Predictors of Bone Density in Women: A Longitudinal Study

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University of Tennessee, Knoxville

Recommended Citation
To the Graduate Council:

I am submitting herewith a dissertation written by Susan G. Munroe entitled "Predictors of Bone Density in Women: A Longitudinal Study." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Human Ecology.

Roy E. Beauchene, Major Professor

We have read this dissertation and recommend its acceptance:

Bill C. Wallace, Dileep S. Sachan, Carol A. Costello

Accepted for the Council:

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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
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[Signatures]

Accepted for the Council:

[Signature]

Associate Vice Chancellor
and Dean of The Graduate School
PREDICTORS OF BONE DENSITY IN WOMEN: A LONGITUDINAL STUDY

A DISSERTATION

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

SUSAN G. MUNROE

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ABSTRACT

This research was a 15-year follow-up study of the relationships of age, physical measurements, diet group (non-vegetarian, NV; vegetarian, V), use of estrogen, dietary intakes of energy, calcium, and bone density in 60 healthy postmenopausal NV (n=31) and V (n=29) women, (age range 53-92). The V group was comprised of 29 lacto-ovo-vegetarian women, the remaining 31 subjects were NV. All subjects had been participants in an earlier (1976-1979) bone density study conducted in these laboratories. Measurements of height, weight, triceps skinfold thickness (TSF), and bone density of the radius and ulna were made (single photon absorptiometry); 7-day dietary records, and 24-hr urine samples, were obtained on each subject. Mean intakes of energy and nutrients were calculated from the dietary records. The urine samples were analyzed for calcium, creatinine, and hydroxyproline (HOP).

Physical parameters for the groups were similar in 1991 except for the NV showing a trend toward greater weight and body mass index (BMI) than the V group. NV had higher intakes of protein, saturated fat, cholesterol and caffeine than V. Conversely, it was the V which demonstrated higher intakes of carbohydrate and fiber than the NV. Of the 8 vitamin intakes studied, NV had the higher intake of vitamin B₁₂ and V had the higher intake of vitamin B₆. Mineral intakes were similar between NV and V except for V having the greater intake of iron.

Decrements were seen in height and TSF in both NV and V over the 15-yr period. The increase in BMI for V was significant. Both NV and V exhibited decrements in
intakes of energy, protein, fat, saturated fat, and cholesterol. NV caffeine intake also showed a decrement. A nonsignificant increase in fiber intake was seen in both NV and V. No changes were noted for vitamin intakes, except for decrements in vitamin B\textsubscript{12} of both groups. Intakes of sodium and iron showed decrements from 1976 to 1991. Both groups met RDA’s except for those of calcium and zinc.

Age, energy intake, body weight, dietary calcium, diet group (NV or V), protein intake, and use of estrogen were considered as predictors of bone density of the radius and the ulna; all were significant except energy and protein. Supplemental calcium was not found to contribute to bone density. At age 50 and 80 yr, predicted bone densities of the radius and ulna were greater in the NV than the V group. Use of estrogen by both diet groups increased bone density of the radius and ulna. Urinary calcium was higher in NV than V. HOP excretion did not significantly differ between NV and V. Urinary excretion of HOP and calcium were not found to be correlated current bone density or to bone loss, but creatinine excretion was negatively correlated to loss of bone density in both the NV and V groups. Creatinine excretion was greater in NV than V. It can be concluded that age, body weight, dietary calcium intake, choice of diet group, and use of estrogen are all predictors of bone density in these subjects.
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LIST OF ABBREVIATIONS

Body Mass Index .............................................. BMI
Estrogen Replacement Therapy ............................... ERT
High Density Lipoprotein .................................... HDL
Hydroxyproline .................................................. HOP
Lean Body Mass .................................................. LBM
Low Density Lipoprotein ..................................... LDL
Nonvegetarian ..................................................... NV
Nonvegetarian/Estrogen User ................................. NVE
Parathyroid Hormone .......................................... PTH
Recommended Dietary Allowance .......................... RDA
Recommended Nutrient Intakes .............................. RNI
Triceps Skinfold Thickness .................................... TSF
University of Tennessee Medical Center-Knoxville .... UTMC-K
Vegetarian ........................................................ V
Vegetarian/Estrogen User ..................................... VE
CHAPTER I

INTRODUCTION

Osteoporosis, the loss of bone mass, is the major skeletal disease in Western countries. This condition affects 15 to 20 million Americans (1). Vertebral fracture incidence increases dramatically in women age 65+ yr. By age 90 yr, one-third of the women suffer hip fracture (2). Many of these women fail to recover normal activity; mortality rates are reported to range from 12 to 20%. For many of those who survive, long term nursing care is required with total direct and indirect costs of osteoporosis estimated to be between $7 to 10 billion in 1986 (3). The incidence and cost of osteoporosis doubtlessly will increase because of the increase in our elderly population. The Surgeon General's Report (3) on nutrition and health indicated a lack of understanding as to why certain individuals develop osteoporosis while others do not. Genetics, nutrition and lifestyle all have been related to this condition (4). Bone mass is highly correlated with bone strength (5) and thus is related to fracture risk (6).

Factors that increase the risk of women developing osteoporosis include: thin small frame, early or surgically induced menopause, low calcium intake, Caucasian or Asian race, low level of exercise, cigarette smoking, alcohol and caffeine consumption, and glucocorticoid therapy (7). The role and interplay of these and countless other factors in the development and loss of bone mass needs further examination.

Because osteoporosis is obviously a slow developing multifactorial disease, it has been difficult to prove casual relationships from epidemiological studies. In his
comprehensive review of the nutritional aspects of osteoporosis, Schaafsma et al. (8) noted the importance of the impact of dietary factors on osteoporosis only will be revealed by longitudinal surveys in which serial measurements are made on the same individual at 2 or more points in time.

Key Year 2000 National Health Objectives (9) targeting improvements in health of older adults include: reducing hip fractures in elderly Caucasian women and increasing to at least 90% the proportion of perimenopausal women on estrogen replacement therapy (ERT) for prevention of osteoporosis.

The National Research Council’s Diet and Health (10) review on osteoporosis concluded 1200 mg/d calcium should be recommended for women considered to be at risk for osteoporosis. But in its summary statement the review stated the benefits of calcium intakes above 800 mg/d, the Recommended Dietary Allowance (RDA), are not well documented.

The American Medical Women’s Association (11) produced a position statement on osteoporosis in 1990 that stressed increased awareness of all factors contributing to osteoporosis if patients are to be counseled properly. Another annual conference jointly sponsored by National Institute of Arthritis and Musculoskeletal Diseases, European Foundation for Osteoporosis and Bone Disease, and the American National Osteoporosis Foundation cited an urgent need for more research on osteoporosis including: better understanding of its’ relationship to nutritional factors, improved measuring techniques to assess bone density, and expansion of knowledge about osteoporosis in general (12). Smith (13) noted that a main message of the Third
International Symposium on Osteoporosis was the recent failures to prove dietary calcium and physical activity influence bone mass represents a triumph of science over common sense. This laboratory was in the unique position of possessing previous dietary records and bone density measurements on V and NV women who indicated a willingness to participate in a follow-up study.

In 1976-79 nutrient intakes and bone density measurements were performed on approximately 200 lacto-ovo-vegetarian (V) and nonvegetarian (NV) women ages 40 to 90 years (14). Seven-day dietary records were used to calculate nutrient intakes using nutrient values from Handbook 8 (15). Bone mineral content of the radius and ulna was determined using the Norland-Cameron single photon absorption technique (16).

Protein intakes were higher in NV than V; more than 99% of the subjects consumed 100% or more of the RDA. Nevertheless, there was a wide range in protein intake (minimum 26g/d, maximum 154g/d). In addition, there were obvious differences in the types of protein consumed. Mean calcium intakes equaled the RDA; however, calcium intakes ranged from a low of 252 mg/d to a high of 2953 mg/d. Bone mineral content, height, and weight of the 2 groups did not differ significantly; however, both groups showed significant age associated decrements (17,18). The significance of these data in relation to the current research was: a) the instrumentation utilized to assess bone mineral content was sensitive to the age-associated decrements in bone mass and b) the consumption of nutrients among subjects varied sufficiently to allow testing whether past nutrient intakes were related
to subsequent bone mass. The present study was conducted in an effort to better understand the relationships of osteoporosis to nutritional factors and to characterize a model which best predicts bone density from factors including: diet group (NV or V), age, weight, use of estrogen replacement therapy, and intakes of calories, protein, and calcium. Specifically, this study investigated the relationships of dietary and nutrient intake, anthropometric measurements, estrogen use, and age on the bone mineral content of V and NV women and their changes over a 15-year time period.

The hypotheses of the study were:

1) Intakes of various nutrients will decrease with age over the 15-year period. Age-associated changes in nutrient intake will be different between V and NV subjects. Age-associated changes in nutrient intake will result in several nutrients not meeting the RDA.

2) Bone density of the radius and ulna will demonstrate age-associated decrements. Bone loss will be greater in NV than in V subjects.

3) Energy and dietary calcium intake will be associated positively with bone density. Protein will be associated negatively with bone density. Calcium supplementation will not improve bone density.

4) Body weight will be related positively to bone density.

5) Estrogen use will be related positively to bone density.
CHAPTER II

REVIEW OF LITERATURE

BONE PHYSIOLOGY

Bone is differentiated as either cortical or trabecular. Cortical bone is dense and has a slow turnover. It forms the outer shell and is predominant in the shaft of long bones. Trabecular bone is spongy, and has a high turnover rate (2). Trabecular bone forms the internal support network for the cortical shell in the bone ends and vertebrae. Each bone in the body is composed of both types of bone but relative proportions differ. The structure of bone consists of protein matrix into which calcium and phosphorus crystals are deposited. Remodeling of bone, the process of breakdown and reformation occurs throughout life. The cells that break down bone are called osteoclasts; those that build bone are called osteoblasts. Until mid-adulthood, osteoblastic activity predominates resulting in an accumulation of bone mass. By age 30-35 yr, peak bone mass is attained. The bone remodeling activity of the osteoclast predominates through the following decades and loss of bone mass results. The initial loss is about 0.3-0.6% per year (4). A post-menopausal bone loss of 1-1.5% which gradually slows to the previous rate of 0.3-0.6% loss per year by age 70-75 years has been reported (4). Although both sexes lose bone with advancing age, the fact
remains that women lose from 10 to 20% bone in critical portions of their skeleton in the 10 to 15 yr following menopause.

**AGE-ASSOCIATED CHANGES IN ENERGY AND NUTRIENT INTAKE**

The National Research Council publication Diet and Health (10) noted that the dietary needs of the elderly are largely unknown. Nutrient needs of older individuals may differ from those of "younger" adults because of age-related physiological changes. Yet, RDAs based on data from younger subjects are used due to lack of data for the needs of the elderly.

Comprehensive nutritional surveys of both V and NV have reported that many women over 65 yr of age consumed diets low in certain nutrients (19, 20, 21, 22). Studies of energy, vitamins, and minerals intakes have been conducted and varying results have been reported.

Several surveys assessing the nutritional status of elderly have reported an age-associated decrease in energy intake (19, 20, 21, 22, 23, 24). Scythes et al. (25) reported mean energy intakes ranged 8-21% below the Recommended Nutrient Intakes (RNI) for Canadian females 60-64, 65-69, and 70+ years. A study of independently living elderly patients of a U.S. gastroenterology practice noted caloric intake of females as "consistently below recommended levels" (26). Other surveys found energy intakes of healthy elderly to be adequate (27) or only slightly below the RDA for energy (22, 28, 29, 30). Unless nutrient dense foods are selected when energy intake
is decreased, intake of nutrients will be decreased. This potentially jeopardizes metabolic homeostasis, e.g., maintenance of bone mineral, of particular interest in this study.

Energy intakes were found to be low, with fat intake high in a study of 619 independently living elderly Canadian men and women (25). In a 25-yr longitudinal study of aging men total fat intake and saturated fat intake significantly decreased from 1960 to 1985 (31). Although desirable dietary changes were observed, it was reported that diets of these NV men failed to meet the recommendations of the Nutrition Council in the Netherlands. In contrast, percentages of energy from protein, fat, and carbohydrate in healthy V men and women (13%, 37%, and 50% respectively) were close to Dutch Guidelines of 10-15% protein, 30-35% fat and 55% carbohydrate (31). Hunt et al. (30) reported V subjects consumed lower intakes of total fat and saturated fat and higher intakes of dietary fiber than NV subjects.

In general, protein intake of elderly persons who do not have any chronic illness is thought to be adequate in both V and NV women (29). A study of protein intakes of healthy elderly women in Hong-Kong aged 60+ years reported mean protein intake of 1.2 g/kg body weight which is well above the RDA of 0.8 g/kg body weight (32). A recent study of American elderly also reported protein intake in excess of the RDA (26). Protein intakes for Canadian women ranging in age from 60 to 70+ years were reported in excess of the RNI (25). The Dutch Nutrition Surveillance System Study of Adequacy of V Diets at Old Age reported mean protein intakes of 54.4g for females
Energy intakes of V and NV American women were reported to be similar and adequate (26). In several recent studies comparing dietary intakes of V and NV subjects vitamin intakes generally met two-thirds RDA except for vitamin B₆ (28, 30, 33). Although intakes of vitamin D have been reported to be adequate, the requirement for vitamin D may be higher for the elderly than younger adults. Aging may reduce the capacity of the skin to produce vitamin D from 7-dehydrocholesterol thus increasing the amount of vitamin D required from the diet (34). Folic acid was reported to be less than two-thirds RDA in V and NV subjects (30) but Nieman et al. (28) reported folic acid to be below RDA only in NV subjects. In general, mineral intake of the elderly appeared to be adequate except for those of zinc and calcium (21). Several studies showed both V and NV to have intakes of zinc less than two-thirds RDA (28, 29, 30).

Of particular concern are low calcium intakes. Wide variations in calcium intake have been reported. The Surgeon General’s report on nutrition and aging noted mean calcium intakes reported in national surveys to be 540-590 mg/day for older women (3). This is well below the RDA of 800 mg for calcium. Median calcium intakes of independently living elderly females in Toronto ranged from 684-769 mg/day (25). Recker and Heaney (35) noted that 25% of U.S. women beyond age 30 yr consume less than 300 mg calcium on "any given day". Studies by Brants et al. (29) and Hunt et al. (30) reported mean calcium intakes of both V and NV male and female subjects to meet RDA, while Neiman et al. (28) reported mean daily calcium intakes of both V and NV to be two-thirds of the RDA (630 mg). Heaney et al. (35) reported current
calcium intakes were not a good estimators of past intakes of calcium for premenopausal women.

Iron and phosphorus intakes are reported to be two-thirds or more of the RDA in several studies (25, 26, 28, 30). Normally the intake of phosphorus and its absorption are adequate. But iron intakes have been found to be below recommended levels in aged subjects (29).

**DIETARY FACTORS RELATED TO BONE**

**CALCIUM**

Calcium is a threshold nutrient that Heaney (4) states relates to bone mass as iron relates to hemoglobin mass. This relationship exists when a variable (such as calcium) "exerts a permissive effect" on another variable (such as bone density). There is a point or "threshold" below which the two variables are related, i.e., an increase in calcium leads to increased bone density, but above which they are not. Low intakes of dietary calcium, failure to absorb calcium efficiently, and high obligatory excretory losses of calcium all result in the body treating bone as a "seemingly limitless reservoir of calcium upon which it can draw" to support the level of calcium required by the extracellular fluid (4). Concern regarding calcium intake should be twofold: calcium sufficiency for the development of optimal peak bone mass during the first 3 to 4 decades of life (36) and for the prevention of bone loss in later life (6,38).
Recent studies of calcium metabolism during skeletal modeling concluded higher calcium intakes during childhood and adolescence might optimize peak bone mass, within genetic boundaries (38, 39, 40). Results of a study of mother-daughter pairs suggested inheritance of bone mass may have two components, one influencing peak bone mass and one relating to rate of bone loss at menopause (37). In a similar study Matkovic et al. (40) found that by age 16 yr, daughters had accumulated 90-97% of the bone mass of their mothers. Studies such as these demonstrate the permissive role of calcium in allowing peak bone mass to reach its full genetic potential. Daughters of women with osteoporosis were found to have reduced bone mass (41).

Sandler et al. (42) found that increased frequency of milk consumption during childhood and adolescence was associated with higher bone densities in postmenopausal women. Matkovic et al. (43) reported levels of calcium intake were important in determining bone mass in young adults but they noted calcium intake had little effect on bone loss in older women. A report on the Third International Symposium on Osteoporosis-1990 emphasized the importance of adequate calcium intake to the development of peak bone mass and its limiting effect on genetically determined skeleton mass as being demonstrated only when it is in short supply (13).

Supplementation of suboptimal calcium intakes has been suggested as a means of minimizing bone resorption. The bone density of the radius increased in subjects given oyster shell electrolysate for 24 mo while bone density fell significantly in elderly female control subjects (44). A 2-year, placebo-controlled, double blind study of calcium supplementation in postmenopausal women found women 6 or more years
beyond menopause with calcium intakes less than 400 mg/d benefitted significantly in spine, hip and radius density if calcium intake was increased to at least 800 mg/d. Women in early menopause did not seem to benefit from calcium supplementation (45, 46). In a double blind study, Smith et al. (47) reported 1500 mg calcium supplementation countered bone loss attributable to menopause.

Other studies have found little or no relationship between calcium and bone density in postmenopausal women (48, 49, 50). These inconclusive and conflicting results led to Kanis and Passmore (51, 52) to conclude there is no case for calcium supplementation while Nordin and Heaney (53) concluded calcium supplementation to be justified.

**PHOSPHORUS**

Concern about dietary phosphorus arises because high levels of the nutrient and/or low dietary Ca/P ratios have been shown to result in bone loss in experimental animals (54). In balance studies with humans, however, increasing dietary phosphorus levels threefold did not increase calcium losses (55, 56). Freudenheim et al. (57) found that high levels of dietary phosphorus were associated with decreased rates of bone loss in post-menopausal women consuming normal diets.
Walker and Linkswiler (58) found that increased levels of dietary protein resulted in increased urinary calcium and a negative calcium balance in young men. In a study of premenopausal women, Heaney and Recker (55) reported a 50% increase in dietary nitrogen resulted in sufficient loss of body calcium to account for the loss of skeletal mass seen in post-menopausal women. Kersteller & Allen (59) linked dietary protein to urinary calcium and calcium balance at "usual" levels of calcium and phosphorus intakes. When intakes of dietary calcium and phosphorus were high (dairy products), the influence of dietary protein on calcium balance was found to be less pronounced. With protein levels above the RDA, more calcium was lost in the urine and calcium balance tended to be negative. Work of Spencer and Kramer (56, 60, 61) does not support the idea that protein intakes greater than the RDA lead to hypercalcuria and negative calcium balance. They have consistently reported no significant increase in urinary calcium with high protein meat diets. The phosphorus content of the meat diet reportedly blunts the calciuretic effect of the protein.

Animal proteins contain a higher percentage of sulfur-containing amino acids than plant proteins. Zemel (62) concluded that the hypercalcuria seen in meat-eaters might be caused in part by the catabolism of sulfur-containing amino acids to sulfate and its subsequent excretion in the urine. Marsh et al. (63) reported a higher rate of bone loss in omnivorous than in V post-menopausal women. Tylavsky and Anderson (64) were unable to demonstrate a difference in bone mass in V and NV groups once bone
indices were age adjusted. Similarly, Hunt et al. (30) did not find bone mineral content to be different between omnivorous and V women.

In "letters to the editor", Spencer and Kramer (65) noted 24 controlled studies supporting the lack of relationship between dietary protein and hypercalcuria. In response, Kerstetter and Allen (66) mentioned review of 16 studies which support an overall relationship between dietary protein and urinary calcium. Obviously the literature is inconclusive.

VITAMIN D

Vitamin D is essential for normal calcium, phosphorus and bone metabolism. The vitamin is obtained from the diet and it is also produced by the action of sunlight on precursors in the skin. Aging reduces the efficiency of ultraviolet conversion of 7-dehydrocholesterol to vitamin D$_3$. It is possible that inadequate production of vitamin D in the skin and inadequate dietary intake sources could contribute to bone loss (67).

Parfitt et al. (68) reviewed the literature on the relationship of vitamin D to bone health. They state the vitamin D requirement of the elderly is 50-100% higher than that of young adults and recommend 600-800 IU of dietary vitamin D for elderly people living mainly indoors.

The most active form of the vitamin in the body is 1,25-(OH)$_2$D$_3$. Serum levels of this metabolite (hormone) have been found to be reduced 30% in osteoporotic women (67). DeLuca (34) suggested that osteoporosis is not due to a vitamin D
deficiency. but a failure of the vitamin D endocrine system to regulate calcium metabolism.

Riggs and Melton (69) described 2 distinct types of osteoporosis: postmenopausal osteoporosis was termed Type I while senile osteoporosis was termed Type II. In reviews of osteoporosis, Heaney et al. (70) and Gallagher (71) postulated a differing pathogenesis of Type I and II osteoporosis. In postmenopausal osteoporosis, estrogen deficiency permits increased bone resorption resulting in a slight elevation in serum calcium which leads to decreased secretion of parathyroid hormone (PTH), decreased 25-(OH) D-1 renal alpha-hydroxylase activity, decreased production of 1,25 (OH) D and ultimately, decreased calcium absorption. The end result being negative calcium balance.

Those with Type II osteoporosis are much older (average age 80) and decreased renal function plays a key role in the pathogenesis. Inadequate production of 25-(OH) D in the skin results in reduced circulating levels. Age-related decrements in kidney function result in reduced hydroxylation of 25-(OH) D$^3$ to 1,25-(OH) D$^3$. Reduced absorption of calcium from the gut leads to hypocalcemia which would stimulate PTH secretion and increase bone turnover.

Pathogenesis of both Type I and Type II osteoporosis include a reduced absorption of calcium and reduced calcitriol (1,25-(OH) D) levels. The major difference between the 2 types lies in the level of PTH.

Himmelstein et al. (72) found Vitamin D supplementation of elderly nursing home residents resulted in an increased circulating 25-(OH)D but not that of the renal 1,25-
(OH)$_2$D, and concluded that long term effects of vitamin D supplementation on bone metabolism was as yet, unclear. In women not deficient in vitamin D, it was concluded that a disturbance of vitamin D metabolism was not likely to play a role in early postmenopausal bone loss (73). In contrast, DeLuca (67) reported treatment of postmenopausal women with osteoporosis with 1,25-(OH)$_2$D$_3$ as a promising means of restoring bone mass and of reducing fracture rate. Gallagher and Riggs (74) found 1,25-(OH)$_2$D$_3$ therapy in postmenopausal osteoporotics improved calcium balance and reduced vertebrae fracture rate at dietary calcium levels at or below the RDA. Future research will be required to clarify the exact role of vitamin D in the development and treatment of osteoporosis.

**DIETARY FIBER AND CAFFEINE**

Although there is no conclusive evidence that high fiber diets cause osteoporosis, they may influence absorption and utilization of calcium and alter vitamin D metabolism (75,76). It has been reported dietary fiber may depress serum levels of androgens which would ultimately result in decreased trabecular bone density (76).

Increased intake of caffeine has been reported to be associated with increased levels of urinary calcium (77). Recently, Yano et al. (78) found a slight inverse correlation between current caffeine intake and bone mineral content of the radius and ulna in elderly Japanese-American women. Prospective data suggested caffeine consumption increased the risk of osteoporotic fracture in middle aged women (79). A
double-blind, placebo-controlled, crossover study of caffeine effects on calcium economy of premenopausal women found no significant effects of caffeine on fractional calcium absorption, endogenous fecal calcium, or urinary calcium (80). A recent study of premenopausal women found caffeine intake of V and NV had a positive effect on urinary calcium excretion but no association was observed between bone density and caffeine intake (81). Here again, the literature is controversial.

ANTHROPOMETRIC MEASUREMENTS

LEAN BODY MASS

Lean body mass (LBM) has been found to peak in the third decade, fall slowly for the next 2 decades, and then to decrease more rapidly (82, 83, 84, 85). The pattern of LBM loss is similar to that of bone loss in women (86). In conjunction with the decrease in LBM with age, an increase in body fat has been reported with advancing age (82, 83, 84).

In a study of institutionalized males bone density was found to be directly correlated with body weight but not height (87). Subscapular skinfolds, calf circumference, and biacromial width were found to be significant predictors of bone mass in adult females even when height, weight, and age were included in the models (88). Conversely, in a study of the relationship of menopause to skeletal and muscle
mass, Aloia et al. (89) found no evidence that adiposity plays a role in protecting against bone loss.

**BODY FAT**

BMI and TSF are commonly used as measures of adiposity in research studies. The 85th percentiles of BMI and TSF are used to quantify obesity (85). It has been hypothesized that body fat increases with age as LBM declines (82, 83, 84), BMI reflects total body weight, without any consideration of composition, as a function of height (kg/m\(^2\)). TSF is an indirect method for estimating body fat. Although the relationship of subcutaneous fat to internal fat is not established, it is presumed subcutaneous fat can predict body fat. TSF and BMI are strongly and consistently correlated among age and sex groups (90). The correlation is weaker in males and thought to be due in part to greater muscularity in males than females as reflected by BMI. BMI at the 85th percentile calculated using NHANES I data is reported to be 31.70 kg/m\(^2\) for white females age 70-74 yrs (91).

From age 50 through age 74 yr TSF declines. TSF at the 85th percentile for medium framed females, were reported to be 40mm at age 58 yrs. and 33mm at age 67 yrs (85). It has been proposed that small framed women are at greater risk for development of osteoporosis than larger women. Beyond the added physical stress excess weight has on the bone, if adipose tissue aromatizes androgens to estrogen, the result could increase plasma estrogen concentrations which are known to have a role in maintenance of bone density.
URINARY CONSTITUENTS AS RELATED TO BONE METABOLISM

Urine is the principal excretory route for nitrogenous waste products. It contains various metabolites which are derived from either dietary or endogenous protein. Measurement of compounds such as creatinine have been used as markers of LBM.

Animal proteins contain a higher percentage of sulfur-containing amino acids than do plant proteins. When sulfur-containing amino acids are present in the diet in excess of what the body utilizes, they are metabolized to sulfates. It is hypothesized that the urine of an NV is more acidic than that of a V (92). The results of this hypothesis would suggest that V would have greater bone mineralization than NV. Marsh et al. (63) reported findings that support this hypothesis, i.e. older V women had greater bone mineral mass than NV. Others have found no such difference between diet groups (30,81).

Urinary creatinine, has been reported to be reflective of LBM and endogenous protein metabolism (93, 94, 95). In humans, urinary creatinine excreted appears to be of both dietary and endogenous sources. This reflects the fact that creatine (physiological precursor of creatinine) is found primarily in muscle.

It has been hypothesized that urinary hydroxyproline (HOP) excretion is related to metabolism of skeletal collagen (96, 97). Thus, changes in collagen metabolism would be reflected by changes in HOP excretion. HOP excretion has been reported to be relatively constant from age 21 to 70 yr and to decrease thereafter (98).
LIFESTYLE FACTORS

EXERCISE

Bone is continuously remodeled to accommodate the mechanical stresses of weight-bearing and muscle contraction. Weight bearing and/or muscle contraction stresses places bone in a "hyperdynamic" state, and results in increased bone mass. Immobilization and/or weightlessness result in increased bone loss (99). The habitual stimulus to weight-bearing bones (legs and spine) is much greater than that to non-weight-bearing bones (ribs, arms, skull). Tennis players have been reported to have one-third more cortical bone thickness in the dominant than in the non-dominant arm (100). The mechanism by which bone responds to stress is not clear. Active older women have higher energy and calcium intakes than less active women (101,102). Exercise may also lead to decreased fracture risk indirectly in older people by reducing their risk of falling through improved coordination, balance, and muscular strength (103).

A 2-year longitudinal study of premenopausal runners showed runners maintained their bone density (104). A 12- month modified Nautilus program yielded nonsignificant changes in bone density (105).

Perhaps exercise alone is not enough to maintain bone mass (106). A 1- year supervised walking program and 831/mg/d calcium supplement altered bone density of postmenopausal women at different sites depending on the turnover rate of cortical or trabecular bone (107). No effect on the distal radius was reported. In post-
menopausal women with low bone density, bone loss was slowed or prevented by exercise and calcium supplementation or ERT. Although the exercise-ERT regimen increased bone mass more than exercise-calcium supplementation, exercise-ERT did exhibit adverse complications such as bleeding (108). The effect of high level physical activity and ERT on bone mass in postmenopausal subjects suggested both contribute to the maintenance of bone mass. Physical activity/ERT groups were evaluated to see if combinations of these factors influenced bone mass. There was a trend for the combined regimen to produce a more positive effect on bone than either factor alone (109). Further investigation as to the intensity, duration, and synergistic relationship of exercise to other bone mass contributors is required.

SMOKING

Increased incidence of bone fracture in smokers has been reported (110). Since smokers are reported to have decreased body weights, it is difficult to single out direct effects of smoking on bone mass (111). In addition it has been reported that female smokers experience menopause on the average 1.2 years earlier than non-smokers and that they have lower serum estrogen levels when on ERT (112). Smoking may influence other facets of bone maintenance if smoking induced vasoconstriction is identified as calcium-mediated process (113).
HORMONES

Various regulatory mechanisms control extracellular calcium levels within the required limits for cellular functioning. Bone mineral homeostasis is hormonally mediated in part by the interactions of PTH, the vitamin D endocrine system, and calcitonin (114).

Initially, normalization of low extracellular calcium levels is achieved by the stimulation of PTH secretion, which acts on the kidney to enhance renal calcium conservation and activation of 25 hydroxyvitamin D to 1,25 (OH)$_2$D. PTH and 1,25-(OH)$_2$D levels also act at the level of the intestine to stimulate transport of calcium across the intestinal mucosa and into the blood stream. As serum calcium levels are restored to normal, further bone resorption is blocked by the thyroid's secretion of calcitonin, a "powerful inhibitor" of bone resorption. The inhibitory action of calcitonin is thought to be direct, through calcitonin receptors on the surface of the osteoclasts (115). Calcitonin may serve as mediator of estrogen's effect on bone.

ESTROGEN

Accelerated bone loss with the onset of menopause long has been recognized. Reduced estrogen production results in reduced intestinal calcium absorption and increased bone resorption (116).
Johnston and Longcope (117) noticed that between the time that peak bone mass has been attained and menopause, little bone loss occurs in most women. Nordin et al. (118) found early menopause to be associated with a self-limiting bone loss which did not progress until aging processes exerted their effects. They concluded that the significance of early menopause as a risk factor for osteoporosis has been overstated. Conversely, in a study of 225 premenopausal women, Rodin et al. (119) concluded significant amounts of trabecular bone are lost from the spine and femoral neck before menopause.

Decreases in bone density among women with differing exercise habits correlated with asymptomatic disturbances of ovulation and not physical activity (120). Better understanding of the association of bone loss seen with abnormalities in ovulation is needed. In a comparative study of menstrual differences due to V and NV diets it was concluded that specific dietary nutrients may have direct effects or exert their effects by modulating circulatory sex steroid status (121). In studies on dietary and hormonal relationships in men (122) and women (123, 124) plasma concentrations of estradiol-17 were found in both studies to be significantly lower in V than NV Seventh Day Adventist subjects. In a study of hormonal concentrations in meat eating women who changed to a V diet, little effect of diet was seen on total hormonal concentrations but estradiol was found to be significantly decreased upon switching to the V diet (124).

Dietary fiber has been reported to significantly reduce estrogen excretion in the urine of young Finnish women (125). However, it has been reported that V have increased fecal excretion of estrogen, resulting in 15-20% decrement in plasma
estrogen concentrations (126, 127). A recent study of dietary and hormonal interrelations found that although omnivores consumed significantly more protein, total and saturated fats, and cholesterol than V subjects, hormonal status and binding capacity of sex-hormone-binding-globulin were similar in both diet groups (128).

There appear to be 2 independent effects of estrogen withdrawal on bone and calcium metabolism. First, there is the loss of a "virtual compartment of bone that is sustained by estrogen in the pre-menopausal years", which is estimated to be on the order of 15% of total skeleton mass (129). Bone is lost irrespective of calcium intake when estrogen is removed. The second effect of estrogen withdrawal is a deterioration of calcium conserving mechanisms resulting in an increase in the calcium requirement (70,71). In a review of estrogen-calcium interactions Heaney (130) noted calcium deficiency if present, is masked during the early years of estrogen withdrawal bone loss. Except in severe deficiency states, it is unlikely that calcium supplementation can reduce bone loss much in the period immediately following menopause. Five to 8 yr beyond menopause, an underlying calcium deficiency, if present, becomes apparent and if uncorrected greatly augments the bone loss due to estrogen withdrawal.

**ESTROGEN REPLACEMENT THERAPY**

In a general overview on the "myths of menopause", Davis (131) noticed that although most women could get relief from postmenopausal symptoms with estrogen
supplements, only 10% take them. Replacement hormonal therapy, usually involving estrogen with or without added progestin has been proposed as a treatment for a variety of changes associated with menopause. Postmenopausal bone loss may be prevented by ERT. ERT has been associated with a 60% reduction in rate of hip fracture (132). All types and administration forms are effective as long as sufficient serum concentration is obtained (133, 134). A 10-year prospective study by Lindsay et al. (135) found 38% of oophorectomized women without ERT had vertebral fractures compared with 4% of women receiving ERT.

Christiansen et al. (136) noted the greatest benefit of ERT is obtained if instituted immediately following menopause when bone loss is most rapid. Estrogen also will arrest bone loss when instituted well beyond the postmenopausal period (133, 137). Lindsay and Tohme (138) concluded ERT to be an effective therapy even for those with established osteoporosis. Intervention was associated with a significant increase in bone mass compatible with reduced skeletal turnover. Comprehensive reviews as to protocols and benefits of ERT in maintaining bone mass recently have been published by Lichtman (134) and also, Noyes and Demmler (139).

In a recent randomized double-blind crossover studies of healthy postmenopausal women, the use of low dose ERT favorably altered LDL and HDL levels suggesting ERT may also protect women against atherosclerosis (140). A monumental 10-year follow up study on 48,000+ nurses found current ERT associated with a reduction in the incidence of coronary heart disease and mortality from cardiovascular disease. ERT was not found to be associated with any altered risk of stroke (141). Since
incidence of osteoporosis and ischemic breast disease increase with age, the potential benefit of ERT is of tremendous interest in overall women’s health.

There is risk associated with ERT. In a call for action, Goldman and Tosteson (142) cite pooled data of a six-fold increase in incidence of endometrial cancer due to endometrial hyperplasia in women receiving only estrogen. An estrogen-progestin regimen has been suggested to minimize endometrial proliferation. The effects of added progestins on the risk of ischemic heart disease are not clear. Progestins blunt the rise in HDL cholesterol (143). Thus, the beneficial effect of ERT in preventing heart disease might be reduced when combined with progestins. But a major reduction in incidence of heart disease with ERT could greatly outweigh the effects of most other factors on life expectancy.

In summary, little consensus is to be found as to which factors influence bone density and to what degree. Data from few longitudinal studies of nutrition and bone density are available. Further research will be required to clarify which factors play which role in the maintenance of bone. Although bone mass may be genetically determined, nutritional intakes and use of ERT may be risk factors for bone loss that could easily be modified to minimize bone loss.
CHAPTER III

DATA COLLECTION PROCEDURES

SUBJECTS

This laboratory was in the unique position of possessing previous 7-day dietary records and bone density measurements of 101 V and 107 NV women. All of the subjects previously participated in one or more surveys conducted by these laboratories from 1976-1979. After an interval of between 11-15 yr, a resurvey of bone density and dietary intakes was made of 31 of the original NV and 29 of the original V subjects agreeing to participate in this follow-up study. Attrition in part, was related to the subjects' nonavailability due to change of address, change in health status, or to death. As there was no significant difference between the groups in terms of the number of subjects able and willing to participate, it was felt that these subjects although they are "survivors" are representative of the original participants. Data were collected using procedures similar to those employed in the original study as described below (17, 18). Subjects again were asked to sign a consent form approved by the Committee on Research Participation, University of Tennessee (see appendix A).
Dietary information was obtained from 7-day diet records. Forms identical to those in the original study were provided (see appendix A). Written and oral instructions were provided by a registered dietitian on the measuring and recording of food portion sizes. The 7-day dietary records were returned by mail in a postage paid envelope provided to the subject. Originally mean nutrient intakes were calculated from the 7-day records using nutrient values from Handbook 8 (15). Along with current records, these previous 7-day records were re-analyzed using Nutritionist III-6.0 dietary analyses software (144). This was done to eliminate any analytical differences between methods of nutrient analysis.

PHYSICAL ACTIVITY AND MEDICAL HISTORY

Interview and questionnaires (see appendix A) were used to assess general medical status (as related to this research), physical activity, use of nutrient supplements, drugs, hormone replacements, and/or tobacco. Information was coded and recorded for statistical analyses. If a subject received ERT for more than one year, she was classified as an estrogen user (NVE or VE).
ANTHROPOMETRIC MEASUREMENTS

Height, weight, and TSF measurements were made when the subject reported to receive instructions for diet records. Height was made without shoes to the nearest 1/4 inch. Weight was recorded to the nearest 1/4 pound with the subjects wearing indoor clothing but no shoes. Height and weight were converted to cm and kg, respectively. TSF was measured to the nearest mm using Lange calipers.\(^1\) BMI or Quetelet's Index was calculated by dividing weight in Kg by the square of height in M.

BONE MEASUREMENTS

Following previous protocol, the distal radius and ulna of the non-dominant arm were scanned at the "one-third" site using a Norland-Cameron bone mineral analyzer (145). The distance from the styloid process of the ulna to the olecranon on the non-dominant arm was measured with a ruler and marked with a pen at a point one-third the distance. This instrumentation uses the principle of single photon absorptiometry to determine bone mineral content. An \(^{125}\) source and detector unit simultaneously pass below and above, respectively, a scan site on the radius or ulna. The quantity of energy absorbed is proportional to the amount of bone mineral. Bone width also is computed by the instrument. Bone mineral, expressed in terms of g/cm and bone width in cm are displayed digitally by the instrument. Bone mineral content is

\(^1\)Cambridge Scientific Industries, Inc., Cambridge, MD.
expressed as g/cm². This value is calculated by dividing the bone mineral (g/cm) by the bone width (cm). The precision level for a single scan is SD ± 0.006 g/cm on adult radii. Agreement of different instruments scanning the same bone has been reported to be better than 1% (145).

URINE COLLECTION AND STORAGE

Subjects were given verbal and written instructions (following previous protocol) for collecting a 24-hour urine sample (see appendix A). Acid-rinsed (concentrated hydrochloric acid diluted 1:7 with water) polyethylene bottles containing 3-4 thymol crystals as a preservative were supplied to each subject. Upon completion, samples were picked up by the researcher. Each sample was thoroughly mixed, the volume measured, and aliquots frozen for analysis of urinary calcium, HOP, and creatinine. Urinary creatinine and calcium were analyzed at The University of Tennessee Medical Center - Knoxville (UTMC-K) using Kodak Ektachem 700XR dry slide methods. Creatinine was assessed by a 2-point rate measurement of creatinine conversion to creatine by creatinine-aminohydrolase (146). Calcium determinations were made by reflectance techniques using azo-3-dye (147). Urinary total HOP was measured spectrophotometrically at UTMC-K using a modification of the method described by Bergman and Loxley (148).
The SAS (149) and GLMM (150) software packages were used to perform statistical analyses. GLMM does a mixed model analyses. In the present analysis of age, bone density, anthropometric and dietary variables were treated as fixed effects and subject as a random effect. Differences between mean values were tested using, student’s paired and non-paired t-tests (as appropriate) and significance determined at a level of \( p < 0.05 \). Levels of \( p > 0.05 \) and \( < 0.10 \) were designated as demonstrating a trend towards significance. Correlation coefficients reported were Pearson \( r \)-values.

In determining the single regression model to predict bone density, the same variables were used to predict the density of both the radius and the ulna. Variables were selected on an a priori basis. An initial model was fitted to the data with the following terms: age, energy, weight, calcium, diet group (NV or V), protein, and use or nonuse of estrogen (see appendix B). Terms in the model were tested on a Type I (sequential) and Type III (partial) basis. Variables which were determined to be nonsignificant (\( p > 0.27 \)) were removed from the model; (protein and energy), and the quadratic term involving age by age and the interaction term age by estrogen were added to the model. The regression equation was recalculated using this revised model. Again, terms which were nonsignificant were again removed (see appendix B). At this point, both the linear and quadratic terms for age, as well as interactions with these terms were included to form a final model (see appendix B). Once a final model was achieved, contrast statements (in GLMM-Predict Statements) were used to
make comparisons between the diet groups (NV or V) and estrogen supplemented and nonsupplemented groups.
CHAPTER IV

RESULTS

Out of the approximately 100 NV subjects participating in the original 1976-79 study, 31 were recruited for the follow-up study. Likewise, 29 V subjects out of the approximately 100 original V agreed to participate. These V subjects had not consumed meat for a mean of 58.9 yrs. Twenty-three of the subjects were estrogen users, 12 NV and 11 V. To be classified as an estrogen user, the subject had to indicate the use of estrogen for at least 1 yr. The average years of use was 8.6 (range 1-18 yrs).

Comparison of 1991 physical parameters for NV and V are reported in Table I. No significant differences in age, height and TSF were found between groups. There was a trend for the NV to weigh more and have a larger BMI than V subjects.

Significant differences were found between the NV and V mean intakes of cholesterol, fiber and caffeine (Table II). NV mean dietary intakes of protein, saturated fat, cholesterol, and caffeine were 61.3, 16.0 g/d, 173 and 122.7 mg/d, respectively, were significantly higher than corresponding intakes of V (50.4, 10.4 g/d, 73, and 6.3 mg/d). V carbohydrate (226 g/d) and fiber (7.2 g/d) mean intakes were significantly higher than those of NV (190 and 4.6 g/d, respectively). Mean protein intakes for both groups met or exceeded the RDA. Caloric composition of the NV group was 52% carbohydrate, 16% protein, and 32% fat while the percentages for the V group were 60, 20, and 30%, respectively.
Table I

COMPARISON OF PHYSICAL PARAMETERS OF NONVEGETARIAN AND VEGETARIAN WOMEN IN 1991

<table>
<thead>
<tr>
<th>Physical Parameter</th>
<th>Nonvegetarian(^1)</th>
<th>Vegetarian(^2)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71.0 ± 1.9(^3)</td>
<td>70.0 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.9 ± 1.4</td>
<td>161.0 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.4 ± 2.3</td>
<td>60.4 ± 2.3</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25.4 ± 1.0</td>
<td>23.4 ± 1.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Triceps Skinfold (mm)</td>
<td>23.6 ± 1.2</td>
<td>20.6 ± 1.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^1\)n=28-31.  
\(^2\)n=28-29.  
\(^3\)Values are means ± SEM.
Table II

COMPARISON OF DIETARY INTAKES IN 1991

<table>
<thead>
<tr>
<th>Dietary Component</th>
<th>RDA¹</th>
<th>Nonvegetarian Women (n=31)</th>
<th>Vegetarian Women (n=29)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kcalories/d</td>
<td>--</td>
<td>1450 ± 64²</td>
<td>1465 ± 60</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>--</td>
<td>190 ± 8</td>
<td>226 ± 10</td>
<td>0.007</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>50</td>
<td>61.3 ± 2.9</td>
<td>50.4 ± 2.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Fat (total g/d)</td>
<td>--</td>
<td>52.2 ± 3.2</td>
<td>46.8 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Saturated fat (g/d)</td>
<td>--</td>
<td>16.0 ± 1.4</td>
<td>10.4 ± 0.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>--</td>
<td>173 ± 14</td>
<td>73 ± 11</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>--</td>
<td>4.6 ± 0.3</td>
<td>7.2 ± 0.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Caffeine (mg/d)</td>
<td>--</td>
<td>122.7 ± 24.7</td>
<td>6.3 ± 2.7</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

¹Recommended Dietary Allowances for women age 51+ yrs.
²Values are means ± SEM.
Table III reports a comparison of mean vitamin intakes of NV and V subjects. Vitamin B₁₂ intake was significantly higher in NV (mean 3.79 µg/d) than in V (2.09 µg/d). V mean intake of 1.84 mg/d Vitamin B₆ was significantly higher than that of NV (1.54 mg/d). V also showed trends towards having higher mean intakes of Vitamin A and thiamin than NV. RDAs for all vitamins were essentially met.

Both NV and V groups failed to meet mean intakes of 12 mg zinc and 800 mg calcium, the RDA’s for women age 51+ yrs. (Table IV). The V group had a higher mean iron intake (13.5 mg/d) than the NV group (11.0 mg/d).

Longitudinal changes in physical parameters for both NV and V subjects are reported in Table V. Over the 15-yr period mean height and TSF declined significantly in both NV (1.3 cm and 5.2 mm) and V (1.6 cm and 4.4 mm) groups. Although no significant increase in mean weight was found, BMI significantly increased for V subjects (1 kg/m²). Significant decrements in intakes of calories, protein, fat, saturated fat, and cholesterol were found over the 15-yr period in both the NV group and V group (Table VI). The 118 mg/d decrement seen in the NV mean intake of caffeine was also significant.

The decrements observed in vitamin intakes in both groups were nonsignificant except for that of vitamin B₁₂ (Table VII). NV subjects demonstrated a significant decline in Vitamin B₁₂ intake (5.96 µg/d); V subjects also showed a significant decrement (0.80 µg/d).
### Table III

**COMPARISON OF VITAMIN INTAKES IN 1991**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>RDA¹</th>
<th>Nonvegetarian Women (n=31)</th>
<th>Vegetarian Women (n=29)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (µgRE/d)</td>
<td>800</td>
<td>1670 ± 251²</td>
<td>2450 ± 345</td>
<td>0.07</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>60</td>
<td>118 ± 10</td>
<td>139 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Niacin (mg/d)</td>
<td>13³</td>
<td>17.3 ± 1.3</td>
<td>14.9 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Thiamin (mg/d)</td>
<td>1.0</td>
<td>1.39 ± 0.09</td>
<td>1.6 ± 0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Riboflavin (mg/d)</td>
<td>1.2</td>
<td>1.61 ± 0.12</td>
<td>1.54 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>B₆ (mg/d)</td>
<td>1.6</td>
<td>1.54 ± 0.11</td>
<td>1.84 ± 0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>B₁₂ (µg/d)</td>
<td>2.0</td>
<td>3.79 ± 0.62</td>
<td>2.09 ± 0.34</td>
<td>0.02</td>
</tr>
<tr>
<td>Folic Acid (µg/d)</td>
<td>180</td>
<td>237 ± 15</td>
<td>275 ± 21</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹Recommended Dietary Allowances for Women age 51+ yrs.
²Values are means ± SEM.
³Niacin equivalents.
<table>
<thead>
<tr>
<th>Mineral</th>
<th>RDA</th>
<th>Nonvegetarian Women (n=31)</th>
<th>Vegetarian Women (n=29)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mg/d)</td>
<td>---</td>
<td>2019 ± 91&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1966 ± 133</td>
<td>NS</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>10</td>
<td>11.0 ± 0.6</td>
<td>13.5 ± 0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>12</td>
<td>7.7 ± 0.7</td>
<td>7.5 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>800</td>
<td>691 ± 57</td>
<td>601 ± 44</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>800</td>
<td>1047 ± 66</td>
<td>1035 ± 55</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup>Recommended Dietary Allowances for women 51+ yrs.
<sup>2</sup>Values are means ± SEM.
<table>
<thead>
<tr>
<th>Physical Parameter</th>
<th>Year</th>
<th>Difference</th>
<th>p¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1976</td>
<td>1991</td>
<td></td>
</tr>
<tr>
<td>Nonvegetarian Women²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>56.1</td>
<td>71.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.2</td>
<td>161.9</td>
<td>-1.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.9</td>
<td>66.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>25.1</td>
<td>25.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Triceps Skinfold (mm)</td>
<td>28.8</td>
<td>23.6</td>
<td>-5.2</td>
</tr>
<tr>
<td>Vegetarian Women³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>55.0</td>
<td>70.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.6</td>
<td>161.0</td>
<td>-1.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.3</td>
<td>60.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>22.4</td>
<td>23.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Triceps Skinfold (mm)</td>
<td>25.0</td>
<td>20.6</td>
<td>-4.4</td>
</tr>
</tbody>
</table>

¹Differences and p values calculated by "paired t-test".
²In general n=31.
³In general n=29.
### Table VI
LONGITUDINAL CHANGES OF MEAN DIETARY INTAKES FROM 1976 TO 1991 CALCULATED FROM 7-DAY DIET RECORDS

<table>
<thead>
<tr>
<th>Dietary Factor</th>
<th>1976</th>
<th>1991</th>
<th>Difference</th>
<th>p&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonvegetarian Women (n=31)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kcalories/d</td>
<td>1608</td>
<td>1450</td>
<td>-158</td>
<td>0.004</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>195</td>
<td>190</td>
<td>-5</td>
<td>NS</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>69.7</td>
<td>61.3</td>
<td>-8.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Fat (total g/d)</td>
<td>62.8</td>
<td>52.2</td>
<td>-10.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Fat (satd, g/d)</td>
<td>22.3</td>
<td>16.0</td>
<td>-6.3</td>
<td>0.0002</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>302</td>
<td>173</td>
<td>-129</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>4.4</td>
<td>4.6</td>
<td>0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Caffeine (mg/d)</td>
<td>240.7</td>
<td>122.7</td>
<td>-118.0</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>Vegetarian Women (n=29)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kcalories/d</td>
<td>1634</td>
<td>1465</td>
<td>-169</td>
<td>0.03</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>226</td>
<td>226</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>56.8</td>
<td>50.4</td>
<td>-6.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Fat (total g/d)</td>
<td>62.9</td>
<td>46.8</td>
<td>-16.1</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fat (satd, g/d)</td>
<td>14.3</td>
<td>10.4</td>
<td>-3.9</td>
<td>0.006</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>114</td>
<td>73</td>
<td>-41</td>
<td>0.01</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>6.7</td>
<td>7.2</td>
<td>0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Caffeine (mg/d)</td>
<td>11.8</td>
<td>6.3</td>
<td>-5.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup>Differences and p values calculated by "paired t-test".
Table VII
LONGITUDINAL CHANGES OF MEAN VITAMIN INTAKES FROM 1976-1991 CALCULATED FROM 7-DAY DIET RECORDS

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Year</th>
<th>Difference</th>
<th>p 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1976</td>
<td>1991</td>
<td></td>
</tr>
<tr>
<td>Nonvegetarian Women (n=31)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A (µgRE/d)</td>
<td>2106</td>
<td>1670</td>
<td>-436</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>122</td>
<td>118</td>
<td>-4</td>
</tr>
<tr>
<td>Niacin (mg/d)</td>
<td>19.3</td>
<td>17.3</td>
<td>-2.0</td>
</tr>
<tr>
<td>Riboflavin (mg/d)</td>
<td>2.0</td>
<td>1.61</td>
<td>-0.39</td>
</tr>
<tr>
<td>Thiamin (mg/d)</td>
<td>1.4</td>
<td>1.39</td>
<td>-0.01</td>
</tr>
<tr>
<td>B6 (mg/d)</td>
<td>1.58</td>
<td>1.54</td>
<td>-0.04</td>
</tr>
<tr>
<td>B12 (µg/d)</td>
<td>9.75</td>
<td>3.79</td>
<td>-5.96</td>
</tr>
<tr>
<td>Folic Acid (µg/d)</td>
<td>257</td>
<td>237</td>
<td>20</td>
</tr>
<tr>
<td>Vegetarian Women (n=29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A (µgRE/d)</td>
<td>2705</td>
<td>2450</td>
<td>-255</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>149</td>
<td>139</td>
<td>-10</td>
</tr>
<tr>
<td>Niacin (mg/d)</td>
<td>16.0</td>
<td>14.9</td>
<td>-1.1</td>
</tr>
<tr>
<td>Riboflavin (mg/d)</td>
<td>1.68</td>
<td>1.54</td>
<td>-0.14</td>
</tr>
<tr>
<td>Thiamin (mg/d)</td>
<td>1.78</td>
<td>1.6</td>
<td>-0.18</td>
</tr>
<tr>
<td>B6 (mg/d)</td>
<td>1.76</td>
<td>1.84</td>
<td>0.08</td>
</tr>
<tr>
<td>B12 (µg/d)</td>
<td>2.89</td>
<td>2.09</td>
<td>-0.80</td>
</tr>
<tr>
<td>Folic Acid (µg/d)</td>
<td>283</td>
<td>275</td>
<td>-8</td>
</tr>
</tbody>
</table>

1Difference and p values calculated by "paired t-test".
The decrement in mean sodium intake of NV (351 mg/d) was significant and that of V (243 mg/d) followed this trend (Table VIII). Iron intake significantly declined in NV subjects (2 mg/d). No significant changes in the intakes of calcium, zinc, and phosphorus were observed over the 15-yr period. Mean intakes of zinc were below the RDA of 12 mg/d at both the initial and follow up measurements for both V and NV subjects. Calcium intake of the NV group met the RDA in 1976 (801 mg/d) but fell below in 1991 (691 mg/d). The V mean calcium intake was also below the RDA in 1976 (689 mg/d) and again in 1991 (601 mg/d).

The regression equations developed using GLMM (150) to predict bone density for either the radius or ulna are shown in Figures 1 and 2, respectively. Of the original variables (age, energy, body weight, dietary calcium, diet group (NV or V), protein, and use of estrogen) energy and protein were nonsignificant and thus were deleted from the model, leaving age, weight, calcium, diet group (NV or V), and use of estrogen as significant predictor variables for bone density of the radius and ulna (see appendix B for ANOVA TABLES). Both linear and quadratic terms for age, as well as interactions with other terms were included to form the final model. Addition of supplemental calcium (non-food calcium) to the model produced Type I sums of squares which were nonsignificant, indicating calcium supplements did not contribute to the prediction of bone density beyond the contribution of dietary calcium. Intercepts were increased in both diet groups (NV and V) by estrogen use and in both estrogen and non-estrogen use groups by consumption of meat.

Bone density of NV subjects also was influenced by age by diet and age by age by diet interactions, that did not influence V bone density. Thus, the simplest equation is for the V and the most complex equation is for NVE.
TABLE VIII
LONGITUDINAL CHANGES OF MEAN MINERAL INTAKES FROM 1976-1991 CALCULATED FROM 7-DAY DIET RECORDS

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Year</th>
<th></th>
<th>Difference</th>
<th>p^1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1976</td>
<td>1991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonvegetarian Women (n=31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>2370</td>
<td>2019</td>
<td>-351</td>
<td>0.01</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>13.0</td>
<td>11.0</td>
<td>-2.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>9.9</td>
<td>7.7</td>
<td>-2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>801</td>
<td>691</td>
<td>-110</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>1181</td>
<td>1047</td>
<td>-134</td>
<td>NS</td>
</tr>
<tr>
<td>Vegetarian Women (n=29)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>2209</td>
<td>1966</td>
<td>-243</td>
<td>0.09</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>13.6</td>
<td>13.5</td>
<td>-0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>7.4</td>
<td>7.5</td>
<td>0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>689</td>
<td>601</td>
<td>-88</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>1049</td>
<td>1035</td>
<td>-14</td>
<td>NS</td>
</tr>
</tbody>
</table>

^1Differences and p values calculated by "paired t-test".
**Vegetarian/Non-estrogen User**

Bone Density = 0.4726 + age (0.0082) - (age * age) (0.0001) + body weight (0.0011) + calcium (0.00003)

**Vegetarian/Estrogen User**

Bone Density = 0.4726 + age (0.0082) - (age * age) (0.0001) + body weight (0.0011) + calcium (0.00003) + 0.0391

**Nonvegetarian/Non-estrogen User**

Bone Density = 0.4726 + age (0.0082) - (age * age) (0.0001) + body weight (0.0011) + calcium (0.00003) + 0.7410 - (age * diet) (0.0236) + (age * age * diet) (0.0002)

**Nonvegetarian/Estrogen User**

Bone Density = 0.4726 + age (0.0082) - (age * age) (0.0001) + body weight (0.0011) + calcium (0.00003) + 0.7410 - (age * diet) (0.0236) + (age * age * diet) (0.0002) + 0.0391

**Figure 1.** Regression equations for bone density (g/cm$^2$) of the radius in postmenopausal women including: age (yrs), body weight (kg), dietary calcium (mg/d), diet (1 for nonvegetarians; 0 for vegetarians), and use of estrogen (1 for those receiving ERT; 0 for those not receiving ERT).
**Vegetarian/Non-estrogen User**

Bone Density = 1.0503 - age (0.0091) + (age * age) (0.00002) + body weight (0.0009) + calcium (0.00001)

**Vegetarian/Estrogen User**

Bone Density = 1.0503 - age (0.0091) + (age * age) (0.00002) + body weight (0.0009) + calcium (0.00001) + 0.0264

**Nonvegetarian/Non-estrogen User**

Bone Density = 1.0503 - age (0.0091) + (age * age) (0.00002) + body weight (0.0009) + calcium (0.00001) + 0.5993 - (age * diet) (0.019) + (age * age * diet) (0.00015)

**Nonvegetarian/Estrogen User**

Bone Density = 1.0503 - age (0.0091) + (age * age) (0.00002) + body weight (0.0009) + calcium (0.00001) + 0.5993 - (age * diet) (0.019) + (age * age * diet) (0.000015) + 0.0264

Figure 2. Regression equations for bone density (g/cm$^2$) of the ulna in postmenopausal women including: age (yrs), body weight (kg), dietary calcium (mg/d), diet (1 for nonvegetarians; 0 for vegetarians), and use of estrogen (1 for those receiving ERT; 0 for those not receiving ERT).
Figure 3A depicts the bone density (g/cm²) of the radius of postmenopausal women age 50-80 yrs., assuming a reference weight of 65 kg and calcium intake of 800 mg for the 4 groups (NVE, NV, VE, V) using regression equations presented previously in Figure 1. Figures 3 A1 - A4 are individual curves ± SD for each group, plus a plot of the actual data points for each group.

Similar curves are presented in Figure 4 for bone density of the ulna. Contrast statements (in GLMM these are called "Predict Statements") were made between the 4 groups at age 50, 60, 70, and 80 yrs for both the radius and ulna. At age 50 yr, predicted bone density of the radius was significantly greater in the NV than the V group. The ulna demonstrated a similar trend at age 50 yr. At age 80 yr, NV bone density was also significantly higher than that of V for both bones. Use of ERT by both diet groups significantly increased bone density of the radius. The ulna exhibited a similar but nonsignificant rise in bone density with estrogen use. At age 80 yr the ulna of the NVE group had a trend for greater bone density than VE at a level of p < 0.07.

Comparisons of mean daily urinary excretion of creatinine, HOP, and calcium for 1991 are reported in Table IX. NV had higher urinary excretion of calcium (2.36 mmol/24 hr) than V subjects (1.38 mmol/24 hr). Mean values of HOP excretion were higher in NV than V, the difference between groups was not significant. There was a trend for NV (7.2 mmol/24 hr) to have creatinine excretion greater than V (5.7 mmol/24 hr).
Figure 3A. Predicted bone density (g/cm²) of the radius versus age (yr) given weight of 65 kg and dietary calcium intake of 800 mg for nonvegetarian (NV), vegetarian (V), estrogen supplemented nonvegetarian (NVE) and estrogen supplemented vegetarian (VE) postmenopausal women.

Figure 3 A1-A4 are Predicted bone density ± SD plus actual data points for each group: A1 = NVE; A2 = NV; A3 = VE; A4 = V.
Figure 4A. Predicted bone density (g/cm²) of the ulna versus age (yr) given weight of 65 kg and dietary calcium intake of 800 mg for nonvegetarian (NV), vegetarian (V), estrogen supplemented nonvegetarian (NVE) and estrogen supplemented vegetarian (VE) postmenopausal women.

Figure 4 A1-A4 are Predicted bone density ± SD plus actual data points for each group: A1 = NVE; A2 = NV; A3 = VE; A4 = V.
<table>
<thead>
<tr>
<th></th>
<th>Nonvegetarian (n=29)</th>
<th>Vegetarian (n=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mmol/24 hr)</td>
<td>7.2 ± 0.6&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.7 ± 0.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Hydroxyproline (mg/24 hr)</td>
<td>11.6 ± 1.9</td>
<td>8.4 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium (mmol/24 hr)</td>
<td>2.36 ± 0.25</td>
<td>1.38 ± 0.19</td>
<td>0.0028</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means ± SEM
Creatinine excretion was found to be negatively correlated ($p < 0.04$) with loss of bone density in both diet groups (NV, ulna; V, radius see Table X). There was a trend ($p = 0.10$) for HOP excretion to be correlated with the current bone density of the ulna in V. In a correlational analyses not presented in table form, urinary HOP and calcium excretion were correlated ($p < 0.03$) in both NV ($r=0.4365$) and V ($r=0.4211$) groups. In NV, urinary HOP and creatinine were correlated ($r=0.6095; p < 0.0004$) and urinary calcium and creatinine showed a trend ($r=0.3230; p < 0.09$) toward being correlated.
### Table X

**Correlation Coefficients Between Bone Density and Urinary Components of Nonvegetarian and Vegetarian Postmenopausal Women**

<table>
<thead>
<tr>
<th></th>
<th>Hydroxyproline</th>
<th>Calcium</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NV1</td>
<td>V2</td>
<td>NV</td>
</tr>
<tr>
<td>1991 Bone Density</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(radius)</td>
<td>0.221</td>
<td>0.318</td>
<td>-0.160</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.10</td>
<td>0.41</td>
</tr>
<tr>
<td>1991 Bone Density</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ulna)</td>
<td>0.091</td>
<td>0.267</td>
<td>-0.116</td>
</tr>
<tr>
<td></td>
<td>0.65</td>
<td>0.17</td>
<td>0.56</td>
</tr>
<tr>
<td>Loss of Bone Density</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(radius)</td>
<td>0.170</td>
<td>0.226</td>
<td>-0.130</td>
</tr>
<tr>
<td></td>
<td>0.38</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>Loss of Bone Density</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ulna)</td>
<td>0.10</td>
<td>-0.016</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>0.62</td>
<td>0.94</td>
<td>0.55</td>
</tr>
</tbody>
</table>

1. n=28
2. n=30
3. r value
4. p value
CHAPTER V

DISCUSSION

PHYSICAL PARAMETERS

Physical measurements of both NV and V subjects were within the range of height, weight, BMI, and TSF described for healthy ambulatory white females age 65-94 yrs. (84). Mean heights of both groups were in close agreement with those of Nieman et al. (28) and Hunt et al. (30) who studied similar populations. Greater body weight and BMI in elderly NV than V women have been reported previously (28). The BMI (25.4 kg/m$^2$) of the NV group is at the 50th percentile for women age 70-74 yrs calculated from NHANES I data (91), while BMI for V (23.4 kg/m$^2$) is below the 50th percentile, neither the NV nor the V group approached the 85th percentile (31.70 kg/m$^2$) used to designate obesity (90). TSF of 19 and 20 mm have been determined to be the 50th percentile for females age 70 with medium and large bone, respectively (85). Although body frame was not measured in those subjects, TSF measurements of NV and V were around the 50th percentile (20 mm). Both BMI and TSF measurements support the conclusion that these NV and V women were neither under nor overweight (obese) in 1991.
DIETARY INTAKES

Energy intakes of NV (1450 kcal/d) and V (1465/d) are similar to those reported by others (28,30), but lower than those reported by Marsh et al. (63), Tylvasky and Anderson (28,30), and Millet et al. (33). Adequate energy intake is important in that it has been hypothesized that decrements in nutrient may be due in part to reduced total energy intake.

Although Marsh et al. (63) found no differences between NV and V intakes for any nutrients, several other studies reported data similar to the findings of this study, i.e., carbohydrate and fiber intakes were higher in the V than NV group (28, 30, 64, 121). Protein intakes are widely reported to be greater in NV than V (28, 30, 64, 121). In studies reviewed (28, 29, 30, 63, 64, 121), the 50 g RDA for protein was met or exceeded by both the NV and V group. Likewise, in this study, protein and energy intakes were adequate in the NV and V groups. Similar to findings of Nieman et al. (28), Pedersen et al. (121) and Marsh et al. (63), no difference between NV and V fat intake was found. In other studies NV fat intake was reported to be greater than that of the V group (29, 30, 64). Similar to this study, cholesterol intake has been reported to be greater in NV than V (28, 30, 64, 121). Caffeine intakes also are consistently reported to be greater in NV than V (28, 30, 121), but this may be more indicative of the specific V population surveyed than representative of all V populations. In this study, and many others, Seventh Day Adventists were used as a source of V population. Not only do many Seventh Day Adventists restrict meat intake, but they
also restrict caffeine consumption as a part of their religious practice. The findings of Nieman et al. (28) for percentage calories from carbohydrate, protein, and fat were identical to those in this study. While Hunt et al. (30) reported saturated fat intakes to be greater in NV than V which supports findings in the present study, Peterson et al. (121) found no such differences between NV and V. Dietary data for the women of the current study seem to be consistent with the findings of others, i.e., intakes of protein, saturated fat, and cholesterol were greater in NV than V, and carbohydrate and fiber intakes were greater in V than NV, suggesting that the subjects in this study are representative of their diet groups.

VITAMIN INTAKES

Little difference was found between NV and V vitamin intakes except for vitamins B₆ and B₁₂. The Vitamin B₆ intake of V was found to be greater than NV, Pederson et al. (121) reported similar findings. Vitamin B₆ intakes have been reported to be below the RDA (1.6 mg/d) for NV (28) and for both NV and V (31,29). Another study's findings reported Vitamin B₆ intake to meet the RDA for both NV and V (121). NV vitamin B₆ intakes are at the RDA level. This RDA was determined for healthy women age 51+ yrs. In older populations atrophic gastritis and/or other age-related malabsorptive conditions may indicate the need for additional age categories beyond that of 51 yrs. Whether or not the RDA would change for females age 70 yr remains to be seen. As Vitamin B₁₂ is found only animal food
sources, it seems reasonable that NV intake would be greater than that of V (29). But
use of Vitamin B$_{12}$ fortified milk and meat analogues may account for some studies
reporting no differences in Vitamin B$_{12}$ intake between NV and V (28, 63). Niacin
(28, 64), thiamin (29, 63, 64), riboflavin, (28, 29, 31, 63) and folic acid (28, 121)
intakes are reported not to differ between NV and V groups. In this study there was a
trend for V to have a larger thiamin intake than NV; Nieman et al. (28) and Millet et
al. (33) reported the larger thiamin intake of V to be significant. Vitamin C intake has
consistently been reported not to differ between NV and V (29, 31, 63, 64). Vitamin
A intakes are consistently reported to be similar for NV and V (29, 31, 63, 64, 121).
There was a trend for Vitamin A intakes to be greater in V than NV in this study;
Nieman et al. (28) reported V intakes of Vitamin A to be significantly lower in NV
than V. Vitamin intakes, of the subjects in the current study are generally adequate,
suggesting these women have nutrient dense caloric intakes, not empty calories as is
often speculated for aging females (21).

**MINERAL INTAKES**

Mineral intakes of NV and V postmenopausal females are consistently reported to
be similar. No significant differences between NV and V were reported in studies by
Nieman et al. (28), Pederson et al. (121), and Tylvasky and Anderson (64). The
intakes of sodium were less than 3g/d and are within the suggested dietary guidelines.
The difference observed in NV and V iron intakes in this study was not expected as others reported no differences between the groups (28, 63, 121, 64). It is particularly unusual that V have the larger iron intake as meat is a major source. The V subjects in this study consumed dark green leafy vegetables, dried fruits, and legumes which are all good iron sources. Although iron sources of V would be non-heme sources, their adequate Vitamin C intake would facilitate optimum utilization non-heme iron.

Although no significant difference was observed in zinc intake of NV and V in this study and in that of others (28, 121), intakes were below the RDA for both groups in this study. Nieman et al. (28) reported both NV and V and Pedersen et al. (121) reported NV to be below RDA. Phosphorous intakes met the RDA and showed no significant differences between NV and V groups (28, 121).

Calcium intakes were reported to be similar in NV and V women in this and other studies (28, 29, 30, 63, 121). While calcium intake met the RDA in some studies (29, 63, 121), findings of calcium intakes below the RDA, similar to that of this study, were reported by others (28, 30). Low calcium intakes are of particular concern in this study. The limiting effects of calcium in the skeleton are exhibited when calcium intake is sub-optimal (4, 70). Low calcium intake has been associated with decreased bone mass (43).

Reduction in sodium intake in NV and V is commendable. Overall decrements in other mineral intakes over the 15-yr period were nonsignificant for NV and V.
Although zinc and calcium intakes did not significantly decline, intakes habitually below the RDA are of concern in this population for both calcium and zinc.
CHANGES

PHYSICAL PARAMETERS

Few longitudinal studies have been made of nutrient intakes, physical parameters, hormone use, and bone density. In this study, similar age-associated decrements were demonstrated for height in NV and V. This loss in stature emphasizes the relationship of the aging process to the maintenance of vertebral bone density. Regardless of group, NVE, NV, VE, and V, bone density of the radius and ulna steadily declined with age. Although NV weight remained constant over the 15 yr period, V demonstrated gain, which coupled with their loss in height yielded a significant increase in BMI. Similar findings of decreased height and increased weight were reported in a twelve year follow-up study of postmenopausal women (6). Body weight has been reported to show significant age-associated decrements (21), perhaps these studies include institutionalized or infirm subjects, or perhaps this decrement occurs beyond age 70.5 yr, the mean age in the current study. In a 25-yr follow-up of NV men (age 65-84 yrs) body weight was reported to significantly increase overall but actually there was no significant changes noted over the last 15 yr study (31).

The increased BMI of the V subjects still does not place subjects in a percentile indicative of obesity (91). TSF significantly declined over the 15-yr period for NV and V. This decline in TSF is similar to that reported by Must et al. (90). Frisancho et al. (85) reports decrements in TSF at the 50th percentile for females (55-74 yrs)
with small, medium, or large frames. The decrements for NV and V are construed to be a normal part of the aging process. Only minor changes in body weight occurred in this study. Change in body composition was evidenced as TSF demonstrated significant decrements in both NV and V.

**DIETARY CHANGES**

Over the 15-yr period the dietary changes made by the NV and V groups were remarkably similar. Energy intakes for both groups showed significant decrements. This is in line with many cross-sectional studies that report energy intakes decrease with age (29, 88). In the Zutphen 25-yr longitudinal study of males (31), energy decrements with age were noted. Despite the decrements noted in the present study, the energy intakes are within the 1400-2000 kcal/d range recommended for women age 51-75 albeit barely, and are actually closer to the 1200-2000 kcal/d range for women age 76+ yrs.

Carbohydrate intakes demonstrated almost no changes over the period of this study for either the NV or V groups. The groups did increase the percentage of their total energy intake from carbohydrate over the 15 yr-period.

Protein intakes for both groups demonstrated decrements. This was not in keeping with the Zutphen Study (31) which reported significant increases in protein intake accounted for by the increased intakes of animal protein. The different outcomes between this and the Zutphen studies could be due to gender or cultural differences.
The Zutphen Study was conducted on males in the Netherlands. Although mean protein intakes declined in the present study, the percentage of total energy intake obtained from protein remained constant in both groups. Protein intake in neither group was excessive and the intake level of the V group only met the RDA for women age 51+ yrs. Continued decrements in protein intake could prove detrimental to this group.

Total fat and saturated fat decrements were significant for both NV and V in this and the Zutphen study (31). In this study, there is a difference in 1976 and 1991 percentage of fat coming from saturated fat for NV and V. The NV group reduced its percentage of fat from saturated fat from 36 to 30%, while the V groups change was from only 23-22%. Of course, the NV group had greater room for improvement as the V group had already consumed less the one-third of its fat in the form of saturated fat in 1976. The greater reduction saturated fat intake of NV as compared to V may explain the larger reduction in cholesterol intake seen in the NV as compared to the V. Increased cholesterol intakes of the Zutphen Study may be related to the increased fat intake reported in their subjects (27).

Although fiber and caffeine intakes demonstrated no significant changes over the 15-yr period for the V group, caffeine did significantly decline in the NV group. Here again, it is the NV group that had the greatest room for change.

The dietary changes made by both groups agreed with national goals. The NV groups decrease in fat intake was favorable in achieving the recommended goal of 58,
12 and 30% of energy needs being supplied by carbohydrate, protein, and fat. V groups dietary changes enabled the group to actually meet those recommendations!

**VITAMIN AND MINERAL CHANGES**

In this study few changes in vitamin and mineral intakes were noted for either group over the 15-yr period. This is in direct contrast to the longitudinal changes seen in the Zutphen Study (31) where intakes of all studied micronutrients significantly declined except Vitamin A which increased and Vitamin C which exhibited no significant change.

Both NV and V groups in this study demonstrated declines in Vitamin B$_{12}$ intake. Vitamin B$_{12}$ normally only occurs in animal food products, coupled with the fact that this decrement corresponds with the decrement in protein intakes seen in both groups, suggesting that the reduction in protein was primarily due to reductions in animal source protein. Of course this only may be true for NV and the V could have just reduced their consumption of Vitamin B$_{12}$ fortified products but this does not seem likely as reductions in egg and milk consumption in those lacto-ovo-vegetarians. The 1976 to 1991 change in Vitamin B$_{12}$ intake for the V group is one that hopefully will not continue as these subjects intakes of Vitamin B$_{12}$ is presently at the RDA and any further reductions could be detrimental to their health.
NV sodium intake significantly declined and V intake exhibited a similar trend. The reduction in sodium intake was in keeping with the dietary guide suggesting excessive sodium intakes be avoided (10).

Although zinc intakes of NV and V subjects did not significantly change, neither group met the RDA in 1976 nor did they meet the RDA in 1991. The chronic insufficiency of dietary zinc, constitutes a threat to these subjects health.

Likewise, failure of both NV and V to increase their calcium intakes is of serious concern. Although NV met the RDA in 1976, the mean calcium intake in 1991 was below the RDA. The decline was not statistically significant, but it is of practical significance as it has been suggested that the RDA itself (800 mg/d) may be too low for optimum nutriment in aging females (70). These chronically low dietary calcium intakes may put these subjects, NV and V, at risk for osteoporosis if calcium is, indeed, a significant predictor of bone density as the results of this study indicate. A recent report on consistency of nutrient intakes concluded current intakes are not a good indicator of past intakes, especially for calcium (36).

Although caloric intake declined in both groups, intakes of vitamins and minerals were maintained, except for NV and V vitamin B12 intake. This suggested that although these subjects were consuming fewer calories, they were nutrient-dense calories, except for 1991 calcium and zinc intakes which remained below the RDA.
PREDICTORS OF BONE DENSITY

In an effort to identify variables which contributed to the prediction of bone density, the contribution of age, energy intake, body weight, dietary calcium intake, diet group (NV or V), protein intake, and use of ERT, all variables which had been either positively or negatively associated with bone density in previous studies were examined using GLMM (150). The terms were used to predict bone density of the radius and ulna with the goal of generating an equation that would best predict bone density for either bone. As the radius and ulna were subjected to the same variables and as both bones were in the nondominant arm of the same individual, it seemed that the generation of one equation would be appropriate. Calorie and protein intakes were found to be non-significant in both bones and thus these terms were discarded in subsequent analyses. Energy intake is often an indicator of nutrient intake such that when intake of calories is low, it predisposes nutrient intakes to be low, also. The results indicate these subjects caloric intakes were nutrient dense and thus not a contributor to the model. Along the same lines, high levels of protein intake, in particular animal protein, have been suggested to increase urinary calcium and thus exert a negative influence on bone density. In this study NV did have considerably higher creatinine excretion than V. Animal protein in the diet of the NV group could in part account for the higher excretion of creatinine seen in NV. NV and V protein intakes were only 20% above (NV) or equal to the RDA (V), thus neither group consumed the levels of protein indicating protein would be a potentially negative
factor on bone density. Hunt et al. (26) also reported that protein was not a significant term in their model for predicting bone density. On the other hand, Tylavsky and Anderson (64) reported protein to be a positive predictor of bone density. In their study NV consumed 69.9 g/d and V 54.6 g/d protein. The researchers had anticipated a negative effect because of the potential of protein-induced hypercalcuria, but they account for the positive effect resulting from a large need for protein in bone formation and overall bone-tissue maintenance. In this study, calcium excretion in the NV group was higher than that of V and this may be the result of the higher protein intake of NV subjects. But, urinary calcium in this study was not correlated to absolute values of bone density or to its loss over the 15-yr period in either NV or V. In fitting the model in the present study, after deletion of calories and protein from the regression model, quadratic terms for age and interaction terms for diet by age and estrogen by age were added. The significant quadratic for age and interaction term for diet and age is consistent with the accelerated bone loss perimenopause.

The final model developed included the terms: age, weight, dietary calcium, diet group (NV or V), and use of estrogen. It was no surprise that age was a predictor as bone density has consistently been shown to decline with age (6, 28, 30, 135).

Body weight also was found to be a significant predictor of bone density in the Hunt et al. study (30). Body weight was hypothesized to be a positive predictor of bone density in this study because a) of the added stress on bone related to increased body weight and b) changes in body composition seen with age whereby LBM decreases and body fat increases (although body weight, itself may not change). Body
fat favors estrogen status as adiposity and estrogen levels are correlated in a positive manner (123).

Controversial results have been previously reported for the relationship of dietary calcium to bone density. Much of the confusion in results may be related to whether current, previous, or lifetime calcium intakes were used, also differences in the site where the bone density measurement was performed, e.g. radius, vertebrae, or hip (31, 34). Even different sites on a given bone may have different bone densities. The distal forearm is approximately 60% trabecular, and the proximal forearm is mostly cortical bone and less than 5% trabecular (108). Thus, each site of the forearm yields a different bone density.

As to the benefit of calcium supplementation on bone density, Heaney (4) noted many studies showed that calcium supplements did not prevent bone loss. In most supplementation studies calcium has been used at the rate of 900-1500 mg/d (44, 45, 47). In a review of controlled clinical calcium supplementation trials, Dawson-Hughes (46) reported the maximal effect of supplementation occurs at doses of approximately 1000 mg/d. She also noted responsiveness of women to calcium supplementation was related to the number of years postmenopause. Women who are more than 6-yr postmenopausal benefit more than women in early menopause (45). It is of interest that the women in Dawson-Hughes’ study (45) had dietary calcium intakes of less than 400 mg (less than one-half the RDA). If calcium is a threshold nutrient (4), 400 mg/d calcium supplementation to 800 mg in these subjects should prove beneficial but supplementation of a dietary calcium of 800-1200 mg/d may not be significantly
beneficial. Calcium also was supplemented 2 yrs. In recent studies of NV and V bone density, current calcium intake was not significantly related to bone density of the radius (64, 30). As dietary calcium remained in the model in this study, supplemental calcium was added as a term in the model and proved to be non-significant. There is an inherent problem with this data as supplement use is not consistent and thus the possible benefits may not be seen due to intermittent use. The mean supplement intake of those taking supplemental calcium in this population was only 120 mg. A higher level of supplementation and, or more consistent use of supplements might have influenced our results. The fact that calcium remained in the model as a predictor is probably the result of longitudinal intake data and that the statistical analyses blocked this variable by subject. Needless to say, the issue of calcium supplementation is not resolved.

While type of diet (NV or V) has been related to bone density, e.g. V have a greater bone density, Hunt et al. (30) reported no difference in NV and V bone density of the radius. In the present study it was the NV rather than the V, who had higher bone density of the radius and ulna. Marsh et al. (63) reported that differences between NV and V are most pronounced at age 80, similar to the findings of this study. In light of recent research concerning vegetarians, hormonal status, and/or breast cancer (121, 122, 123, 124, 126, 128), the findings that NV have higher bone density seems reasonable. The latter studies consistently report plasma estrone and estradiol-17B to be lower in V than NV in pre-and post-menopausal women. While
lower circulating estrogen levels would be beneficial in terms of hormone-sensitive
tumor development, they would be deleterious to maintenance of bone density.

Recent reports have established the relationship of estrogen and bone density (7,
9, 11). Estrogen was a significant term in the model when entered as a "yes" or "no"
variable. Duration of use and level of intake were not included in the model,
indicating estrogen would likely be a stronger predictor of bone density if quantitative
terms were used. ERT is currently recommended to ameliorate the symptoms of
menopause, including prophylaxis of bone loss (9, 131).

A possible reason for the large number of predictor terms that remained in the
multiple-regression equation was probably related to not only their biological
importance to bone density itself, but in addition to the utilization of GLMM statistical
analyses (150). GLMM included an among-subject component or blocked for subject
allowing for recognition of the repeated measures component of this study. This
analyses also limited the standard error term by establishing a correlational structure
rather than considering the error terms independent. The use of improved statistical
techniques such as GLMM provides a more sensitive test of a term’s contribution to
the model.

The hypothesis that intakes of various nutrients would decrease over the 15-yr
period is accepted. Significant decrements in protein, total fat, saturated fat,
cholesterol, and vitamin B_{12} intakes were found in both NV and V groups. Caffeine,
sodium, and iron intakes of NV subjects demonstrated significant decrements that the
V group did not experience. These findings support the hypothesis that changes in
nutrient intakes would be different in the NV and V groups, although the patterns of decrements seen in NV and V were remarkably similar. The hypothesis stating that age-associated decrements in nutrient intakes would result in several nutrients not meeting the RDA is rejected. The only nutrients to not meet the RDA were zinc and calcium and their intake levels did not significantly change over the 15-yr period. Thus the fact that zinc and calcium fall below the level of the RDA is related to habitually low intakes rather than age-related decrements in intake of these nutrients.

The component of the hypothesis regarding bone density demonstrating age-associated decrements is accepted as both the radius and the ulna showed significant decrements over the 15 yr period. Density was greater in NV than in V, thus the hypothesis stating that V bone density would be greater than NV is rejected. It is of interest that the NV group bone density was higher at age 80 yr but not during the 50-70 yr age-period. Perhaps this is why cross-sectional studies made on subjects in this age range did not find differences in bone density between the groups (30, 64).

Rejection of the hypothesis stating that energy and protein are associated with bone density is necessary as energy and protein were not significant predictors of bone density in these subjects. The hypothesis stating dietary calcium would positively be associated with bone density was accepted. Calcium supplements did not contribute to bone density in these subjects.

The hypotheses stating a positive relationship exists for body weight and estrogen to bone density are accepted as both were significant positive contributions to bone density.
The findings of this study are the results of the generosity of 60 postmenopausal women. There is a notable absence of and a great need for longitudinal studies of aging females. Without such research, the controversies as to which diet group (NV or V) and calcium supplementation will not be resolved.
CHAPTER VI

SUMMARY

Sixty women (mean age 70.5 yr) consuming nonvegetarian (NV, n=31) and lacto-ovo-vegetarian (V, n=29) diets were studied as to longitudinal changes occurring in physical parameters including bone density and in nutrient intakes. Significant decrements were observed over a 15-yr period in both NV and V of height and intakes of energy, protein, fat, saturated fat, cholesterol, vitamin B₁₂, and sodium. NV also demonstrated decrements in intakes of caffeine and iron. V demonstrated an increase in BMI. Current age, height, and TSF of the 2 groups did not differ. There was a trend for NV to weigh more and to have a larger BMI than V. Current NV intakes of protein, saturated fat, cholesterol and caffeine were significantly higher than those of V. The V also had higher intakes of carbohydrate and fiber than the NV group. While NV had higher B₁₂ intakes than V, the V group had larger intakes of vitamin B₆ than NV. There were trends for V to have greater intakes of vitamin A and thiamin than NV. Mineral intakes of other nutrients were not significantly different except for iron. RDA's were met or exceeded by both groups in 1991 for all nutrients except zinc and calcium.

Bone density demonstrated age-associated decrements of both the radius and the ulna. Body weight, dietary calcium, diet group (NV or V) and use of estrogen all positively contributed beyond age (a negative predictor) to the prediction of bone
density of the radius and ulna in these subjects. Neither energy, nor protein intake, nor calcium supplementation were significant terms in the model predicting bone density.

The inclusion of dietary calcium in the model as a significant predictor combined with intakes of calcium below the RDA is of concern in this population. Bone density was found to be highest in NVE users and lowest in V non-estrogen users.

Habitually low calcium and zinc intakes were remarkable for NV and V. Chronically low calcium intake coupled with the absence of estrogen seen in menopause, could synergistically function to accelerate the loss of bone associated with the aging process in these NV and V postmenopausal women.
LITERATURE CITED
LITERATURE CITED


APPENDICES
I agree, as indicated by my signature below, that:

(1) I would like to participate in the Nutrition and Bone Density Project approved and administered by the professional staff of the Tennessee Agricultural Experiment Station and the College of Human Ecology, University of Tennessee, Knoxville;

(2) I understand that exposure to ionizing radiation, such as used in this study to assess bone status, may be harmful to me. I also understand that the amount of radiation to which I will be exposed is similar to or less than the amount I would be exposed to by a dentist in obtaining a full mouth set of dental x-rays.

(3) I understand that because of the exposure to the radiation that if there is a possibility I am pregnant I should not participate.

(4) I understand that participation in this program is not likely to harm me and that no specific benefits or effects are guaranteed other than information from the assessment of my bone density and nutrient intake;

(5) It is my understanding that each aspect of the project in which I am asked to participate will be explained to me and that I may withdraw from participation at any time if involvement is unacceptable to me;

(6) All results will be treated with strict confidence, all individuals will remain anonymous in reporting any results, and all results will be handled in a professional manner;

Your continued participation in the University of Tennessee's bone density research is greatly appreciated. As in the past, we will be looking at the role of dietary intake on bone density. Measurements required in this study include:

I. Height, weight, and skin-fold thickness

II. Medical, dietary, and physical activity histories

III. Measurement of bone density
IV. 3- or 7-day dieting records.

(Procedures I through III will be obtained at our initial meeting.) Dietary records (IV) will be returned upon completion.

By my signature, I indicate that the research has been explained to me in detail and that I understand that any further questions that I may have about the project will be answered for me by the project director or some other designated member of the project staff.

Signed: _______________________________________________
Witness: _______________________________________________
Date: _______________________________________________

Roy E. Beauchene, Ph.D.
Professor of Nutrition
College of Human Ecology
The University of Tennessee
Knoxville, TN 37996-1900

Telephone: 974-5445
           974-6255
UNIVERSITY OF TENNESSEE
NUTRITION RESEARCH
INSTRUCTION SHEET FOR RECORDING FOOD INTAKE

We would like a record of what you eat for ___ days.

Please read carefully the instructions below before you start to list the foods you have eaten.

Please record foods and snacks as they are eaten rather than trying to do a recall at the end of the day. If you need more space, use the back of the sheet.

1. WRITE DOWN EVERYTHING THAT YOU EAT.
   If you miss a meal, write "nothing" in the space for that meal.

2. BE SURE TO WRITE DOWN THE KIND OF FOOD YOU EAT (KIND).
   Example: Cereal - Oatmeal, shredded wheat, cornflakes, etc.
   Bread - Whole wheat, white, rye; also commercial or homemade
   Meat - Roast beef, hamburger, veal steak, pork chops, etc.
   Salad - Head lettuce, canned fruit, tuna, cottage cheese, etc.
   Milk - Whole, 2%, skim, canned, etc.

3. DESCRIBE SPECIFICALLY HOW EACH FOOD IS PREPARED (STATE).
   Example: Egg - fried, boiled, scrambled, etc.
   Meat - broiled, breaded, fried, baked, etc.
   Fruits and vegetables - fresh, frozen or canned
   Vegetables - creamed, buttered, mashed, baked, etc.

   If food is not cooked, but eaten raw, write "RAW".

4. WHEN DIFFERENT FOODS ARE COMBINED WRITE DOWN EACH FOOD INCLUDED AND THE AMOUNT OF EACH FOOD.
   Example:
   Raw Salad
   lettuce 1/2 cup Cheese Sandwich
   tomato 1 slice bread
   cucumber 2 slices cheddar cheese
   french dressing 1 tablespoon lettuce
   french dressing 1 tablespoon mayonnaise

5. WHEN YOU EAT OTHER COMBINATION FOODS, SUCH AS CASSEROLES, DISHES, SOUPS, STEWS, PUDDINGS, ETC., WRITE DOWN THE INGREDIENTS IF HOMEMADE OR SIMPLY THE BRAND NAME IF A CONVENIENCE OR STORE-Bought ITEM IS USED.
   Example: Soup - Campbell's Tomato made with water.

6. WRITE DOWN THE AMOUNT OF EACH FOOD YOU EAT. Use a standard measuring cup, teaspoon or tablespoon, and a ruler to "measure" your food. Write down how many level teaspoons (t), tablespoons (T) you eat or whether you eat 1/2 or 1/3 or 1 cup, etc. Write down the number of slices or pieces. For example: pineapple, canned, 1 slice or apple, raw, 1 whole. Do not write down "glasses", "bowls", or "plates" for any foods such as milk, soup, vegetables, etc. Use the utensils provided to determine the amount.
   Example: Soup - Campbell's Tomato, 1 cup.
The ruler should be used for foods that cannot be measured with a measuring cup, teaspoon or tablespoon. Some examples are cake, meat, pancakes, pies, etc. For foods with a round shape such as rolls, pancakes, meat patties, cupcakes, etc., the diameter and thickness should be measured. For all other shapes, length, width and thickness should be measured.

Example: pancake - 1-8" diameter, 1/4" thick
choc. cake - iced, 1 piece, 2" x 3" x 1"
baked ham - 1 slice, 4" x 3" x 1/4"
pie - give measurements in inches, or tell whether it is a 1/4th or 1/8th, etc. of a 8", 9" or 10" pie (diameter of whole pie)

7. BE SURE TO WRITE DOWN THE FOODS YOU ADD TO OTHER FOODS AND THE AMOUNT SUCH AS THE SUGAR, CREAM, OR BUTTER YOU USE.

Example: - the amount of sugar or cream used on cereal, fruit or in tea and coffee
- the amount of butter on vegetables or bread
- the amount of jelly on toast or syrup on pancakes

Remember to record in level teaspoons or tablespoons; then if you want more, take it, just remember to add that amount too.

SAMPLE RECORDINGS:

<table>
<thead>
<tr>
<th>FOOD</th>
<th>KIND AND STATE</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>cereal</td>
<td></td>
<td>3/4 cup</td>
</tr>
<tr>
<td>sugar</td>
<td>oatmeal</td>
<td>2 tablespoons</td>
</tr>
<tr>
<td>cream</td>
<td>half and half</td>
<td>1/4 cup</td>
</tr>
<tr>
<td>pancake</td>
<td>Hungry Jack Pancake Mix</td>
<td>1, 6&quot; diam., 1/4&quot; thick</td>
</tr>
<tr>
<td>egg</td>
<td>fried</td>
<td>1 large</td>
</tr>
<tr>
<td>meat</td>
<td>baked ham</td>
<td>4&quot; x 2&quot; x 1&quot;</td>
</tr>
<tr>
<td>potatoes</td>
<td>mashed, with milk and butter</td>
<td>3/4 cup</td>
</tr>
<tr>
<td>peas</td>
<td>canned</td>
<td>1/2 cup</td>
</tr>
<tr>
<td>butter on peas</td>
<td></td>
<td>1/2 teaspoon</td>
</tr>
<tr>
<td>milk</td>
<td>whole</td>
<td>1 cup</td>
</tr>
<tr>
<td>cake</td>
<td>chocolate, iced</td>
<td>2&quot; x 2&quot; x 1&quot;</td>
</tr>
</tbody>
</table>

8. LIST AMOUNT AND BRAND OF ANY VITAMIN/MINERAL SUPPLEMENTS YOU TAKE.

9. IF YOU HAVE QUESTIONS, PLEASE DO NOT HESITATE TO CALL SUSAN MUNROE AT 974-5445.
<table>
<thead>
<tr>
<th>FOOD</th>
<th>KIND &amp; STATE</th>
<th>AMOUNT</th>
</tr>
</thead>
</table>

**BREAKFAST**

**BETWEEN MEALS**

**NOON MEAL**

**BETWEEN MEALS**

**EVENING MEAL**

**AFTER EVENING MEAL**

**SUPPLEMENTS:**

- **VITAMIN:**
- **MINERAL:**
- **OTHER:**
- **BRAND:**
  
  **AMOUNT:**
PHYSICAL HISTORY

Name___________________ Experiment No._________ Date__________

I. Has your physician diagnosed any chronic illness such as diabetes, arthritis, lupus, etc.?  
   No_______  Yes_______  If yes, what__________________________
   How long ago were you diagnosed?___________________________

II. FRACTURE HISTORY
   Have you had a broken bone? No_______  Yes________
       If yes, which bone_________________________  When____________

III. SMOKING HISTORY
   Have you ever smoked cigarettes? No_______  Yes_______
       If yes: 1) Are you currently a smoker?  
              No_______  Yes_______
              For what length of time were you a smoker?
              a) less than 1 year
              b) 1-3 years
              c) 4-6 years
              d) 7-10 years
              e) 10-15 years
              f) more than 15 years
       In general, how many cigarettes do/did you smoke?
              a) 1/2 pack per day
              b) 1 pack per day
              c) 1-2 packs per day
              d) 2 packs per day

IV. Pharmacological History

   Are you routinely taking any prescription or over the counter medications? No_______  Yes_______
   If yes, please name and dose:
   1.
   2.
   3.
   4.
   5.
V. Reproductive History

Age at menarche__________
Age at menopause_________
Was menopause spontaneous or artificial (circle one)

Do you take Estrogen Replacement Therapy (ERT)?
No_______ Yes_______

If yes:
   a) How many years after menopause did you wait before beginning ERT?_________________________
   b) How many years have you been in ERT?_______
   c) What form of ERT are you on, i.e., pill or patch, etc.?_________________________
   d) What dosage estrogen is prescribed?_______

How many term pregnancies 0 1 2 3 4 5
Did you nurse your babies?  No_______ Yes_______

If yes, for how long?_______ months (estimate time for each lactation period)

Have you ever used oral contraceptives?
No_______ Yes_______

If yes, for how long?_______ Brand_________________

VI. Exercise History

How often do you participate in one or more of the following physical activities: aerobics, tennis, jogging, hiking, walking two or more miles at a time, square dancing, etc.

________ Never
________ Once a month
________ Twice a month
________ Once a week
________ More than once a week
________ Daily
________ Seasonally, twice a month
________ Seasonally, once a week
________ Seasonally, over once a week
________ Seasonally, daily
How often do you participate in one or more of the following physical activities: golf, bowling, swimming, bicycling?

- Never
- Once a month
- Twice a month
- Once a week
- More than once a week
- Daily
- Seasonally, twice a month
- Seasonally, once a week
- Seasonally, over once a week
- Seasonally, daily
Instruction for Collection of a 24-Hour Urine Specimen

On first morning: Void urine, discard it.

During the next 24 hours: Collect all urine excreted, day and night. Pour into containers provided.

Second morning: Void urine completely and save (this should end the 24 hours).

What is collected at this point represents a 24-hour urine specimen. Keep the containers cool; you do not need to refrigerate, just avoid direct heat or sunlight.

Please return completed specimen when you come for your appointment.

Thank you for your cooperation. Should you have any questions, do not hesitate to call Susan Munroe or Roy Beauchene at the University of Tennessee - 974-5445.
Table XI

Analysis of Variance of Selected Predictor Variables of Bone Density of the Radius and Ulna - Using GLMM - (Original Version)

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>f statistic for radius</th>
<th>f statistic for radius</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type I</td>
<td>Type III</td>
</tr>
<tr>
<td>Subject (Diet)</td>
<td>1</td>
<td>5044³</td>
<td>130.9³</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>105.3³</td>
<td>93.03³</td>
</tr>
<tr>
<td>Calories</td>
<td>1</td>
<td>0.189³</td>
<td>0.1212⁵</td>
</tr>
<tr>
<td>Weight</td>
<td>1</td>
<td>8.276⁴</td>
<td>1.934</td>
</tr>
<tr>
<td>Calcium</td>
<td>1</td>
<td>2.738</td>
<td>1.618</td>
</tr>
<tr>
<td>Diet¹ (Subject diet)</td>
<td>1</td>
<td>1.187⁵</td>
<td>2.734</td>
</tr>
<tr>
<td>Protein</td>
<td>1</td>
<td>0.3663⁵</td>
<td>0.6164⁵</td>
</tr>
<tr>
<td>Use of Estrogen²</td>
<td>1</td>
<td>4.778⁴</td>
<td>4.778⁴</td>
</tr>
</tbody>
</table>

¹Denotes nonvegetarian and vegetarian diet effects.
²Denotes use or nonuse of estrogen replacement therapy.
³ p ≤ 0.0001
⁴ p ≤ 0.05
⁵ p > 0.23
Table XII
Analyses of Variance of Selected Predictor Variables of Bone Density of the Radius and Ulna Using GLMM - (Modified Version)

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>f statistic for radius</th>
<th>f statistic for ulna</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type I</td>
<td>Type III</td>
</tr>
<tr>
<td>Subject (Diet)</td>
<td>1</td>
<td>4972$^3$</td>
<td>27.15$^3$</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>104.5$^3$</td>
<td>0.0462$^5$</td>
</tr>
<tr>
<td>Age * Age</td>
<td>1</td>
<td>4.295$^4$</td>
<td>1.277</td>
</tr>
<tr>
<td>Weight</td>
<td>1</td>
<td>6.502$^4$</td>
<td>1.493</td>
</tr>
<tr>
<td>Calcium</td>
<td>1</td>
<td>2.255</td>
<td>1.237</td>
</tr>
<tr>
<td>Diet$^1$ (Subject diet)</td>
<td>1</td>
<td>1.255$^5$</td>
<td>1.963</td>
</tr>
<tr>
<td>Estrogen$^2$</td>
<td>1</td>
<td>4.536$^4$</td>
<td>0.0013$^5$</td>
</tr>
<tr>
<td>Age * Estrogen</td>
<td>1</td>
<td>0.2975$^5$</td>
<td>0.2974$^5$</td>
</tr>
</tbody>
</table>

$^1$Denotes nonvegetarian and vegetarian diet effects.
$^2$Denotes use or nonuse of estrogen replacement therapy.
$^3$p ≤ 0.0001
$^4$p ≤ 0.05
$^5$p > 0.27
Table XIII

Analyses of Variance of Selected Predictor Variables of Bone Density of the Radius and Ulna Using GLMM - (Final Version)

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>Type I</th>
<th>Type III</th>
<th>Type I</th>
<th>Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject (Diet)</td>
<td>1</td>
<td>5322(^3)</td>
<td>31.65(^3)</td>
<td>5359(^3)</td>
<td>156.0(^3)</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>101.0(^3)</td>
<td>0.5713(^3)</td>
<td>178.4(^3)</td>
<td>31.63(^3)</td>
</tr>
<tr>
<td>Age * Age</td>
<td>1</td>
<td>4.224(^4)</td>
<td>0.3864(^5)</td>
<td>4.861(^4)</td>
<td>13.64(^3)</td>
</tr>
<tr>
<td>Weight</td>
<td>1</td>
<td>7.686(^4)</td>
<td>1.915</td>
<td>4.5823(^4)</td>
<td>1.349</td>
</tr>
<tr>
<td>Calcium</td>
<td>1</td>
<td>2.894</td>
<td>1.420</td>
<td>1.371(^4)</td>
<td>0.5016(^5)</td>
</tr>
<tr>
<td>Diet(^1) (Subject diet)</td>
<td>1</td>
<td>1.173</td>
<td>6.444(^4)</td>
<td>0.8286(^5)</td>
<td>8.420(^4)</td>
</tr>
<tr>
<td>Age * Diet</td>
<td>1</td>
<td>0.1838(^5)</td>
<td>6.310(^4)</td>
<td>0.1721(^5)</td>
<td>8.434(^4)</td>
</tr>
<tr>
<td>Age * Age * Diet</td>
<td>1</td>
<td>6.824(^4)</td>
<td>6.4151(^4)</td>
<td>8.827(^4)</td>
<td>8.689(^4)</td>
</tr>
<tr>
<td>Estrogen(^2)</td>
<td>1</td>
<td>4.522(^4)</td>
<td>4.522(^4)</td>
<td>2.036</td>
<td>2.036</td>
</tr>
</tbody>
</table>

\(^1\) Denotes nonvegetarian and vegetarian diet effects.
\(^2\) Denotes use or nonuse of estrogen replacement therapy.
\(^3\) \(p < 0.0001\)
\(^4\) \(p < 0.05\)
\(^5\) \(p > 0.29\)
Table XIV

Analyses of Variance of Selected Predictor Variables of Bone Density of the Radius and Ulna Using GLMM - (Including Calcium Supplementation)

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>f statistic for radius Type I</th>
<th>f statistic for ulna Type I</th>
<th>f statistic for radius Type III</th>
<th>f statistic for ulna Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject (Diet)</td>
<td>1</td>
<td>5203(^3)</td>
<td>5232(^3)</td>
<td>31.97(^4)</td>
<td>158.0(^3)</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>102.7(^3)</td>
<td>179.6(^4)</td>
<td>0.3399(^6)</td>
<td>31.51(^3)</td>
</tr>
<tr>
<td>Age * Age</td>
<td>1</td>
<td>4.146(^5)</td>
<td>4.904(^5)</td>
<td>0.6233(^6)</td>
<td>13.55(^4)</td>
</tr>
<tr>
<td>Weight</td>
<td>1</td>
<td>7.571(^3)</td>
<td>4.338(^5)</td>
<td>2.071</td>
<td>1.330(^5)</td>
</tr>
<tr>
<td>Dietary Calcium</td>
<td>1</td>
<td>0.0723(^5)</td>
<td>0.8627(^6)</td>
<td>1.035(^5)</td>
<td>0.0766(^6)</td>
</tr>
<tr>
<td>Calcium Supplement</td>
<td>1</td>
<td>0.2268(^6)</td>
<td>0.0291(^6)</td>
<td>0.185(^5)</td>
<td>0.0086(^6)</td>
</tr>
<tr>
<td>Diet(^1)</td>
<td>1</td>
<td>1.614</td>
<td>0.9121</td>
<td>6.194(^5)</td>
<td>7.986(^4)</td>
</tr>
<tr>
<td>Subject (Diet)</td>
<td>1</td>
<td>1.614</td>
<td>0.9121</td>
<td>6.194(^5)</td>
<td>7.986(^4)</td>
</tr>
<tr>
<td>Age * Diet</td>
<td>1</td>
<td>0.2857(^6)</td>
<td>0.2101(^6)</td>
<td>6.062(^5)</td>
<td>8.027(^4)</td>
</tr>
<tr>
<td>Age * Age * Diet</td>
<td>1</td>
<td>6.560(^5)</td>
<td>8.361(^4)</td>
<td>6.224(^5)</td>
<td>8.316(^4)</td>
</tr>
<tr>
<td>Estrogen(^2)</td>
<td>1</td>
<td>4.883(^5)</td>
<td>8.361(^4)</td>
<td>4.883(^5)</td>
<td>8.316(^4)</td>
</tr>
</tbody>
</table>

\(^1\) Denotes nonvegetarian and vegetarian diet effects.  
\(^2\) Denotes use or nonuse of estrogen replacement therapy.  
\(^3\) \(p \leq 0.0001\)  \(^4\) \(p \leq 0.0007\)  \(^5\) \(p \leq 0.05\)  \(^6\) \(p > 0.36\)
VITA

Susan Gandy Munroe was born in St. Louis, Missouri and attended Clayton public elementary and junior high schools. She moved to Jacksonville, Florida where she completed high school. She attended Tulane University and received a Bachelor of Science in Home Economics with a major in Nutrition from Florida State University in 1975. In 1978, she received a Master of Science with a major in Clinical Nutrition from Florida State University. After graduating, she taught nutrition on the community college level and served as a consulting registered dietitian in Tallahassee, Florida and Charlotte, North Carolina. In May 1992, she received a Doctor of Philosophy in Human Ecology with a major in Nutrition and a graduate minor in Gerontology. While at The University of Tennessee she served for 2 years as a graduate research assistant and for 1 year as a graduate teaching assistant in the Department of Nutrition.