimum level of calcium intake to maximize peak bone mass (31).

The high calcium intakes (1252-1541 mg per day) reported by our male subjects may have exceeded the threshold where a relationship to TBMC or spine BMD can be shown in a linear model. Still, we cannot explain the negative correlation seen between

calcium intake and spine BMD, and we suspect that other factors, unrelated to calcium intake influenced the significant increase in spine BMD during the study period. In a separate cohort of 44 male MIDN, Armstrong found that TBMC increased 10.4% in four years. (Armstrong abstr 1997). With such profound bone accumulation occurring in Armstrong's cohort early in the third decade of life, calcium intake must play a positive role in this bone accumulation.

Lifestyle changes dramatically upon admission to the USNA, and appears to affect calcium intake patterns differently between the male and female subjects. This is particularly evident in that calcium intake from the Spring 96 FFQ and the Spring 96 twenty four hour recall were highly correlated for the male subjects, but were unrelated for the females. The 24 hour recall and the FFQ were obtained during the same session, but differences in these assessment tools (75,77,78) do not allow substituting one method for the other, and these data were interpreted separately. Examining the relationship between the two methods was due to interest in examining patterns of dietary intake.

The female subjects may change their eating patterns more frequently and significantly than the male subjects thereby increasing the intra-subject variation in calcium intake. In general, the female subjects seemed more conscientious about their dietary intake in an effort to prevent weight gain. This observation is supported by Eating Disorder Inventory (EDI) scores of female MIDN. EDI is a well established tool for screening large groups of individuals for indications of disordered eating (99). Seventy-nine female MIDN and 364 male MIDN completed the EDI early in their first

year at the USNA and again 10 months later. Scores for two sub-scales - Drive for Thinness and Body Dissatisfaction significantly increased in the female MIDN over the first year, but did not change for the males (Armstrong-Submitted).

The female MIDN's scores on the EDI support our observation that they feel more pressure to stay thin than male MIDN, and therefore may engage in restrictive dieting more often. Concerns about their caloric intake, may have made the female subjects more likely to change their dietary intake during the study period, increasing the variation in their diets compared to their male counterparts. This may explain why calcium intake as measured by the 24 hour recall correlated to the FFQ for the male subjects, but not for the female subjects. This raises two points; first, future studies should collect multiple days of food records and/or recalls more frequently throughout the study period in the female subjects. This may allow more accurate estimation of nutrient intakes. Second, female MIDN are more likely to be driven towards thinness and displeased with their body image prompting them to restrict their caloric intake thereby potentially hindering attainment of their maximal peak bone mass.

Weight Bearing Exercise

In the female subjects, a model including body weight, calcium and hours spent in WBE was predictive of TBMC in subjects weighing greater than 60 kg. We initially thought that subjects who exercised for longer periods of time had lower body weights and therefore lower TBMC. This was not substantiated however, since there was no

association between hours spent in exercise and body weight in this population. The mean body weight of the female subjects was 59.75 kg. Subjects weighing less than 60 kg, (n=11) participated in WBE 2 hours per week on average, compared to the 11 heavier subjects who exercised 2.8 hours per week. The heavier group exercised 30% longer than the lighter group--the equivalent of one 49 minute session of WBE per week ($p<.05$). The heavier subjects also had 5.2% greater hip BMD, although this difference did not reach significance likely due to the small number of subjects. In the case of these MIDN, there may have been greater motivation among the heavier subjects to exercise to ensure that they did not exceed the Naval Academy standards for body weight.

These results suggest that a threshold level of weight bearing from body weight and WBE is required to exert an osteogenic effect. Holbrook has noted that men and women who maintain a low body weight such that their lifetime maximum BMI is less than 24, had markedly lower BMD at all sites (100). Ninety percent of the female MIDN in our study had BMIs less than 24, so it seems particularly important that they participate in WBE regularly to reap the associated osteogenic benefits. There were no significant findings in the male subjects related to WBE, likely due to the small sample size.

Strength Training

Small sample size in this study is likely one of the causes for the lack of any significant relationship of the subject's strength training (ST) to their BMD. A study

with similarly aged subjects and methodology reported positive results of ST on BMD relative to other forms of physical activity. Women ages 20-30 who participated in ST activities for more than 1 hour per week in addition to 2.5 hours per week of aerobic activity had significantly greater lumbar spine BMD compared to sedentary controls and to those who only performed aerobic exercise (101). These subjects reported similar amounts of time spent exercising and were similarly aged to our female MIDN who reported performing WBE 2.4 hours per week and ST 1.2 hours per week on the average. Davee's study (101) quantified exercise history using a self administered questionnaire inquiring about exercise history, hobbies, job related physical activity and daily exercise activities. Training exercise hours were classified into aerobic or muscle building activities. This is similar to our method of quantifying hours per week of WBE and ST, although our questionnaire was administered during an interview.

Perhaps we would have identified a positive effect of ST on BMD of the hip and spine in our subjects if we had measured muscular strength rather than time spent in strength training. The subjects likely varied in the intensity of their ST workouts, so it's possible that time spent in strength training did not predict muscular strength-the factor that appears to be most predictive of BMD. Friedlander showed that in a group of 32 young women (ages 20-35), 2 years of an aerobics and strength training regimen increased isokinetic muscle performance which was positively related to spine and femoral neck BMD (102).

A final explanation for the lack of any significant relationship between ST and BMD is that the subjects may have been too homogeneous with regards to muscle

building activity. The range of ST in the subjects was relatively narrow; therefore, contrasts between BMD in subjects with extreme levels of ST were not possible.

Our findings are different than numerous cross sectional (46,103) and longitudinal studies (42,44,16) that have shown consistent positive association between physical activity and BMD. The effect of physical activity that is feasible for the general population appears to increase peak bone mass about 7-8% on the average compared to sedentary subjects. This is almost one SD, and if this bone mass is maintained until the elderly years, risk of osteoporotic fractures should be significantly reduced (104). Currently, there is no consensus on the optimal activity, intensity, frequency and duration of exercise to positively affect bone accretion and maintenance. The optimal activity likely involves weight bearing since weightlessness, and being bedridden have been shown to cause marked skeletal atrophy (50,51,52). Intensity of exercise is likely important too since, postmenopausal women engaging in a walking program three hours per week did not alter rate of bone loss (105). These results, despite being from a different population illustrate the principle that activities must be of sufficient intensity to create an osteogenic stress.

Bone Accretion

The 3.4% increase in TBMC among the female MIDN supports the hypothesis that bone mineral accretion occurs in female MIDN between the ages of 18.5 years and 21.3 years. Since body weights at baseline & follow-up were significantly different, but

heights were not, the accretion of TBMC cannot be attributed to linear growth during this study period. A prospective study (Drake-unpublished) of other female MIDN supports these findings as well. Over a 2.5 year study period, 79 female MIDN had a 4.6% increase in TBMC. This group of subjects gained 1.3 kg more body weight than the 22 females reported here likely accounting for the slightly greater change in TBMC. Site specific increases in BMD ranged from 1.8-3.9% in Drake's group similar to our subjects whose BMD sites increased 2.3-3.3%. These similar results suggest that the female subjects in our study are representative of a larger population of MIDN who increase their TBMC and spine BMD during the first 3 years at the USNA.

The male subject's bone accretion was very similar to the females in that their TBMC increased positively and significantly, despite no increase in height during the study period. This supports cross sectional (13,14) and longitudinal studies (15,16) that bone mineral accretion occurs after cessation of linear growth in males and female. The extent of change in TBMC in our subjects is quite remarkable since Matkovic and Ilich (29) reported that increases in peak bone mass of 5-10% are associated with a 50% reduction in hip fracture rate in the elderly years. Promotion of the lifestyle factors that may increase bone accretion appears to be a worthy effort in the goal to maximize the peak bone mass of MIDN.

Both male and female subjects exhibited strong correlations of body weight to TBMC with the former predicting up to 63% of the variation in TBMC in the female subjects. This result is in agreement with the literature, where body weight is consistently documented as one of the major variables affecting bone accretion

(103,106). The proposed mechanism for the positive correlation between body weight and TBMC is that increased body weight acts to load the skeleton, and stimulates osteoblast activity. Although there is no consensus as to what BMI reduces the risk of osteoporosis, BMIs between 26-29 are known to have some protective affect against fracture, while BMIs between 22-24 are associated with increased fracture risk (106). In this study, the female MIDN's mean BMI was 22.2 at the study follow-up point, placing more than 85% of the subjects in the range of BMI's that are associated with increased fracture risk. This is not to suggest that higher BMI's are more desirable in MIDN or others, but rather to show contrast that unlike many other chronic diseases, risk of osteoporosis is associated with lower BMI's, and is therefore a relevant preventive health issue in MIDN.

Since it is well established that body weight is positively associated with bone mass, (107) current studies are examining the relative importance of the fat and lean components of body weight to bone mineral content (BMC). Early results are contradictory with regards to which component-fat or lean mass is the strongest predictor of BMC (108,109,110).

A cross sectional multi-center study of more than 1600 post-menopausal women found both fat mass and lean mass to be significant, independent predictors of BMC, and further showed that the relative influences of the two components depended on the bone site studied (107). Body weight positively influenced both weight bearing and non-weight bearing sties suggesting that the mechanism by which body weight affects BMC is not solely explained by mechanical loading. The influence of lean and fat components

at various bone sites did not parallel that of body weight. Sites rich in trabecular bone such as Ward's triangle (a region of the femoral neck) and the ultradistal radius were heavily influenced by fat mass possibly due to mechanical loading and the conversion of adrenal androgens to estrogen in the adipose tissue (107). Trabecular bone is more metabolically active, and thus may be more responsive to hormonal influence than cortical bone sites.

Lean mass-50% of which is comprised of muscle tissue, influences BMC likely secondary to dynamic loading from muscular contraction. Hypertrophy in radial BMC in the dominant arm of tennis players supports this hypotheses (111). Other studies have made similar conclusions regarding the effects of fat mass and lean mass on BMC (112), however these studies share a common limitation--that DEXA is not without flaws for measuring lean and fat mass. Measurement errors can occur if subjects are dehydrated, sick, very young or very old (64).

Thirty-one percent of the male and female subjects in our study experienced hip BMD loss over the study period. Seven of the 22 female subjects had negative changes in their hip BMD during the study, preventing the total change in hip BMD from reaching significance. The Norland DEXA used to measure BMD in these subjects maintains excellent precision with a coefficient of variation of 1.5% for in vivo measures, so it is unlikely that measurement error is the primary explanation for the lack of significant hip BMD accretion. In fact, losses in the hip BMD in 4 of the female subjects far exceeded the 1.5% coefficient of variation, (mean 6% range 2.3-11.3%) suggesting true bone loss. These subjects ranged in weight from 56-64 kg (mean 61kg) placing

most of them below the mean weight (59.75 kg) of the female subjects, however they did not lose weight during the study period. Baseline hip BMD was positively predicted by body weight although changes in hip BMD were not associated with weight changes.

We speculate that sub-clinical menstrual disturbances early in the female MIDN's tenure at the USNA caused the loss of hip BMD. None of the 22 female subjects were ammenorrheic during the study period, however, the number of menstrual cycles per year was significantly lower in the first year. This time period is concurrent with the plebe summer and first academic year at the USNA when physical training and psychological stress are most intense. In a cohort of 182 females at the USNA who were divided into quintiles by number of menstrual cycles, a small group of women who were ammenorrheic had a 2.4% loss of femoral neck BMD (FN BMD) (87). These subjects' FN BMD was significantly lower than MIDN with normal menstrual cycles and those taking oral contraceptives.

At the 2 year follow-up of this cohort, the group who had previously been ammenorrheic no longer had significantly lower FN BMD, but appeared to be recovering from the bone losses that occurred in the first year of the study. In this cohort, modest menstrual irregularity and oligomenorrhea appeared to affect hip bone accretion in a small group of midshipmen, but these effects may not be irrecoverable (87). On the other hand, losses of hip BMD due to menstrual disturbances in this cohort may have been minimized due to high weight bearing activity compensating for the estrogen deficit. Non-weight bearing sites may not have fared as well according to a study in ballet dancers. This study suggested that non-weight bearing sites and weight bearing

sites comprised mostly of trabecular bone, were more adversely affected by menstrual disturbances than cortical weight bearing sites (88). Given this evidence, and that from another study (89) menstrual disturbances should not be ignored as a benign condition simply because there is preservation of bone mass at weight bearing sites in active, oligo and ammenorrheic young women.

The small number of females in our study did not allow for dividing the subjects into tertiles based on their menstrual cycles. However, we suspect that a trend similar to that in Drake's cohort (87) may be occurring in our subjects since 18 of the females gained hip BMD during the last study year when the number of menstrual cycles was most normal. Hip BMD increased modestly for male and female subjects during the last study year, and there were trends towards correlations to changes in calcium intake over the same time period. Notably, the female subject's calcium intake increased more than 100 mg per day over this time period, suggesting that the increase in hip BMD may be related to more normal menstrual status and increases in calcium intake.

Another theory exists that may explain the losses of hip BMD in the female and male subjects in this study. Woo et al reports that moderate treadmill exercise in young pigs with growing bones resulted in increased cortical thickness and increased cross sectional area, but no changes in BMD (90). Young bones have a greater potential for cortical expansion than elderly bones allowing them to increases the area in the bone into which mineral can be deposited in response to increased activity (91). Ultimately this results in a larger bone of equal or greater density better equipped to tolerate mechanical stress (92). This remodeling process by which new mineral is deposited to fill in the

larger area may occur slowly in subjects who are no longer growing. It could be that the intense physical activity in the MIDN during the first study year results in cortical expansion, but that the mineral deposition process occurs slowly due to the subjects' ceasing linear growth. Therefore, the BMD of the hip appears to decrease. Currently, there is no method to accurately assess volumetric bone density to test this theory.

Strengths and Limitations

The small number of subjects in this study reduced the statistical power of various analyses. Subsequently, this serves more as preliminary work useful in planning future, larger studies. Additionally, the dietary methodology in future studies would likely benefit from replacing the 24 hour dietary recalls with 3 day food records or a combination of a 24 hour recall followed by a 2 day food record--especially in the female subjects. The 3 day food records are known to have less intra-subject variation than a single day's recall, making it more feasible to evaluate, in addition to calcium, nutrients and dietary components such as sodium, phosphorus, protein, caffeine and alcohol in conjunction with changes in the bone indices.

This study was conducted on a convenience sample of volunteers rather than randomly selected MIDN. Volunteers for a health related study may be more health conscious, so the results of this study must be cautiously interpreted.

If the same methods for collecting exercise data are used in future studies, it may be useful to validate them by comparing data collected from exercise questionnaires with

data determined by accelerometers worn by a portion of the subjects. It may also be helpful to measure muscular strength as a predictor of BMD instead of time spent in ST.

Conclusions

Body weight, calcium intake and WBE are factors with positive roles in increasing peak bone mass in 18-22 year old MIDN. In our study, calcium intake was only an independent predictor of the female MIDN's TBMC at baseline. The effect of calcium intake on TBMC weakened over the study period and was dependent on body weight at follow-up. Changes in calcium intake over a one year period showed a trend towards a positive relationship to changes in BMD of the hip in male and female MIDN. In light of these findings and the absence of any known deleterious effects, MIDN should be encouraged to consume calcium rich foods to ensure an average intake of 1200 mg per day.

Subjects with a low body weight did not gain an osteogenic benefit from exercising 2 hours per week while WBE appeared to increase peak bone mass in 18-22 year old female MIDN weighing greater than 60 kg. These findings suggest that a threshold level of weight bearing (from body weight and exercise) is necessary to stimulate osteogenesis. Regular WBE should be promoted to all MIDN especially those who may be at risk for osteopenia due to low body weight. Unhealthy dieting practices and maintenance of very low body weight should be discouraged due to their deleterious effects on BMD. Weight loss regimens for MIDN who are significantly overweight and

out of USNA standards should be supervised by a registered dietitian to ensure nutritional adequacy of dietary intake while gradual weight loss is achieved. Menstrual disturbances especially during the first year at the USNA may result in losses of hip BMD and should be promptly evaluated.

Chapter VII.

APPENDICES

Appendix A

Date _____

NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND

Consent Form for Males

Consent for Voluntary Participation in a Clinical Investigation Study

1. I, _____, have been asked to voluntarily participate in a research project entitled, "The Association of Bone Mineral Density and Menstrual Dysfunction with Stress Fractures in USNA Midshipmen" being conducted at the Naval Branch Clinic, Bancroft Hall, U.S. Naval Academy, Annapolis, Maryland, and the National Naval Medical Center, Bethesda, Maryland.

2. The purpose of this research project has been explained to me. Stress fractures and related orthopedic injuries are a common problem in Midshipmen during training. Risk for stress fracture may be related to variations in bone mineral density (BMD) (i.e., the amount of calcified bone material present in a given volume of bone) among individuals. This study will seek to determine if the risk for stress fractures in USNA Midshipmen is related to decreased BMD.

3. I understand my participation in this research project will be for a period of four (4) years or for the duration of my enrollment at the USNA.

4. The procedures have been explained to me as follows: Upon entering this study, after providing written informed consent, I will be medically evaluated by a physician participating in the study. I will have a complete medical history and a basic physical examination, as well as yearly interval medical history.

Within two to three months of entry into the study, and then each year thereafter for four years, a bone mineral density and body composition measurement (percent body fat and percent body water) will be performed utilizing a dual energy x-ray bone densitometer, which is a machine that measures bone mineral density and body composition with a painless scan technique requiring only a few minutes to perform while I am lying down on the scan table. I understand that the procedure is painless, requires approximately 30-45 minutes or less of total time, and involves a minimal exposure to ionizing radiation - less than that of a routine standard chest x-ray.

My percent body fat measurement will also be validated by caliper measurements of my skinfold thickness each year.

Patient Initials _____

Upon entry into the study and yearly thereafter for four years, I will have blood drawn and will collect urine samples as described below for routine laboratory testing and for examination of biochemical markers of bone metabolic activity and endocrine (hormonal) function. I will have approximately three (3) tablespoons (45 cc) of blood drawn initially for the baseline laboratory tests and up to 4 times per year follow-up blood draws of approximately two (2) tablespoons (30 cc). The urine samples will consist of giving a first morning fasting urine sample, up to 4 times per year, collected by urinating into a urine sample container just after awakening in the morning, before eating breakfast, and after fasting (no food or drink except water) after 2000 hours the night before.

Other procedures that I perform will include a 24 hour diet recall interview. I will be asked to remember everything I have eaten or drunk in the previous 24 hours 4 times per year. I understand that this 24 hour diet recall will be analyzed to determine my caloric and nutrient intake, with a copy of the analysis provided to me as well as to the study investigators.

In addition, I will be evaluated in the Orthopedic Department in the Bancroft Hall Branch Clinic for any orthopedic complaints or injuries. After evaluation by an orthopedic physician, further studies such as routine x-rays or bone scanning will be performed as medically indicated.

Periodically, about 4 times per year I may be asked to maintain a 3 day activity log and wear an instrument, about the size of a wrist-watch, on my wrist to record my activity patterns.

In the event of any unusual test results I may be referred for further testing, evaluation, and treatment.

5. Specifically, I am aware that the <u>experimental part</u> of this study is the measurement of my bone density and percent body composition yearly for four years, the analysis of information (to test for statistical relationships) concerning any orthopedic injuries I may experience while at the USNA, and the analysis of information from my medical history, mood, diet history, activity patterns, blood tests, and urine tests.

6. A total of 480 subjects are expected to participate in this project.

7. The <u>risks or discomforts</u> which are possible are as follows:

A. For blood drawing: Pain and inconvenience, as well as possible "black and blue" bruise marks, infection and/or fainting spells.

Patient Initials _____

B. For bone mineral density and body composition measurement: Inconvenience and minimal exposure to ionizing radiation (the skin doses of 2-5 mrem or less per scan are a little above background solar radiation and are significantly less than the 10-40 rem exposure of a typical routine chest x-ray).

C. For urine collection: Inconvenience.

D. For orthopedic evaluation of injuries: Possible discomfort of routine orthopedic examination.

E. For giving of a complete medical history: Inconvenience.

F. 24 hour diet recall interview: Inconvenience.

G. Skinfold thickness measurement: Possible mild discomfort.

H. Activity log and monitor. Inconvenience.

I understand and accept these risks. Patient Initials_____

8. I understand that the research may or may not help me personally but that the results may help the investigator learn about risk factors for stress fractures and decreased bone mineral density or aid in the prevention or treatment of these conditions in other patients.

9. The alternate treatment, should I decline enrollment into this study, has been explained as follows: My nonparticipation in this study will not affect the care and evaluation I receive in the Orthopedics Clinic at the USNA for any orthopedic injuries or complaints I may have while at the USNA. This study does not otherwise involve a medical treatment. Therefore, there is no alternative treatment that would be advantageous to me.

10. I am aware that this study may involve risks to me which are currently unforeseeable.

11. The investigator may terminate my participation in this project for the following reasons: If I am discharged from the USNA.

12. I understand that I may withdraw from this study at any time without prejudice to my future care. I understand that my withdrawal from this project may result in decreased information being available concerning possible further changes in my bone density; however, I will not lose any benefits to which I am otherwise entitled.

13. Any new significant findings developed during the course of the research which may affect my willingness to participate further will be explained to me.

Patient Initials _____

14. In all publications and presentations resulting from this research project, my anonymity will be protected to the maximum extent possible; although, I realize that authorized Navy Medical Department personnel may have access to my research file in order to verify that my rights have been safeguarded. Otherwise my research file will be maintained in a secure location accessible only to the study investigators.

15. If I suffer any physical injury as a result of my participation in this study, immediate medical treatment is available at the National Naval Medical Center, Bethesda, Maryland and/or the Bancroft Hall Branch Medical Clinic. I understand that although no compensation is available, any injury as a result of my participation will be evaluated and treated in keeping with the benefits or care to which I am entitled under applicable regulations.

16. I have been informed that there will not be additional cost to me if I choose to participate in this research project. However, should I require hospitalization at the National Naval Medical Center arising from participating in this research project, I will be required to pay the customary fees for hospital meals to National Naval Medical Center based on my Department of Defense beneficiary status.

17. If I have any questions regarding this research project, I may contact Dr. A.J. Drake, III, MC, USN at (301) 295-5165. If I have any questions regarding my rights as an individual while participating in a research project at the Naval Branch Clinic, Bancroft Hall, USNA and the National Naval Medical Center, Bethesda, I can contact one of the Research Administrators, Clinical Investigation Department, at (301) 295-2275. He/she will answer my questions or refer me to a member of the Committee for the Protection of Human Subjects for further information. If I believe I have been injured as a result of this project I may call the Legal Office at (301) 295-2215.

18. I understand that my participation in this project is voluntary and that my refusal to participate will involve no penalty or loss of benefits to which I am entitled under applicable regulations. If I choose to participate, I am free to ask questions or to withdraw form the project at any time. If I should decide to withdraw from the research project, I will notify Dr. A.J. Drake, III, MC, USN at (301) 295-5165, to ensure an orderly termination process. My withdrawal will involve no loss of benefits to which I am entitled.

Patient Initials_____

I certify that I have received a copy of this consent form.

Patient Initials _____

_____ _____

Date Signed Volunteer Signature

_____

Volunteer Printed Name-Rank-SSN

_____ _____

Witness' Signature and Date Investigator Signature and Date

_____ _____

Witness' Printed Name-Rank-SSN Investigator Printed Name-Rank-SSN

I have reviewed the above document and hereby authorize my son _____ to participate in this research project.

_____ _____

Signature Date

PRIVACY ACT STATEMENT

1. Authority. 5 USC 301

2. Purpose. Medical research information will be collected to enhance basic medical knowledge, or to develop tests, procedures, and equipment to improve the diagnosis, treatment, or prevention of illness, injury or performance impairment.

3. Use. Medical research information will be used for statistical analysis and reports by the Departments of the Navy and Defense, and other U.S. Government agencies, provided this use is compatible with the purpose for which the information was collected. Use of the information may be granted to non-Government agencies or individuals by the Chief, Bureau of Medicine and Surgery in accordance with the provisions of the Freedom of Information Act.

4. Disclosure. I understand that all information contained in this Consent Statement or derived from the experiment described herein will be retained permanently at National Naval Medical Center, Bethesda, Maryland and salient portions thereof may be entered into my health record. I voluntarily agree to its disclosure to agencies or individuals identified in the preceding paragraph and I have been informed that failure to agree to such disclosure may negate the purposes for which the experiment was conducted.

_____ _____

Subject/Guardian Signature Signature of Witness

_____

Printed Name, Grade or Rank

_____

Date of Birth

Date _____

NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND

Consent Form for Females

Consent for Voluntary Participation in a Clinical Investigation Study

1. I, _____, have been asked to voluntarily participate in a research project entitled, "The Association of Bone Mineral Density and Menstrual Dysfunction with Stress Fractures in USNA Midshipmen" being conducted at the Naval Branch Clinic, Bancroft Hall, U.S. Naval Academy, Annapolis, Maryland, and the National Naval Medical Center, Bethesda, Maryland.

2. The purpose of this research project has been explained to me. Stress fractures and related orthopedic injuries are a common problem in Midshipmen during training. Risk for stress fracture may be related to variations in bone mineral density (BMD) (i.e., the amount of calcified bone material present in a given volume of bone) among individuals. In addition, in females, menstrual irregularities are frequent in the setting of increased physical and mental stress such as that at the USNA. There is evidence in the medical literature that persistent menstrual abnormalities can result in decreased bone mineral density in females, and subsequent increased risk for stress fracture. This study will seek to determine if the risk for stress fractures in USNA Midshipmen is related to decreased BMD and if the risk in female Midshipmen in particular is related to subtle abnormalities in menstrual function and bone density.

3. I understand my participation in this research project will be for a period of four (4) years or for the duration of my enrollment at the USNA.

4. The procedures have been explained to me as follows: Upon entering this study, after providing written informed consent, I will be medically evaluated by a physician participating in the study. I will have a complete medical history and a basic physical examination, as well as yearly interval medical history.

Within two to three months of entry into the study, and then each year thereafter for four years, a bone mineral density and body composition measurement (percent body fat and percent body water) will be performed utilizing a dual energy x-ray bone densitometer,

Patient Initials _____

which is a machine that measures bone mineral density and body composition with a painless scan technique requiring only a few minutes to perform while I am lying down on the scan table. I understand that the procedure is painless, requires approximately 30-45 minutes or less of total time, and involves a minimal exposure to ionizing radiation - less than that of a routine standard chest x-ray.

My percent body fat measurement will also be validated by caliper measurements of my skinfold thickness each year.

Upon entry into the study and yearly thereafter for four years, I will have blood drawn and will collect urine samples as described below for routine laboratory testing and for examination of biochemical markers of bone metabolic activity and endocrine (hormonal) function. I will have approximately three (3) tablespoons (45 cc) of blood drawn initially for the baseline laboratory tests and follow-up blood draws up to up to 4 times per year, of approximately two (2) tablespoons (30 cc).

Other procedures that I perform will include a 24 hour diet recall interview. I will be asked to remember everything I have eaten or drunk in the previous 24 hours up to 4 times per year. I understand that this 24 hour diet recall will be maintained on a log provided for that purpose and will then be analyzed to determine my caloric and nutrient intake, with a copy of the analysis provided to me as well as to the study investigators.

In addition, I will be evaluated in the Orthopedic Department in the Bancroft Hall Branch Clinic for any orthopedic complaints or injuries. After evaluation by an orthopedic physician, further studies such as routine x-rays or bone scanning will be performed as medically indicated.

Periodically, about 4 times per year I may be asked to maintain a 3 day activity log and wear an instrument, about the size of a wrist-watch, on my wrist to record my activity patterns.

I understand that I must keep monthly menstrual calendars for the duration of my participation in the study. These menstrual calendars will be collected from me by the study investigators periodically during each year of the study.

During two (2) monthly menstrual cycles each year, for up to 45 days each, I will also collect daily morning urine samples and turn them in to the study investigators. Convenient containers will be provided for the daily morning urine collections and the urine will be tested for hormonal markers of menstrual function. I understand that all information concerning my menstrual function and timing of my menstrual cycles will be maintained in my study file in a secure fashion and will not be accessible to individuals other than the study investigators.

In the event of any unusual test results, I may be referred for further testing, evaluation, and treatment.

Patient Initials _____

5. Specifically, I am aware that the experimental part of this study is the measurement of my bone density and percent body composition yearly for four years, the analysis of information (to test for statistical relationships) concerning any orthopedic injuries I may experience while at the USNA, the analysis of information from my medical history, mood, diet history, activity patterns, blood tests, and urine tests, and the analysis of information concerning my menstrual cycles.

6. A total of 480 subjects are expected to participate in this project.

7. The risks or discomforts which are possible are as follows:

A. For blood drawing: Pain and inconvenience, as well as possible "black and blue" bruise marks, infection and/or fainting spells.

B. For bone mineral density and body composition measurement: Inconvenience and minimal exposure to ionizing radiation (the skin doses of 2-5 mrem or less per scan are a little above background solar radiation and are significantly less than the 10-40 mrem exposure of a typical routine chest x-ray).

C. For urine collection: Inconvenience.

D. For orthopedic evaluation of injuries: Possible discomfort of routine orthopedic examination.

E. For keeping of menstrual calendars: Inconvenience.

F. For giving of a complete medical history: Inconvenience.

G. 24 diet recall interview: Inconvenience.

H. Activity log and monitor. Inconvenience.

I. Skinfold thickness measurement: Possible mild discomfort.

I understand and accept these risks. Patient Initials_____

8. I understand that the research may or may not help me personally but that the results may help the investigator learn about risk factors for stress fractures and decreased bone mineral density or aid in the prevention or treatment of these conditions in other patients.

Patient Initials _____

9. The alternate treatment, should I decline enrollment into this study, has been explained as follows: My nonparticipation in this study will not affect the care and evaluation I receive in the Orthopedics Clinic at the USNA for any orthopedic injuries or complaints I may have while at the USNA. This study does not otherwise involve a medical treatment. Therefore, there is no alternative treatment that would be advantageous to me.

10. I am aware that this study may involve risks to me (or to the embryo or fetus, if I become pregnant) which are currently unforeseeable. I am aware that I should promptly advise the investigator if I become pregnant.

11. The investigator may terminate my participation in this project for the following reasons: If I am discharged from the USNA or if I become pregnant.

12. I understand that I may withdraw from this study at any time without prejudice to my future care. I understand that my withdrawal from this project may result in decreased information being available concerning possible further changes in my bone density; however, I will not lose any benefits to which I am otherwise entitled.

13. Any new significant findings developed during the course of the research which may affect my willingness to participate further will be explained to me.

14. In all publications and presentations resulting from this research project, my anonymity will be protected to the maximum extent possible; although, I realize that authorized Navy Medical Department personnel may have access to my research file in order to verify that my rights have been safeguarded. Otherwise my research file will be maintained in a secure location accessible only to the study investigators.

15. If I suffer any physical injury as a result of my participation in this study, immediate medical treatment is available at the National Naval Medical Center, Bethesda, Maryland and/or the Bancroft Hall Branch Medical Clinic. I understand that although no compensation is available, any injury as a result of my participation will be evaluated and treated in keeping with the benefits or care to which I am entitled under applicable regulations.

16. I have been informed that there will not be additional cost to me if I choose to participate in this research project. However, should I require hospitalization at the National Naval Medical Center arising from participating in this research project, I will be required to pay the customary fees for hospital meals to National Naval Medical Center based on my Department of Defense beneficiary status.

Patient Initials _____

17. If I have any questions regarding this research project, I may contact Dr. A.J. Drake, III, MC, USN at (301) 295-5165. If I have any questions regarding my rights as an individual while participating in a research project at the Naval Branch Clinic, Bancroft Hall, USNA and the National Naval Medical Center, Bethesda, I can contact one of the Research Administrators, Clinical Investigation Department, at (301) 295-2275. He/she will answer my questions or refer me to a member of the Committee for the Protection of Human Subjects for further information. If I believe I have been injured as a result of this project I may call the Legal Office at (301) 295-2215.

18. I understand that my participation in this project is voluntary and that my refusal to participate will involve no penalty or loss of benefits to which I am entitled under applicable regulations. If I choose to participate, I am free to ask questions or to withdraw from the project at any time. If I should decide to withdraw from the research project, I will notify Dr. A.J. Drake, III, MC, USN at (301) 295-5165, to ensure an orderly termination process. My withdrawal will involve no loss of benefits to which I am entitled.

Patient Initials _____

I certify that I have received a copy of this consent form.

Patient Initials _____

_____ _____
Date Signed Volunteer Signature

_____
Volunteer Printed Name-Rank-SSN

_____ _____
Witness' Signature and Date Investigator Signature and Date

_____ _____
Witness' Printed Name-Rank-SSN Investigator Printed Name-Rank-SSN

I have reviewed the above document and hereby authorize my daughter _____ to participate in this research project.

_____ _____
Signature Date

PRIVACY ACT STATEMENT

1. Authority. 5 USC 301

2. Purpose. Medical research information will be collected to enhance basic medical knowledge, or to develop tests, procedures, and equipment to improve the diagnosis, treatment, or prevention of illness, injury or performance impairment.

3. Use. Medical research information will be used for statistical analysis and reports by the Departments of the Navy and Defense, and other U.S. Government agencies, provided this use is compatible with the purpose for which the information was collected. Use of the information may be granted to non-Government agencies or individuals by the Chief, Bureau of Medicine and Surgery in accordance with the provisions of the Freedom of Information Act.

4. Disclosure. I understand that all information contained in this Consent Statement or derived from the experiment described herein will be retained permanently at National Naval Medical Center, Bethesda, Maryland and salient portions thereof may be entered into my health record. I voluntarily agree to its disclosure to agencies or individuals identified in the preceding paragraph and I have been informed that failure to agree to such disclosure may negate the purposes for which the experiment was conducted.

_____ _____

Subject/Guardian Signature Signature of Witness

_____

Printed Name, Grade or Rank

_____

Date of Birth

Appendix B

STUDY ID NUMBER _____

MEDICAL HISTORY FORM

Date _____

Class Year _____

I. Demographic Data:

a. Age: _____ Years

b. Sex: _____

c. Race: _____ (White, Black, Hispanic, Asian, Native American)

d. Ethnic Extraction: _____ (Example: Northern European, African, Italian, Spanish, Chinese, etc.)

e. Ethnic Origin of Grandparents (For use only in determination of your predominate ethnic origin, which is important because of its impact on normal bone density:

Paternal Grandfather _____

Paternal Grandmother _____

Maternal Grandfather _____

Maternal Grandmother _____

II. Physical Data:

a. Height: _____ Inches

b. Weight: _____ lbs.

III. Past Medical History:

a. Do you have any known medication allergies?

Yes _____ No _____

If yes, please list

_____

_____

_____

_____

b. Please list all prescription and nonprescription medications which you are taking (including vitamins and any calcium supplements).

_____

_____

_____

c. Do you smoke? Yes _____ No _____

If yes, how much per day?

_____ Packs per day or

_____ cigars per day or

_____ "bowls" of pipe tobacco per day.

For how long? _____ Years

d. How much alcohol, if any, do you consume in an average week?

_____ ounces per week (assuming one mixed drink, one beer, and one glass of wine <u>each</u> is equal to <u>one ounce</u> of alcohol).

e. Do you have any chronic medical problems (such as thyroid disease, asthma, kidney problems, etc.)?

Yes _____ No _____

If yes, please list. _____

_____

_____

_____

_____

IV. <u>Surgical History</u>:

a. Have you had any surgery in the past? Yes ____ No ____

If yes, please list procedures you have had with the dates they were done_____

_____

_____

_____

b. Have you ever had or passed a kidney stone?

Yes _____ No _____

If yes, how many and when? _____

_____

_____

c. Have you ever had a broken bone? Yes _____ No _____

If yes, list bones broken, with dates and cause of the injury. _____

_____

_____

_____

_____

_____

V. Family History:

a. Have any of your direct family members had osteoporosis (thin bones) before age 65? Yes _____ No _____ Don't Know _____

If yes, in whom and at what approximate age was it discovered?

_____

_____

_____

_____

b. Have either of your parents or any of your grandparents had hip, spine or forearm fractures before age 70?

Yes _____ No _____ Don't Know _____

If yes, please list family member, bone fractured, approximate age at fracture, and cause of fracture if known (fall, auto accident, etc.).

_____

_____

_____

Appendix C

VI. Exercise History:

a. Please list the three most frequent types of exercise you have engaged in for fitness over the past year along with the average number of hours engaged in per week for each:

(Please list exercise activity type such as running, walking, aerobics, swimming, rowing, biking, or weight lifting, etc. rather than particular sport played such as "baseball", "track", "crew", "lacrosse" etc.)

Exercise Activity	Average Hours Per Week
1) _____	_____
2) _____	_____
3) _____	_____

b. If you run, how many miles per week do you average?

_____

c. Have you ever had to limit your exercise due to a stress fracture? Yes _____ No _____

If yes, list bone in which stress fracture occurred, date, sport being played at the time and duration of limited activity resulting _____

_____

_____

_____

d. Have you had problems with "shin splints"? (persistent pain in the lower leg brought on by exercise) Yes _____ No _____

If yes, please list the date of occurrence, sport being played or activity it was associated with, and the duration of activity limitation resulting:

_____

_____

_____

VII. Dietary History:

a. Are you currently on a "diet" or on any dietary restriction?

Yes _____ No _____

If yes, please list the type of diet and your reason for being on it:_____

_____

_____

_____

_____

b. Have you had a weight change of greater than 10 pounds in the past year? Yes _____ No _____

If yes, please list amount gained or amount lost and briefly list the circumstances surrounding the weight change.

_____ Pounds lost.
_____ Pounds Gained.

Circumstances: _____

_____

_____

_____

c. Have you ever had an eating disorder such as anorexia nervosa or bulemia? Yes _____ No _____

If yes, please list the type of disorder and dates it began and ended._____

_____

_____

d. How many glasses of milk do you usually drink?

_____ Per Day

_____ Per Week

e. How many servings of other dairy products (ice-cream, yogurt, cheese, milk shakes) do you usually consume?

_____ Per Day

_____ Per Week

Appendix D

1 3 ______

8 ___

HEALTH HABITS AND HISTORY QUESTIONNAIRE

This form asks you a variety of questions about your background, environment, and habits, which may affect or be related to your health. The information you provide will help scientists to understand more about the causes of disease.

This questionnaire will take about 40 minutes to complete. Please fill in the information requested, or place a check in the appropriate space. A few questions may be similar to ones you have answered before, but please do not skip any questions for this reason. If you are not sure about an answer, please estimate.

If you have any questions or would like help filling it out, please call ________________ at ______-________. Please return this questionnaire by ______________________. We thank you for your time and your contribution to this research.

THIS SPACE FOR OFFICE USE

TODAY'S DATE: Month [ ] - Day [ ] - Year [ ] (11–16)

Please PRINT YOUR NAME (name of study participant)

[ ] LAST (17) [ ] FIRST (31) [ ] MIDDLE (40)

FEMALES: [ ] MAIDEN (49–63)

In what STATE (or country, if not U.S.) were you born? ______________________ 64 __ State Code

SOCIAL SECURITY NUMBER: [ ] - [ ] - [ ] (66–74)

This information is completely voluntary. It will be used only to refer to statistical records maintained by the National Center for Health Statistics, in order to determine how health practices may be related to how long people live. For studies conducted by the National Institutes of Health, this information is collected under the authority of section 405(b)(1)(A) of the Public Health Service Act, 42 U.S.C. 284(b)(1)(A).

A 79 80

1-10*

ADDRESS: [ ] STREET (11–34)

[ ] CITY (35–49) [ ] STATE [ ] ZIP (52–61) 62 __ State Code

TELEPHONE: ([ ]) - [ ] - [ ] (64–73)

What is your relationship to the person enrolled in the study?

1____Self 2____Spouse 3____ Relative 4____Other

74

B 79 80

Version 02.1, March, 1987. GEN.ALL.YR.2 -1-

THIS PAGE FOR OFFICE USE

PLEASE GO TO NEXT PAGE

Information for coders:

Columns 1–10 are identical on each "card". They are omitted after page 2, but should be repeated on each card.
Col. 80 is blank on each card.
Enter number of the response which was checked (e.g., 1 for male, 2 for female).
For those questions in which a quantity is entered (e.g., years), code as entered.
"9" = Not Stated or Don't know. Leave no blanks. (Blanks are permitted in name and address fields on p. 1, and occupation field on p. 9).
P. 1, Col. 64–65, Col. 62–63: Use state codes shown below.
P. 3, Col. 11–17: Include century of birth: MM DD YYY.
P. 3, Q 10: For each vitamin, code # pills in first two columns; code day, week, etc., in third column (1 = day, 2 = week, 3 = month, 4 = year); code mg/pill in fourth column, using codes shown at bottom of p. 3. If more than one "other vitamin" is checked, code = 8.
P. 5–6: Code as shown on p. 4.
P. 7, Q 14: Code first two columns of each food using codes at bottom of p.7, or additional codes from codebook or database. Code remaining four columns as shown at bottom of p.4.
P. 8, Col. 71–18: No–Yes in 1st column; # times in 2nd col. (8 = 8 or more); age in 3rd–4th col.
P. 8, Col. 46–47, 50–51: Use codes at bottom of p. 8.

State codes:

01 AL Alabama	13 ID Idaho	25 MS Mississippi	37 OK Oklahoma	49 WV West Virginia
02 AK Alaska	14 IL Illinois	26 MO Missouri	38 OR Oregon	50 WI Wisconsin
03 AZ Arizona	15 IN Indiana	27 MT Montana	39 PA Pennsylvania	51 WY Wyoming
04 AR Arkansas	16 IA Iowa	28 NE Nebraska	40 RI Rhode Island	52 PR Puerto Rico
05 CA California	17 KS Kansas	29 NV Nevada	41 SC South Carolina	53 VI Virgin Islands
06 CO Colorado	18 KY Kentucky	30 NH New Hampshire	42 SD South Dakota	54 GU Guam
07 CT Connecticut	19 LA Louisiana	31 NJ New Jersey	43 TN Tennessee	55 Canada
08 DE Delaware	20 ME Maine	32 NM New Mexico	44 TX Texas	56 Cuba
09 DC District of Col.	21 MD Maryland	33 NY New York	45 UT Utah	57 Mexico
10 FL Florida	22 MA Massachusetts	34 NC North Carolina	46 VT Vermont	59 Remainder of World
11 GA Georgia	23 MI Michigan	35 ND North Dakota	47 VA Virginia	99 Unknown or blank
12 HI Hawaii	24 MN Minnesota	36 OH Ohio	48 WA Washington	

Information for proper use of analysis program:

For use with the Personal Computer analysis program, the questionnaire must be keyed in 80-column lines, with the ID field in columns 1–10 of each line, and a line-identifying letter in column 79 of each line, starting with "A" and progressing evenly upward. For use with the mainframe program, the ID and line-ID requirements are less rigid. See Health Habits and History Questionnaire information package for further instructions.

Version 02 of this questionnaire (this version) differs slightly from earlier versions. To use the diet analysis program with this version, you must select the "Nonstandard" option ("STANDQ = N"), and provide the program with the following information, when prompted:

Number of characters = 960

Position of variables:	Card	Col.		Card	Col.		Card	Col.
Name	A	17	Amt. of weight change	J	67	Type of cooking fat	J	54
Age	C	18	First special diet	C	70	Fat on vegetables	J	56
Sex	C	20	Second special diet	C	71	Intake of vitamins	C	43
Height (ft.)	L	43	Whether eats skin	J	47	Intake of multiple vits.	C	44
Height (in.)	L	44	Whether eats fat	J	48	Intake of single vits.	C	53
Weight	L	46	Freq. of cooking fat	J	51	Intake of other vits.	C	69
Weight change	J	68	Unit of cooking fat	J	53	Types of restaurants	C	72

In addition, if you set VEGADJ = Y, tell it J61 when prompted.
In addition, if you set ADDSALT = Y, tell it J49 when prompted.
In addition, if you set COLDCER = Y, tell it J58 when prompted.
In addition, if you set FRTADJ = Y, tell it J64 when prompted.

Number of food fields = 12

Field	Card	Col.	# foods	Field	Card	Col.	# foods	Field	Card	Col.	# foods
1	D	11	15	5	F	11	17	9	G	59	5
2	D	75	1	6	G	11	4	10	H	11	17
3	E	11	12	7	G	31	7	11	I	11	7
4	E	63	4	8	E	59	1	12	I	43	5

All 98 foods included? No
Number not included = 3. Which ones = 6 8 20

Number of extra foods = 3

1 Food: Card D col. 71 Food code: Card I col. 67
2 Food: Card G col. 27 Food code: Card I col. 69
3 Food: Card I col. 39 Food code: Card I col. 71

Number of open-ended foods = 6 Open-ended information starts in Card J col. 11.

If you modify this questionnaire, you must change the above variables to correspond with your revised version.

PERSONAL INFORMATION, HABITS

1. When were you born? ____/____/____ (Month Day Year)

2. How old are you? ____ years

3. Sex: 1 ___ Male 2 ___ Female

4. Race or ethnic background:
   1 ___ White, not of Hispanic origin
   2 ___ Black, not of Hispanic origin
   3 ___ Hispanic
   4 ___ American Indian/Alaskan native
   5 ___ Asian
   6 ___ Pacific Islander

5. Please circle the highest grade in school you have completed:
   1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16+

6. What is your marital status? 1 ___ Single 2 ___ Married 3 ___ Widowed 4 ___ Divorced/Separated

7. How many times have you moved or changed residences in the last ten years? ___ times

8. Have you smoked at least 100 cigarettes in your entire life? 1 ___ No 2 ___ Yes If Yes,

   IF YES: About how old were you when you first started smoking cigarettes fairly regularly?
   ____ years old
   On the average of the entire time you smoked, how many cigarettes did you smoke per day?
   ____ cigarettes per day
   Do you smoke cigarettes now? 1 ___ No 2 ___ Yes
   IF NO: How old were you when you stopped smoking? ____ years old
   IF YES: On the average, about how many cigarettes a day do you smoke now? ____ cigarettes

9. Have you ever smoked a pipe or cigars regularly? 1 ___ No 2 ___ Yes If Yes,

   IF YES: For how many years? ____ years
   About how much? ____ pipes or cigars per _____ (day or week)

10. During the past year, have you taken any vitamins or minerals?
    1 ___ No 2 ___ Yes, fairly regularly 3 ___ Yes, but not regularly If Yes,

What do you take fairly regularly?	# of PILLS per DAY, WEEK, etc.	How many milligrams or IUs per pill?
Multiple Vitamins		
One-a-day type	____ pills per _____	
Stress-tabs type	____ pills per _____	
Therapeutic, Theragran type	____ pills per _____	
Other Vitamins		
Vitamin A	____ pills per _____	____ IU per pill
Vitamin C	____ pills per _____	____ mg per pill
Vitamin E	____ pills per _____	____ IU per pill
Calcium or dolomite	____ pills per _____	____ mg per pill

Other (What?) 1 ___ Yeast 2 ___ Selenium 3 ___ Zinc 4 ___ Iron 5 ___ Beta-carotene
6 ___ Cod liver oil 7 ___ Other _____

Please list the brand of multiple vitamin/mineral you usually take: _____

1 3 8 11 18 20 21 22 24 25 27 28 30 32 33 35 37 38 40 43 44 47 50 53 57 61 65 69

FOR OFFICE USE
Q10, mg or IU: 1=50-100 2=200-250 3=400-500 4=1000 5=5000 6=10,000 7=20,000-25,000 8=50,000 9=Unk.

11. Are you on a special diet?
1 ___ No 2 ___ Weight loss 3 ___ For medical condition 4 ___ Vegetarian 5 ___ Low salt
6 ___ Low cholesterol 7 ___ Weight gain

OFFICE USE
70 ___

12. How often do you eat the following foods from *restaurants* or *fast food places?*

RESTAURANT FOOD	1 Almost every day	2 2-4 times a week	3 Once a week	4 1-3 times a month	5 5-10 times a year	6 1-4 times a year	7 Never, or less than once a year	
Fried chicken								72 ___
Burgers								73 ___
Pizza								74 ___
Chinese food								75 ___
Mexican food								76 ___
Fried fish								77 ___
Other foods								78 ___

13. This section is about your *usual* eating habits. Thinking back over the past year, how often do you usually eat the foods listed on the next page?

C ___
79 80

First, check (✓) whether your usual serving size is small, medium or large. (A small portion is about one-half the medium serving size shown, or less; a large portion is about one-and-a-half times as much, or more.)

Then, put a *NUMBER* in the most appropriate column to indicate *HOW OFTEN*, on the average, you eat the food. You may eat bananas *twice a week* (put a 2 *in the "week"* column). If you never eat the food, check "Rarely/Never." Please DO NOT SKIP foods. And please BE CAREFUL which column you put your answer in. It will make a big difference if you say "Hamburger once a day" when you mean "Hamburger once a week"!

Some items say "in season." Indicate how often you eat these just in the 2-3 month time when that food is in season. (Be careful about overestimating here.)

Please look at the *example* below. This person

1) eats a medium serving of cantaloupe once a week, in season.
2) has ½ grapefruit about twice a month.
3) has a small serving of sweet potatoes about 3 times a year.
4) has a large hamburger or cheeseburger or meat loaf about four times a week.
5) never eats winter squash.

EXAMPLE:

	Medium Serving	Your Serving Size S	M	L	How often? Day	Week	Month	Year	Rarely/ Never
Cantaloupe (in season)	¼ medium		✓			1			
Grapefruit	(½)		✓				2		
Sweet potatoes, yams	½ cup	✓						3	
Hamburger, cheeseburger, meat loaf	1 medium					4			
Winter squash, baked squash	½ cup								✓

PLEASE GO TO NEXT PAGE

FOR OFFICE USE

On the following two pages, code the four characters for each food as follows:

___ S-1 M-2 L-3 NS-9

___ ___ No. Times NS-99

___ Da-1 Wk-2 Mo-3 Yr-4 Nev-5 NS-9

If respondent places a checkmark in the "How often" columns, **do not** impute "01", once. Instead, code "99", Not Stated. If respondent does not check a portion size, **do not** impute medium, but code "9".

	Medium Serving	Your Serving Size	How often?					OFFICE USE
			Day	Week	Month	Year	Rarely/ Never	
FRUITS & JUICES		S M L						
EXAMPLE – Apples, applesauce, pears	(1) or ½ cup	✓		4				
Apples, applesauce, pears	(1) or ½ cup							11
Bananas	1 medium							15
Peaches, apricots (canned, frozen or dried, whole year)	(1) or ½ cup							19
Peaches, apricots, nectarines (fresh, in season)	1 medium							23
Cantaloupe (in season)	¼ medium							27
Watermelon (in season)	1 slice							31
Strawberries (fresh, in season)	½ cup							35
Oranges	1 medium							39
Orange juice or grapefruit juice	6 oz. glass				1			43
Grapefruit	(½)							47
Tang, Start breakfast drinks	6 oz. glass							51
Other fruit juices, fortified fruit drinks	6 oz. glass							55
Any other fruit, including berries, fruit cocktail	½ cup							59
VEGETABLES		S M L	Da	Wk	Mo	Yr	Nv	
String beans, green beans	½ cup							63
Peas	½ cup							67
Chili with beans	¾ cup							71
Other beans such as baked beans, pintos, kidney beans, limas	¾ cup							75
Corn	½ cup							11
Winter squash, baked squash	½ cup							15
Tomatoes, tomato juice	(1) or 6 oz.							19
Red chili sauce, taco sauce, salsa picante	2 Tblsp. sauce							23
Broccoli	½ cup							27
Cauliflower or brussel sprouts	½ cup							31
Spinach (raw)	¾ cup							35
Spinach (cooked)	½ cup							39
Mustard greens, turnip greens, collards	½ cup							43
Cole slaw, cabbage, sauerkraut	½ cup							47
Carrots, or mixed vegetables containing carrots	½ cup							51
Green salad	1 med. bowl							55
Salad dressing, mayonnaise (including on sandwiches)	2 Tblsp.							59
French fries and fried potatoes	¾ cup							63
Sweet potatoes, yams	½ cup							67
Other potatoes, including boiled, baked, potato salad	(1) or ½ cup							71
Rice	¾ cup							75
Any other vegetable, including cooked onions, summer squash	½ cup							11
Butter, margarine or other fat on vegetables, potatoes, etc.	2 pats							15
MEAT, FISH, POULTRY & MIXED DISHES		S M L	Da	Wk	Mo	Yr	Nv	
Hamburgers, cheeseburgers, meat loaf	1 medium							19
Beef—steaks, roasts	4 oz.							23
Beef stew or pot pie with carrots, other vegetables	1 cup							27
Liver, including chicken livers	4 oz.							31
Pork, including chops, roasts	2 chops or 4 oz.							35
Fried chicken	2 sm. or 1 lg. piece							39
Chicken or turkey, roasted, stewed or broiled	2 sm. or 1 lg. piece							43
Fried fish or fish sandwich	4 oz. or 1 sand.							47
Tuna fish, tuna salad, tuna casserole	½ cup							51
Shell fish (shrimp, lobster, crab, oysters, etc.)	(5) ¼ cup or 3 oz.							55
Other fish, broiled, baked	4 oz.							59
Spaghetti, lasagna, other pasta with tomato sauce	1 cup							63
Pizza	2 slices							67
Mixed dishes with cheese (such as macaroni and cheese)	1 cup							71

	Medium Serving	Your Serving Size S	M	L	How often? Day	Week	Month	Year	Rarely/ Never	OFFICE USE
LUNCH ITEMS										
Liverwurst	2 slices									75 ____ F 79 80
Hot dogs	2 dogs									11 ____
Ham, lunch meats	2 slices									15 ____
Vegetable soup, vegetable beef, minestrone, tomato soup	1 med. bowl									19 ____
Other soups	1 med. bowl									23 ____
BREADS / SALTY SNACKS / SPREADS		S	M	L	Da	Wk	Mo	Yr	Nv	
Biscuits, muffins, burger rolls (incl. fast foods)	1 med. piece									27 ____
White bread (including sandwiches), bagels, etc., crackers	2 slices, 3 cracks									31 ____
Dark bread, including whole wheat, rye, pumpernickel	2 slices									35 ____
Corn bread, corn muffins, corn tortillas	1 med. piece									39 ____
Salty snacks (such as chips, popcorn)	2 handfuls									43 ____
Peanuts, peanut butter	2 Tblsp.									47 ____
Butter on bread or rolls	2 pats									51 ____
Margarine on bread or rolls	2 pats									55 ____
Gravies made with meat drippings, or white sauce	2 Tblsp.									59 ____
BREAKFAST FOODS		S	M	L	Da	Wk	Mo	Yr	Nv	
High fiber, bran or granola cereals, shredded wheat	1 med. bowl									63 ____
Highly fortified cereals, such as Product 19, Total, or Most	1 med. bowl									67 ____
Other cold cereals, such as Corn Flakes, Rice Krispies	1 med. bowl									71 ____
Cooked cereals	1 med. bowl									75 ____ G 79 80
Sugar added to cereal	2 teaspn.									11 ____
Eggs	1 egg = small, 2 eggs = medium									15 ____
Bacon	2 slices									19 ____
Sausage	2 patties or links									23 ____
SWEETS		S	M	L	Da	Wk	Mo	Yr	Nv	
Ice cream	1 scoop									27 ____
Doughnuts, cookies, cakes, pastry	1 pc. or 3 cookies									31 ____
Pumpkin pie, sweet potato pie	1 med. slice									35 ____
Other pies	1 med. slice									39 ____
Chocolate candy	small bar, 1 oz.									43 ____
Other candy, jelly, honey, brown sugar	3 pc. or 1 Tblsp.									47 ____
DAIRY PRODUCTS		S	M	L	Da	Wk	Mo	Yr	Nv	
Cottage cheese	½ cup									51 ____
Other cheeses and cheese spreads	2 slices or 2 oz.									55 ____
Flavored yogurt	1 cup									59 ____
Whole milk and bevs. with whole milk (not incl. on cereal)	8 oz. glass									63 ____
2% milk and bevs. with 2% milk (not incl. on cereal)	8 oz. glass									67 ____
Skim milk, 1% milk or buttermilk (not incl. on cereal)	8 oz. glass									71 ____
BEVERAGES		S	M	L	Da	Wk	Mo	Yr	Nv	
Regular soft drinks	12 oz. can or bottle									75 ____ H 79 80
Diet soft drinks	12 oz. can or bottle									11 ____
Beer	12 oz. can or bottle									15 ____
Wine	1 med. glass									19 ____
Liquor	1 shot									23 ____
Decaffeinated coffee	1 med. cup									27 ____
Coffee, not decaffeinated	1 med. cup									31 ____
Tea (hot or iced)	1 med. cup									35 ____
Lemon in tea	1 teaspn.									39 ____
Non-diary creamer in coffee or tea	1 Tblsp.									43 ____
Milk in coffee or tea	1 Tblsp.									47 ____
Cream (real) or Half-and-Half in coffee or tea	1 Tblsp.									51 ____
Sugar in coffee or tea	2 teaspn.									55 ____
Artifical sweetener in coffee or tea	1 packet									59 ____
Glasses of water, not counting in coffee or tea	8 oz. glass									63 ____
										67 0 2 2 0 6 9 . . . I 79 80

14. Think about your diet over the last year and the responses you have just made on this questionnaire. Are there any foods not mentioned which you ate *at least once a week*, even in small quantities, or ate frequently in a particular season? Consider other meats, breakfast foods, catsup, green chilies or jalapenos, avocado (guacamole), Mexican dishes, Chinese or other ethnic foods, other fruits or vegetables, as well as nutritional supplements (bran, etc.). *Please take a look at the list of foods at the bottom of the page.*

FOOD	Your Serving Size S | M | L	How Often? Day	Week	OFFICE USE Code	Amounts
				11	
				17	
				23	
				29	
				35	
				41	

	1 Seldom/Never	2 Sometimes	3 Often/Always		
15. How often do you eat the skin on chicken?				47	—
How often do you eat the fat on meat?				48	—
How often do you add salt to your food?				49	—
How often do you add pepper to your food?				50	—

16. How often do you use fat or oil in cooking?
For example, in frying eggs, meat or vegetables? _____ times per _____ (day, week, month) — 51 ———

17. What do you *usually* cook with? 1 ___ Don't know or don't cook 2 ___ Soft margarine
3 ___ Stick margarine 4 ___ Butter 5 ___ Oil 6 ___ Lard, fatback, bacon fat
7 ___ Pam or no oil — 54 ——

18. What kind of fat do you *usually* add to vegetables, potatoes, etc?
1 ___ Don't add fat 2 ___ Soft margarine 3 ___ Stick margarine 4 ___ Butter
5 ___ Half butter, half margarine 6 ___ Lard, fatback, bacon fat — 56 ——

19. If you eat cold cereal, what kind do you eat most often? _____ — 58 ———

20. Not counting salad or potatoes, about how many vegetables do you eat per day or per week? _____ (vegetables) per _____ (day, week) — 61 ———

21. Not counting juices, how many fruits do you usually eat per day or per week? _____ (fruits) per _____ (day, week) — 64 ———

22. Have you gained or lost more than five pounds in the past year? (You may check more than one answer.)
1 ___ No 2 ___ Lost 5-15 lbs. 3 ___ Lost 16-25 lbs. 4 ___ Lost more than 25 lbs.
5 ___ Gained 5-15 lbs. 6 ___ Gained 16-25 lbs. 7 ___ Gained more than 25 lbs. — 67 —

68 9 9

DO YOU EAT THESE ONCE A WEEK?

veal, lamb	01	pancakes, waffles	21	onions	41	Hi-C	63
tofu	03	instant breakfast, metrecal	22	summer squash	42	cranberry juice cocktail	64
mixed dish w/meat	04	pudding	23	asparagus	43	grapes	65
mixed dish w/chicken	05	milkshake	24	sweet green peppers	44	mangoes	66
Chinese dishes	06	other diary product	25	sweet red peppers	45	papayas	67
Mexican dishes	07	other dessert, sweet	26	bean sprouts	46	honeydew or cassaba melon	68
seafood creole	08	sour cream, dips	31	avocado, guacamole	47	lemons or lemon juice	69
refried beans or bean burritos	09	diet salad dressing	32	beets	48	nuts and seeds	70
Polish or Italian sausage	10	catsup	33	pineapple or pineapple juice	61	bran	71
cream soups	11	green chilies, jalapenos	34	prunes or prune juice	62	other vegetable/fruit	79
noodles	12					other not mentioned here	88

MEDICAL INFORMATION

OFFICE USE

23. In the past five years, how many times have you been hospitalized? (if female, omit childbirths) ___ times — 70 —

24. Have you ever had any of the following tests or treatments?

	1 NO	2 YES	IF YES, HOW MANY TIMES?	IF YES, AGE AT FIRST TREATMENT	OFFICE USE
X-ray treatments for acne, ringworm, enlarged tonsils, adenoids, thymus					71
Treatment with radium, cobalt, or other radioactive isotopes					75 / 79 80
Upper GI series (x-ray of stomach after drinking white liquid)					11
Lower GI series (Barium enema)					15

25. Have you ever been told by a doctor that you had any of the following conditions?

	1 NO	2 YES	DON'T KNOW	OFFICE USE
Heart disease or angina				19
Heart attack				21
High blood pressure				23
Stroke				25
Tuberculosis				27
Chronic bronchitis or emphysema				29
Asthma				31
Hay fever				33
Diverticulosis				35
Rectal/colon polyps				37
Chronic colitis				39
Diabetes				41
Thyroid condition				43
Kidney disease				45
Bladder disease				
Liver cirrhosis				
Hepatitis				
Stomach ulcers				
Rheumatoid arthritis				
Other arthritis				
Osteoporosis				
Fractured hip				
Prostate trouble				
Abnormal Pap smear				
Skin cancer				
Leukemia				
Other cancer				

If yes to leukemia, skin, or other cancer, fill in below:

What kind of cancer? (Lung, breast, etc.) _____ Year 1st Diagnosed _____ — 46 —

What kind of cancer? (If you had a second) _____ Year 1st Diagnosed _____ — 50 _____ (See codes below)

26. In the past year, have you had

	1 NO	2 YES	OFFICE USE
Bleeding or sore gums			54
Bruise easily			56
Nosebleeds			58
Difficulty seeing in the dark			
Frequent or chronic fever			
Frequent constipation or hemorrhoids			

PLEASE GO TO NEXT PAGE

FOR OFFICE USE

Ca ___ Yr ___

01—Bladder
02—Bone
03—Brain
04—Breast
05—Cervix
06—Colon
07—Esophagus
08—Kidney
09—Liver
10—Leukemia
11—Lung, bronchus
12—Lymphoma, including Hodgkins
13—Mouth, oral
14—Ovary
15—Pancreas
16—Prostate
17—Rectum
18—Skin—Melanoma
19—Skin—Not melanoma (Basal or squamous)
20—Skin—Not specified
21—Stomach
22—Thyroid
23—Uterus
24—Other

OCCUPATIONAL INFORMATION

OFFICE USE

27. What is your current employment status? Check the one that applies to the greatest percent of your time.

1 ___ Employed
2 ___ Homemaker
3 ___ Retired
4 ___ Disabled, unable to work
5 ___ Unemployed
6 ___ Student
7 ___ Other

60 ___

28. What has been your usual occupation or job — the one you have worked at the longest? (For example, carpenter, executive, salesman, foreman, waitress, truck driver)

Job/occupation _____ 61 _____

Years in this job _____ 70 ___

In your work, did you spend more time 1 ___ indoors 2 ___ outdoors? (Please check one.) 72 ___

29. In your work, have you ever been exposed *for a year or more* to any of the following?

	1 NO	2 YES	DON'T KNOW
Asbestos			
Radiation			
Welding			
Coal tar, soot, pitch, creosote, asphalt			
Mineral, cutting or lubricating oil			
Benzidine, beta-naphthylamine			
Benzene			
Isopropyl oil			
Dyestuffs			
Arsenic			

	1 NO	2 YES	DON'T KNOW	
Iron foundry				73 ___
Nickel smelting				75 ___
Underground mining				77 ___ K 79 80
Lumber industry, or heavy wood dust				11 ___
Rubber or cablemaking industry				13 ___
Chemical or plastics industry				15 ___
Pesticides, herbicides				17 ___
Mustard gas				19 ___
Chromium				21 ___
Cadmium, beryllium, vinyl chloride				23 ___

FAMILY HISTORY

30. Have any close relatives had cancer? 1 ___ No 2 ___ Yes 25 ___

IF YES, please fill this out for each blood relative who had cancer. Include your natural parents, sisters and brothers, daughters and sons, grandparents.

One RELATIVE per line (Mother, son, etc.)	Circle one 1	2	If Alive, give age	If Dead, give age at death	Type of Cancer	Age at Diagnosis
	Alive	Dead				
	Alive	Dead				
	Alive	Dead				
	Alive	Dead				
	Alive	Dead				

26 See below

PLEASE GO TO NEXT PAGE

FOR OFFICE USE

M-1 Sn-5 Dis. codes:
F-2 Dt-6 See p. 8
B-3 GF-7
S-4 GM-8

No. Rel.	Rel.	Al Dd.	Age	Dis.	Age
26	—	—	— —	— —	— —
35	—	— —	— —	— —	

OTHER HEALTH FACTORS

OFFICE USE

31. How tall are you? ____ feet ____ inches 32. How much do you weigh? _____ pounds — — — — — — (43)

33. What is the most you have ever weighed? _____ pounds (49) — — —

34. About how many times have you gone on a diet to lose weight?

(1) ___Never (2) ___1-2 (3) ___3-5 (4) ___6-8 (5) ___9-11 (6) ___12 or more times (52) —

35. How many hours of sleep do you usually get at night?

(1) ___6 hours or less (2) ___7 hours (3) ___8 hours (4) ___9 hours or more (53) —

36. How often do you feel under stress which makes you tense or worried, or causes physical problems such as stomach or back trouble or headaches?

(1) ___Every day (2) ___Several times a week (3) ___Several times a month (4) ___Several times a year (5) ___Rarely or never (54) —

37. Here is a list of active things that people do in their free time. How often do you do any of these things?

	1 MORE THAN ONCE A WEEK	2 ABOUT ONCE A WEEK	3 A FEW TIMES A MONTH	4 A FEW TIMES A YEAR	5 RARELY OR NEVER		
Active sports	_____	_____	_____	_____	_____	(55)	—
Doing physical exercises	_____	_____	_____	_____	_____	(56)	—
Jogging or running	_____	_____	_____	_____	_____	(57)	—
Swimming or taking long walks	_____	_____	_____	_____	_____	(58)	—
Gardening, fishing, hunting	_____	_____	_____	_____	_____	(59)	—
Something else_____	_____	_____	_____	_____	_____	(60)	—

38. How many close friends do you have? (People that you feel at ease with, can talk to about private matters, and can call on for help.)

(1) ___None (2) ___1 or 2 (3) ___3 to 5 (4) ___6 to 9 (5) ___10 or more (61) —

How many relatives do you have that you feel close to?

___None ___1 or 2 ___3 to 5 ___6 to 9 ___10 or more (62) —

How many of these friends or relatives do you see or talk to at least *once a month*?

___None ___1 or 2 ___3 to 5 ___6 to 9 ___10 or more (63) —

39. How often do you participate in the following groups or activities?

	1 MORE THAN ONCE A WEEK	2 ABOUT ONCE A WEEK	3 A FEW TIMES A MONTH	4 A FEW TIMES A YEAR	5 RARELY OR NEVER		
Go to church or temple	_____	_____	_____	_____	_____	(64)	—
Participate in group meetings or activities (such as clubs, PTA, professional, labor or service groups)	_____	_____	_____	_____	_____	(65)	—

Please take a moment to fill in any questions you may have skipped.

Version # (75) 0 (76) 2

THANK YOU VERY MUCH for taking the time to fill out this information. The answers you have given will be very useful in interpreting the results of this study, and in helping to understand and control disease. Your participation is sincerely appreciated.

Coder: (77) — (78) —

(79) L (80) —

Reviewed by _____ ___

Appendix E

INTERVAL MEDICAL and EXERCISE QUESTIONNAIRE

Study ID_____ Date_____

Weight _____

Height _____

I. Orthopedic History:

Have you sustained a stress fracture while at the Academy?

YES NO

Please list, with dates, any orthopedic injuries you have sustained at the Academy:

Have you been assigned to "light-duty" or "no-duty" because of an orthopedic injury?

YES NO

How many days in light-duty or no-duty?_____ Date: _____

Do you exercise by voluntarily running or jogging?

YES NO

If yes, how many miles per week do you run or jog?_____

How may hours per week do you spend running or jogging? _____

How many minutes per mile do you run when exercising? _____

Other than running, what exercise activities do you regularly perform?

Exercise Activity	Hours per week	Date of Last PFT:
_____		
1._____	_____	1.5 mile run time:
_____		
2._____	_____	# Push-ups:
_____		
3._____	_____	# Sit-ups: _____

Have your ever run a Marathon? YES NO

If yes, what was your best time?_____

Are you on a sports team?

YES NO

List the sport:	Jr. Varsity	Varsity	Other
1._____	_____	_____	_____
2._____	_____	_____	_____
3._____	_____	_____	_____

Which wing and deck did/do you live on:		WING	DECK
	Plebe Summer	_____	_____
	Plebe Year	_____	_____
	Present Time	_____	_____

Has your weight changed by more than 10 pounds while at the academy?

YES NO

Net Pounds Gained_____ Lost_____

Have you been placed on a restricted diet while at the Academy?

YES NO

Do you drink milk? YES NO

If yes, how many 8 ounces servings of milk do you drink?_____ DAY
WEEK

How many servings of other dairy products do you consume?_____ DAY
WEEK

Have you taken any nutritional supplements while at the Academy?

YES NO

If yes, please list the type and amount taken daily:

Have you taken any drugs prescribed by a medical doctor while at the Academy?

YES NO

If yes, please list the drug, dose and dates taken:

Thank you, we appreciate your assistance with this study.

Tear Off

Study questions?

Principal Investigator:
LCDR Al Drake 301-295-5165 (Work) 0830-1630

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Chapter VIII.

GLOSSARY

Analysis of Covariance-a form of analysis of variance that tests the significance of the different effects of sample groups on the dependent variable after controlling for differences between the group means for another independent variable--the covariate (112).

Analysis of Variance-a test to determine whether two or more sample means have been obtained from populations with the same parametric mean (113).

Bone Mineral Content-a quantitative assessment of bone mineral expressed in grams per centimeter (113).

Bone Mineral Density-a quantitative assessment of bone mineral expressed in grams per square centimeter; sometimes referred to as areal density (114).

Body Mass Index-a ratio of height to weight calculated as Weight (kg) / Height (m^2) (114).

Dual Energy X-Ray Absorptiometry-a precise method of bone densitometry that uses x-rays to estimate bone mineral content and bone mineral density (114).

Dual Photon Absorptiometry-a method of bone densitometry that uses ^{153}Gd to estimate bone mineral in the spine, femur and total body (114).

Eating Disorders Inventory-EDI is a well established tool for screening large groups of individuals for indications of disordered eating (99).

Food Frequency Questionnaire-a dietary assessment method in which the questions relate to how frequently a given list of foods are consumed (115).

Health, Habits and History Questionnaire-a health assessment tool developed by the National Cancer Institute which includes a food frequency questionnaire (71).

Linear Regression Analysis-an estimate of the relationship of one variable with another by expressing the one in terms of a linear function of the other (113).

Midshipmen-a male or female student and Naval officer candidate at the United States Naval Academy

Pearson's Correlation-a measurement of the amount of association between two variables. (113)

Recommended Dietary Allowance-the levels of intake of essential nutrients considered, in the judgment of the Committee on Dietary Allowances of the Food and Nutrition Board on the basis of available scientific knowledge, to be adequate to meet the known nutritional needs of practically all healthy persons. (27)

Total Body Bone Mineral Content-a quantitative assessment of the mineral content of the whole body expressed in grams, and measured by a bone densitometer.

T-Tests-a method of hypothesis testing using the t distribution to determine if two samples are from the same parametric mean.

Usual Intake-ones habitual dietary intake of a given food or nutrient

Chapter IX.

REFERENCES

1. Melton LJ. How Many Women Have Osteoporosis now? J Bone Miner Res 10(2) 175-177 1995.

2. Ray N, Chan J, Thamer M, Melton J. Medical expenditures for the treatment of osteoporotic fractures in the United States in 1995: report from the National Osteoporosis Foundation. J Bone Miner Res. 12:24-35, 1997.

3. Melton LJ, III. Hip fractures: a worldwide problem today and tomorrow. Bone 14: S1-8 1993.

4. Maggi S, Kelsey JL, Litvak J, Heyse SP. Incidence of hip fractures n the elderly: a cross-national analysis. Osteoporosis Int 1:232-41 1991.

5. Praemer A, Turner S, Pice DP. Costs of musculokeletal conditions in the United States. Park Ridge: American Academy of Orthopaedic surgeons. 143-70 1993.

6. Cooper C, Campion G, Melton LJ, III. Hip fractures in the elderly: a world-wide projection. Osteoporosis Int 2: 285-9 1992.

7. Ostlere SJ, Gold RH. Osteoporosis and Bone Density Measurement Methods. Clin Orthop 271(10) 149-163 1991.

8. Riggs BL, Melton LJ. Evidence for two distinct syndromes of involutional osteoporosis. Am J Med. 75:899-907 1983.

9. Kanis JA, Melton LJ III, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 9:1137-1141. 1994.

10. Raisz LG, Johannesson A. Pathogenesis, prevention and therapy of osteoporosis. J Med 15:267-274. 1984.

11. Kroger H, Kotaniemi A, Kroger L, Alhava E Development of bone mass and bone density of the spine and femoral neck--a prospective study of 65 children and adolescents. Bone Miner 23(3)171-82. 1993.

12. Slosman D, Rizzoli R, Pichard C, Donath A, Bonjour JP. Longitudinal measurements of regional and whole body bone mass in young healthy adults. Osteoporosis Int 4(4): 185-190 1994.

13. Rico H, Revilla M, Gonzales-Riola J, Villa LF, Alvarez de Buergo M. Bone mineral content and anthropometric variables in men a cross sectional study of 324 normal subjects. Clin Rheumatol 12(4) 485-9 1993.

14. Pumarino H, Lillo R, Oviedo S, Gonzalez D. Bone mineral content and density by dual photon absorptiometry in healthy male subjects. Abstract Rev Med Chil 121(9) p. 1006. 1993.

15. Ott SM, Attainment of Peak Bone Mass. J Clin Endocrinol Metab 71(5):1082-1087 1990.

16. Recker RR, Davies M, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. J Am Med Assoc. 268(17):2403-2408. 1992.

17. Maggio D, Pacifici R, Cherubini A, Aisa MC, Santucci C Appendicular cortical bone loss after age 65: sex dependent event? Calcif Tissue Int 56(5): 410-4 1995.

18. Heaney RP, Matkovic V. Inadequate peak bone mass. In: Riggs BL, Melton LS, eds. Osteoporosis. Etiology, diagnosis, and management, 2nd ed. New York: Raven Press. 1994.

19. Krall EA, Dawson-Hughes B. Osteoporosis Chapter 89 p1565-6. In: Shils ME, Olson J, Shike M. eds. Modern Nutrition in Health and Disease. 8th ed. Philadelphia, PA: Lea & Fabiger, 1994.

20. Evans RA, Marel GM, Lancester EK, Kos S, Evans M, Stanlyey YP. Bone mass is low in relatives of osteoporotic patients. Ann Intern Med 109:870-873. 1988.

21. Pollitzer W, Andersen J Ethnic and genetic differences in bone mass: a review with a hereditary vs. environmental perspective. Am J Clin Nutr 50: 1244-1259. 1989.

22. Pocock N, Eisman J, Hopper J, Yeates M, Sambrook P Ebert S. Genetics determinants of bone mass in adults. J Clin Invest 80:706-710. 1987.

23. Wardlaw G, The effects of diet and life-style on bone mass in women. J Am Diet Assoc 88:17-22. 1988.

24. Wardlaw G, Putting osteoporosis in perspective. J Am Diet Assoc 93(9):1000-1006. 1993.

25. Jelic T, Wardlaw GM, Ilich JZ, et al. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. J Bone Mine Res 7S:187-192. 1992.

26. Matkovic V, Kostial K, Simonovic I, Buzina R, Brodarec A, Nordin BEC. Bone status and fracture rates in two regions of Yugoslavia. Am J Clin Nutr 32:540-9. 1979.

27. Recommended Dietary Allowances, 10th edition. 1989 National Academy of Ssciences. National Academy of Sciences Press, Washington, DC.

28. Johnson RK, Johnson DG, Wang MQ, Smickiklas-Wright H, Guthrie HA. Characterizing nutrient intakes of adolescents by sociodemographic factors. J Adolesc Health 15(2): 149-54. 1994.

29. Matkovic V, Ilich JZ. Calcium requirements for growth: Are current recommendations adequate? Nutr Rev 51(6) 171-180. 1993.

30. Forbes RM, Weignartner KE, Parker HM, Bell RM, Erdman JW Jr. Bioavailability to rats of zinc, magnesium, and calcium in casein, egg and soy protein containing diets. J Nutr 1; 109: 1652-60, 1979

31. Matkovic V, Heaney RP. Calcium balance during human growth: evidence for the threshold behavior. Am J Clin Nutr 55: 992-996, 1992.

32. Mertz W. Use and misuse of balance studies. J Nutr 117: 1811-1813. 1987.

33. Optimal Calcium Intake. NIH Consensus Statement. June 6-8 1994. 12(4):1-31.

34. Alaimo K, McDowell MA, Briefel RR, Bischof AM, Caughman RC, Loria CM, Johnson, CL. Dietary Intake of Vitamins, Minerals and Fiber of Persons Ages 2 Months and Over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988-91. Advance Data from the National Center for Health Statistics Number 258, Nov 1994.

35. Fleming KH, Heimbach JT Consumption of calcium in the U. S: food sources and intake levels. J Nutr 124 (8 Suppl): 1426S-1430S 1994.

36. Clarkson PM, Haymes EM. Exercise and mineral status of athletes: calcium, magnesium, phosphorus and iron. Med Sci Sports Exerc 27(6): 831-842. 1995.

37. King N, Fridlund KE, Askew EW. Nutrition issues of military women J Am Coll Nutr 12(4): 344-8 1993.

38. Nieves JW, Golden AL, Siris E, Kelsey JL, Lindsay R. Teenage and current calcium intake are related to bone mineral density of the hip and forearm in women aged 30-39 years. Am J Epidemiol 141(4):342-351. 1995.

39. Metz JA, Anderson J, Gallagher PN. Intakes of calcium, phosphorus and protein, and physical activity level are related to radial bone mass in young adult women. Am J Clin Nutr. 58:537-42 1993.

40. Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, Peacock M. Calcium supplementation and increases in bone mineral density in children. N Engl J Med 327(2):82-7. 1992.

41. Lloyd T, Andon MB, Rollings N, Martel JK, Landis JR, Demers LM, Eggli DF, Kieselhorst K, Kulin HE. Calcium supplementation and bone mineral density in adolescent girls J Am Med Assoc. 270(7):841-4. 1993.

42. Valimaki MJ, Karkkainen M, Lamberg-Allardt C, Laitinen K, Alhava E, Heikkinen J, Impovaara O, Makela P, Palmgren J, Seppanen R, et al. Exercise, smoking and calcium intake during adolescence and early adulthood as determinants of peak bone mass. Cardiovascular Risk in Young Finns Study Group. BMJ 309(7) 230-5 1994.

43. Welten DC, Kemper HC, Post GB, Van Staveren WA, A meta-analysis of the effect of calcium intake on bone mass in young and middle aged females and males. J Nutr 125(11): 2802-13 1995.

44. Welten DC, Kemper HC Post GB, Van Mechelen W, Twisk J, Lips P Teule GJ. Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. J Bone miner Res 9:1089-96. 1994.

45. Heaney RP, Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. J Bone Min Res 10(1) 172. 1995.

46. Kanders B, Dempster D, Lindsay R. Interaction of calcium nutrition and physical activity on bone mass in young women. J Bone Miner Res. 3:145-149. 1988.

47. Kanis JA. Calcium requirements for optimal skeletal health in women. Calcif Tissue Int 49:S33-S41. 1991.

48. Holbrook TL, Barrett-Connor E. An 18-year prospective study of dietary calcium and bone mineral density in the hip. Calcif Tissue Int 56:364-367. 1995.

49. Harris A, Martin BJ, Exercise Physiology, Chapter 32 in: Tanner GA, Rhoades RA eds. Medical Physiology Little, Brown and Co. New York, NY p 624 1995.

50. Krolner B, Toft B. Vertebral bone loss: an unheaded side effect of therapeutic bed rest. Clin Sci 64:537-540. 1983.

51. Nishimura Y, Fukuoka H, Kiriyama M, Suzuki Y, Oyama K, Ikawa S, Higurashi M, Gunji A. Bone turnover and calcium metabolism during 20 days bed rest in young healthy males and females. Acta Physiol Scand Suppl 616: 27-35 1994.

52. Lane HW, Smith SM, Rice BL, Bourlad CT. Nutrition in space: lessons from the past applied to the future. Am J Clin Nutr 60:801S-5S. 1994.

53. Toss G, Effect of calcium intake vs other life-style factors on bone mass J Int Med 231:181-6 1992.

54. Casez JP, Stadler H, Stuessi E, Gerber A Delmas PD, Jaeger P. Effects of physical exercise on bone mineral density and serum osteocalcin in military recruits. In: Christiansen C et al. eds. Osteoporosis. 1459-1460. 1990.

55. Kirchner EM, Lewis RD, O'Connor PJ Bone mineral density and dietary intake of female college gymnasts. Med Sci Sports Exerc 27(4): 543-549. 1995.

56. Myburgh KH, Hutchins J, Fataar AB, Hough SF, Noakes TD. Low bone density is an etiologic factor for stress fractures in athletes. Ann Intern Med 113(10): 754-9. 1990.

57. Robinson TL, Snow-Harter C, Taaffe DR, Gillis D, Shaw J, Marcus R. Gymnasts exhibit higher bone mass than runners despite similar prevalence of amenorrhea and oligomenorrhea. J Bone Min Res 10(1) 26-35 1995.

58. Hamdy RC, Anderson JS, Whalen KE, Harvill LM. Regional differences in bone density of young men involved in different exercises. Med Sci Sports Exerc 26(7) 884-8 1994.

59. Lloyd T, Buchanan JR, Waldman CJ, Myers C, Ford BG. Interrelationships of diet, athletic activity, menstrual status, and bone density in collegiate women. Am J Clin Nutr 46:681-4 1987.

60. Heaney RP, Lifelong calcium intake and prevention of bone fragility in the aged. Calcif Tissue Int 49:S42-S45 1991.

61. Nelson ME, Fisher EC, Catsos PD, Meredith CN, Turksoy RN, Evans WJ. Diet and bone status in amenorrheic runners. Am J Clin Nutr 43:910-916 1986.

62. Lohman T. Advances in Body Composition Assessment. Human Kinetics Publishers, Champaign, IL.p26 1994.

63. Gluer CC, Steiger P, Selvidge R, Libesen-Kitcloth K, Hayaski C, Genant HK. Comparative assessment of dual-photon absorptiometry and dual energy radiography. Radiology, 174 223-228. 1990.

64. Roubenoff R, Kehayias JJ, Dawson-Hughes B, Heymsfield SB. Use of dual-energy x-ray absorbtiometry in body-composition studies: Not yet a "gold standard". Am J Clin Nutr 58:589-91 1993.

65. Gibson RS, Principles of nutritional assessment. Oxford University Press, New York, NY p44 1990.

66. Zulkifli SN, Yu SM. The food frequency method for dietary assessment. J Am Diet Assoc. 92: 681-685. 1992.

67. Musgrave KO, Giambalvo L Leclerc HL, Cook RA. Validation of a quantitative food frequency questionnaire for rapid assessment of dietary calcium intake. J Am Diet Assoc. 89:1484-1488. 1989.

68. Brown JL, Griebler R. Reliability of a short and long version of the Block food frequency form for assessing changes in calcium intake. J Am Diet Assoc 93: 784-789, 1993

69. Margetts BM, Cade JE, Osmond C. Comparison of a food frequency questionnaire with a diet record. Int J Epidemiol. 18:868-873. 1989.

70. Krall EA, Dwyer JT. Validity of a food frequency questionnaire and a food diary in a short-term recall situation. J Am Diet Assoc. 87: 1374-1377. 1987.

71. Block G, Hartman AM, Dresser CM, Carrol MD, Gannon J. A data-based approach to diet questionnaire design and testing. Am J Epidemiol. 124:453-469. 1986.

72. Block G, Coyle LM, Hartman AM, Scoppa SM. Revision of dietary analysis software for the Health Habits and History Questionnaire. Am J Epidemiol 139(12)1190-6. 1994

73. Smucker R Block G, Coyle L Harvin A. A dietary and risk factor questionnaire and analysis system for personal computers. Am J Epidmiol 129(2) 445-449 1989.

74. Cummings SR, Block G, McHenry K, Baron RB. Evaluation of two food frequency methods of measuring dietary calcium intake. Am J Epidemiol 126(5): 796-802. 1987.

75. Block G, Thompson FE, Hartman AM Larkin FA, Guire KE. Comparison of two dietary questionnaires validated against multiple dietary records collected during a one year period. J Am Diet Assoc. 92:686-693. 1992.

76. Gibson RS, Principles of nutritional assessment. Oxford University Press, New York, NY 1990 p39.

77. Beaton GH, Milner J, McGuire V, Feather TE, Little JA. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Carbohydrate sources, vitamins, and minerals. Am J Clin Nutr 37: 986-995 1983.

78. Beaton GH. Approaches to analysis of dietary data: a relationship between planned analysis and choice of methodology. Am J Clin Nutr 59(suppl): 253S-61S 1994.

79. Howat PM, Revathi M, Champagne C, Monlezun C, Wozniak P, Bray G. Validity and reliability of reported dietary intake data. J Am Diet Assoc. 94:169-173 1994.

80. Stuff JE, Garza C, O'Brian E, Nichols BL, Montandon CM. A comparison of dietary methods in nutritional studies. Am J Clin Nutr 37:300-306 1983.

81. Taitano RT, Novotny R, Davis JW, Ross PD, Wasnich RD. Validity of a food frequency questionnaire for estimating calcium intake among Japanese and white women. J Am Diet Assoc 95:(7) 804-806 1995.

82. University of Minnesota 24 Hour Dietary Recall. Nutrition Counseling Center. University of Minnesota, Minneappolis, MN.

83. Pate et al. Physical Activity and Public Health. A Recommondation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. J Am Med Assoc 273(5): 402-407 1995.

84. Ainsworth BE, Haskell WL, Leon AS et al. Compendium of physical activities. Med. Sci Sports Exerc 25:71-80 1993.

85. Norland Operators Manual, Norland Instruments, Ft Atkinson, WI.

86. Anderson VL, McLean R. Design of Experiments: A realistic approach. Cambridge University Press p 391, 1988.

87. Drake AJ, Armstrong DW, McDevitt ER, Shakir KMM. Bone mineral density and menstrual status in U.S. Naval Academy female Midshipmen. Abstract The Uniformed Services University of the Health Sciences 1997.

88. Young N, Formica C, Szmukler G, Seeman E. Bone density at weight-bearing and non-weight bearing sites in ballet dancers: The effects of exercise, hypogonadism, and body weight. J Clin Endocrin Metab 78(2) 449-454, 1994.

89. Prior JC, Vigna YM, Barr SI, Kennedy S, Schulzer M, Li DK. Ovulatory premenopausal women lose cancellous spinal bone: a five year prospective study. Bone 18(3) 261-7. 1996.

90. Woo SL, Kuei SC, Amiel D, Gomez M, Hayes WC, White F, Akeson W. The effect of physical training on the properties of long bone: a study of Wolff's Law. J Bone Joint Surg 63A(5):780-785. 1981

91. Forwood MR, Parker AW. Repetitive loading in vivo of the tibiae and femora of rats: effects of repeated bouts of treadmill running. Bone Miner 13:35-46. 1991.

92. Burr DB. Remodeling and the repair of fatigue damage. Calcified Tissue International. 53 (Suppl 1): S75-S81. 1993.

93. Rencken Ml, Chesnut CH, Drinkwater BL. Bone density at multiple skeletal sites in ammenorrheic athletes. J Am Med Assoc. 276(3) 238-240, 1996.

94. Rico H, Revilla M, Villa LF, Alkvarez de Buergo M, Arribas I. Longitudinal study of the effect of calcium pidolate on bone mass in eugonadle women. Calcif Tissue Int 54: 477-480, 1994.

95. Prior JC, Vigna YM, Barr SI, Rexworthy C, Lentle BC. Cyclic medroxyprogesterone treatment incresase bone density: A contorlled trial of women with menstrual cycle disorders. Am J Med 96:521-530, 1994.

96. Baran D, Sorensen A, Grimes J, Lew R, Karellas A, Johnson B, Roche J. Dietary modification with dairy products for preventing vertebral bone loss in premenopausal women.: A three-year stuyd. J Clin Endocrinol Metab 70:264-270, 1989.

97. Smith EL, Gilligan C, Smith PE, Sempos CT. Calcium supplementation and bone loss in middle-aged women. Am J Clin Nutr 50:833-842, 1989.

98. Riggs BL, Wahner HW, Melton III J, Richelson LS, Judd HL, O'Fallon M. Dietary calcium intake and rates of bone loss in women. J Clin Invest 80:979-982, 1994.

99. Garner DM. Self-report measures for eating disorders. Current Contents, Social & Behavioral Sciences, 25(8), 8 Philadelphia: Institute for Scientific Information. 1993.

100. Holbrook TL, Barrett-Connor E. The association of lifetime weight and weight control patterns with bone mineral density in an adult community. Bone Miner 20:141-9, 1993.

101. Davee AM, Clifford JR, Adler RA. Exercise patterns and trabecular bone density in college women. J Bone and Miner Res 5(3) 245-250. 1990.

102. Friedlander AL, Genant HK, Sadowsky S, Byl NN, Gluer CC. A two year program of aerobics and weight training enhances bone mineral density of young women. J Bone and Min Res 10(4): 574-583. 1995.

103. Henderson NK, Price RI, Cole JH, Gutterridge DH, Bhagat Chotoo. Bone density in young women is associated with body weight and muscle strength but not dietary intake. J Bone Miner Res 10:384-393 1995.

104. Vuori I. Peak bone mass and physical activity: A short review. Nutr Rev 54(4) S11-S14. 1996.

105. Cavanaugh DJ, Cann CE. Brisk walking does not stop bone loss in postmenopausal women. Bone 9:201-204, 1988.

106. Wardlaw GM. Putting body weight and osteoporosis into perspective. Am J Clin Nutr 63(suppl):433S-6S. 1996.

107. Hla MM, Davis JW, Ross PD, Wasnich RD, Yates AJ, Ravn R, Hosking DJ, McClung MR. A muticenter study of the influence of fat and lean mass on bone mineral content: evidence for differences in their relative influence at major fracture sties. Am J Clin Nutr 64:354-60, 1996.

108. Reid IR, Ames R, Evans MC, et al. Determinants of total body and regional bone mineral density in normal postmenopausal women-a key role for fat mass. J Clin Endocrinol Metab. 75:45-51, 1992.

109. Lindsay R, Cosman F, harrington BS, Himmelstein S. Bone mass and body composition in normal women. J Bone Miner Res 7:55-63, 1992.

110. Bevier WC, Wiswell RA, Pyka G, et al. Relationship of body composition, muscle strength and aerobic capacity to bone mineral density in older men and women. J Bone Miner Res 4:421-32;1989.

111. Pirnay F, Bodeux M, Crielaard Jm, Franimont P. Bone mineral content and physical activvity. Int J Sports Med 8:331-5, 1987.

112. Kerlinger FN. Foundations of Behavioral Research. 3rd Edition, Harcourt Brace & Company. 1992.

113. Sokal RR, Rohlf FJ. Introduction to Biostatistics. 2nd Edition, W.H. Freeman and Company. 1987.

114. Lohman TG. Advances in Body Composition Assessment. Human Kinetics Publishing. 1992.

115. Mahan LK, Escott-Stump S. Krause's Food, Nutrition, and Diet Therapy. 9th Edition, WB Saunders. 1996.