FOLLOW-UP STUDY ON THE RELATIONSHIP OF DIETARY, ENVIRONMENTAL, AND BIOCHEMICAL FACTORS TO BONE DENSITY IN A POPULATION OF ELDERLY MEN AND WOMEN

by

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ABSTRACT

The purpose of this study was to investigate whether the relationship of dietary, environmental, and biochemical factors to bone density changed over a four year period in a group of normal healthy elderly residents in southeast Kansas. Fifteen of thirty-five male and sixteen of twenty-nine female volunteers between the ages of 67 and 88 who participated in two previous studies completed the study.

Dietary intakes of calcium, phosphorus, ratio, vitamins D and C, animal and vegetable protein, and calories were determined using the Missouri Nutrition Survey. Amount of sunlight exposure and physical activity were determined by questionnaire. Bone density was measured by photon absorption with a bone densitometer. Serum levels of calcium, 25-hydroxyvitamin D, and parathyroid hormone were measured.

Mean dietary intake of calcium, vitamin D, and animal and vegetable protein decreased considerably in the men. Less change in dietary intake was found in the women except for a sizeable increase in intake of vitamins D and C. Mean sunlight exposure and bone density decreased for both men and women; physical activity decreased in the women. Stepwise multiple regression analysis demonstrated a significant relationship between a lower sunlight exposure, higher serum calcium level, and higher calcium intake with a higher bone density in the men; and between a younger

age and higher vitamin C intake with a higher bone density in the women. A regression analysis of the change in values for the factors studied predicted that women who consumed less vegetable protein and engaged in less physical activity had less of a decrease in bone density; no significant associations were found in the men.

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CHAPTER 1

Introduction and Review of Literature

Introduction

Many factors determine the health and longevity of older people. Aging is so modified by disease that a truly uncomplicated course is unknown (1). One of the greatest challenges of modern medicine is to probe and to understand the dynamic interrelationships of nutrition, aging, and health (2). As people age, alterations in food intake, absorption, and physical activity become evident. While the same nutrients are essential for all individuals, physiological changes accompanying the aging process may alter the amount of nutrients required by the elderly (2). Proper nutrition throughout life has been suggested as one of the best means of minimizing degenerative changes and superimposed diseases (1).

Osteoporosis is the most common metabolic disease of bone (3,4,5) and loss of bone with increasing age is a universal phenomenon in humans (4,6,7). Severe bone deterioration affects one in four women and about half that number of men resulting in a devastatingly high susceptibility to bone fractures (8). A number of dietary, environmental, and hormonal factors have been implicated as contributory to the etiology of bone loss in the elderly.

In 1978 and 1979, Tiemann and Goodnight investigated the relationships of dietary and environmental factors to bone density

in a population of elderly postmenopausal women and a population of elderly men (9,10). The factors studied included calcium, phosphorus, calcium:phosphorus ratio, vitamins D and C, protein of both animal and vegetable origin, calories, sunlight exposure, and physical activity. Although no significant relationships were found in the men, the women demonstrated a significant relationship between increased sunlight exposure and increased bone density, and a marginally significant relationship between increased physical activity and decreased bone density. Biochemical values were measured but were not included in the analysis of data in either study. In 1983, a number of persons who participated in the Tiemann and Goodnight studies were still living. A follow-up study of these individuals may provide more insight into factors affecting bone loss in this group of elderly men and women.

Statement of Purpose

The purpose of this study was to investigate whether the relationship of dietary, environmental, and biochemical factors to bone density changed over a four year period in the elderly men and women who participated in the earlier studies by Tiemann and Goodnight.

Review of Literature

Bone Formation, Structure, and Remodeling

The skeleton is the heaviest and most durable part of the body and the most intricate of the materials of which the body is composed (11). In addition to functioning as a stabilizer

of the body, bone plays an important role in furthering mineral metabolism (11).

Bone consists of an organic phase, which is nearly entirely collagen, and an inorganic phase, which is calcium phosphate (12). Collagen is required for the deposition of calcium phosphate crystals to form bone (12). The combined properties and degree of bondage between the collagen fibers and the calcium phosphate provide a bony structure with both extreme tensile and compressional strength (13). As much as 20 to 30 percent of the calcium phosphate salts may remain permanently in the amorphorus form and can be absorbed rapidly when there is need for extra calcium in the extracellular fluid (13).

Bone undergoes continuous remodeling (14). Since old bone becomes relatively weak and brittle, new organic matrix is needed as the old organic matrix degenerates (13). Osteoblasts and osteoclasts are the two cells in bone that are responsible for the bone mass in growing individuals and adults (15). Osteoclasts reabsorb both organic and mineral phases of bone in a sharply defined area after which osteoblasts initiate formation of a section of new bone (16). Normally, except in growing bones, the rates of bone deposition and absorption are equal so that total bone mass remains constant (13) until age 30 when bone resorption may begin to exceed bone formation (6).

The strength and adaptability of bone is accomplished through varying combinations and arrangements of mineral salts and fibrous tissues (11). Cortical or compact bone, which constitutes

over 80 percent of mineralized tissue and provides the mechanical strength of bone (15), is deposited on the outer or periosteal surface and removed or resorbed on the inner or endosteal surfaces (14). To add strength to the cortical bone, there is a spongy network of trabecular bone inside the shaft. In long bones, most of the trabecular bone is at the ends where the shape and pattern are complex. The vertebral and flat bones of the skull, pelvis, and shoulder girdle consist largely of trabecular bone with a relatively thin cortex (14).

Hormonal Control of Bone Metabolism

The various hormone systems and their balance have a vigorous effect on bone and mineral metabolism (8). The endocrine control of calcium homeostasis involves three hormones: vitamin D, parathyroid hormone (PTH), and calcitonin; and three target organs: intestine, bone, and kidney. Figure 1 summarizes the hormonal regulation of extracellular fluid (ECF) calcium concentration (Ca^{++}) by means of negative feedback relationships between the hormones and their secretory cells.

The principal control of ECF calcium concentration is thought to be exerted by PTH along with $1,25\text{-}OH_2D_3$. PTH is secreted in response to low plasma calcium and proceeds to the kidney to stimulate production of $1,25\text{-}OH_2D_3$, the active metabolite of vitamin D. PTH works together with $1,25\text{-}OH_2D_3$ to stimulate bone calcium mobilization and to promote renal reabsorption of calcium. $1,25\text{-}OH_2D_3$ also proceeds to the intestine where it independently

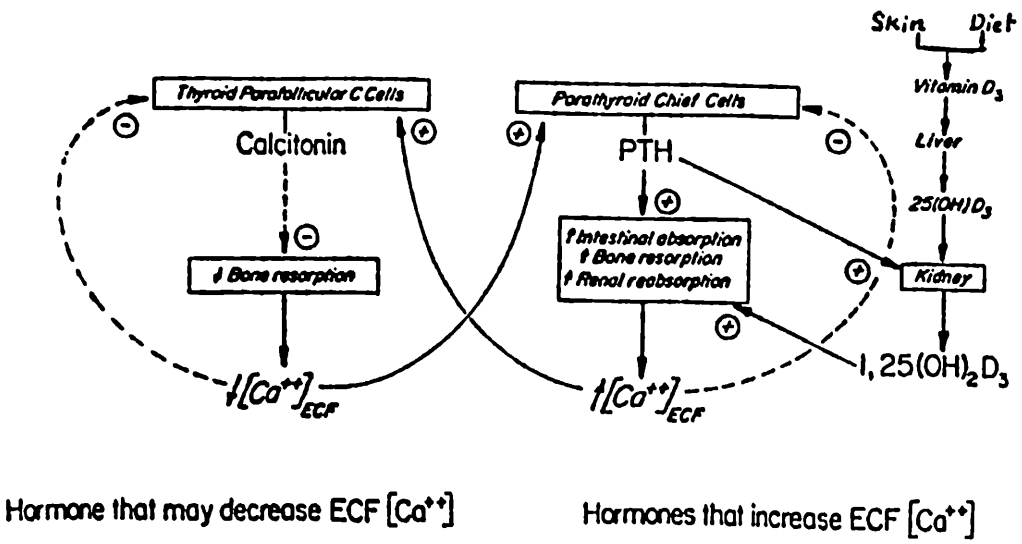

Figure 1. Endocrine Control of Extracellular Fluid (ECF) Calcium Concentration (Ca++). (Inhibitory effects are indicated by a negative sign and dashed arrow; stimulatory effects are indicated by a plus sign and solid line. From Stubbs, D.W., Brown, A.M.: Medical Physiology. John Wiley and Sons, Inc., 1983).

stimulates intestinal absorption of calcium (17,18). Calcitonin is an antihypercalcemic hormone which reduces serum calcium concentration by inhibiting bone resorption (14) although the role of calcitonin in the minute-to-minute regulation of ECF calcium is thought to be less important than PTH and 1,25-OH_2D_3 in humans (19).

Age-Related Bone Loss

Starting at about 30 years of age, human bones become less dense and increasingly porous through an imbalance in bone resorption-bone formation rates (6,20). Although age-related bone loss occurs in all regions, there are two distinctly different patterns of bone loss. In women, loss of bone from the vertebrae

(trabecular bone) begins in young adulthood and continues linearly throughout life, while there is little bone loss in appendicular skeleton (cortical bone) until menopause, at which time loss accelerates until age 65 years after which loss begins to decelerate (6). Albright and Reifenstein first suggested that bone loss associated with menopause is related to estrogen withdrawal resulting in a decrease in stimulation of the osteoblast leading to inadequate bone formation (21). Rate of bone loss is estimated to be four percent per decade in men and eight percent per decade in women (4). From age 40 to 80, bone mass may decrease by one-half (4).

Osteoporosis

Loss of bone with age is an inevitable process resulting in osteoporosis, particularly in females (7). Osteoporosis may be defined as a reduction in the amount of bone without any change in chemical composition (22).

The important aspect of osteoporosis is that as the volume of bone is reduced, mechanical support becomes inadequate and the risk of fracture increases (4). More than 75 percent of hip fractures are due to osteoporosis (4). Albanese has estimated that six million spontaneous fractures occur annually in the United States in persons more than 45 years of age and almost five million of these are women (23). The rate per 1,000 population per year of hip fractures in white women due to minimal trauma increases from 2.0 at ages 50 to 64, to 5.0 at ages 65 to 74, to 10.0 at ages

greater than 75 years (24).

Fractures are usually associated with mild trauma, such as lifting a heavy object, missing a step, or in the case of Colle's fracture, falling on an extended arm (15). Spine and hip fractures are largely caused by trabecular bone loss while wrist fractures are mainly due to cortical bone loss (6). The upper thoracic vertebrae are particularly prone to anterior wedging which produces the dorsal kyphosis known as "dowager's hump" (3). The more symmetrical collapse of the lumbar vertebrae shortens height and may be so excessive that the ribs come to lie on the ileum (3). Loss of height may progress at the rate of 1.5 inches per decade after the onset of menopause (8). Many patients with osteoporosis remain asymptomatic and the diagnosis is made by noting bone demineralization on x-rays taken for unrelated problems (6).

The cause of osteoporosis is still obscure, but the observed change is an increase in bone resorption on the endosteal surfaces of bone with failure of adequate compensatory bone formation (4,6). Reduced bone mass associated with osteoporosis may be the result of an accelerated rate of loss in some individuals after the achievement of peak bone mass; or may be due to a relatively small bone mass in youth with the same percentage of bone loss as nonosteoporotics as age increases (4,5,6). Loss of mineral from the skeleton with age has been attributed to: nutritional deficiencies and general decrease in sunlight exposure and level of physical activity characteristic in many older persons; and changes in physiological function which are concomitant with age.

Factors Affecting the Onset of Osteoporosis

Considerable controversy exists over the relative importance of various dietary, environmental, and biochemical factors involved in the loss of bone mass associated with osteoporosis.

Dietary Factors

Dietary factors have often been implicated in the pathogenesis of osteoporosis. These factors include: calcium, calcium: phosphorus ratio, vitamin D, protein, and others.

Calcium. Calcium deficiency has important implications for the health of aging Americans (25). Calcium is essential for bone mineralization and therefore for skeletal growth and maintenance (23). The body of an adult weighing 70 kilograms contains approximately 1,200 grams of calcium, of which about 99 percent is in the skeleton (23,26). The calcium in body fluids and soft tissues (one percent) is needed for a number of vital functions: biochemical regulatory actions concerned with neuromuscular irritability, blood clotting, muscle contractibility, cell and capillary membrane permeability and cardiac performance (23). In health and with adequate dietary intake of calcium, extracellular calcium concentrations are maintained without loss of bone. However, if adequate calcium is not absorbed from the diet or if excessive amounts are lost from the body, calcium must be provided by the skeleton to maintain the serum calcium at a normal level which is achieved by osteoclastic bone resorption stimulated by parathyroid hormone (15). Although the cause of

age-related calcium loss is unknown, impaired intestinal calcium absorption, reduced endogenous secretion of anticatabolic hormones such as estrogen, increased renal calcium loss, reduced dietary calcium intake, and diminished physical activity probably play a role (4,25).

One of the reasons for bone loss with age may be a decrease in the intestinal absorption of calcium (7). Calcium is absorbed by both active and passive transport systems in the upper small intestine, is mediated by $1,25\text{-}OH_2D_3$, and is saturable (25). Gallagher et al. found a greater reduction in intestinal calcium absorption in elderly people with osteoporotic fractures than in subjects without osteoporosis (27). Some investigators have suggested that impaired calcium absorption with aging may be related to changes in vitamin D metabolites, possibly due to inadequate dietary intake and inadequate sunlight exposure, or impaired renal hydroxylation of $25\text{-}OH\text{-}D_3$ to $1,25\text{-}OH_2D_3$, the active metabolite of vitamin D (28,29). Continued impairment of calcium absorption over the years may significantly contribute to the development of osteoporosis (7).

Superimposed on the effects of age on calcium absorption and calcium requirement is the effect of the menopause (25). There is a deterioration of calcium absorption efficiency and accelerated rate of bone loss when a woman loses estrogen at menopause (30). Estrogen withdrawal leads to a decrease in intestinal absorption efficiency and in renal calcium conservation, both effects causing a need for an increase in calcium intake requirement (25).

Whether a lifelong dietary calcium deficiency can predispose to the development of skeletal demineralization and osteoporosis is uncertain (28). If dietary calcium deficiency results in bone loss, individuals with low levels of calcium consumption should have lower bone mineral content than do those having higher levels of calcium intake (28). Matkovic et al. found clear differences in bone mass in two Yugoslav communities distinguished principally by an approximate two-fold difference in calcium intake (31). Bone mass was higher at all ages in both men and women in the high calcium district. A number of investigators suggest that patients with clinical osteoporosis have a lower calcium intake than patients without osteoporosis (25,26,32). Studies have also shown that calcium intake decreases as age increases (33,34).

Nordin et al. reviewed 212 calcium balance studies in the medical literature and found that the mean value at which input and output of calcium were equal was 578 milligrams in normal adults (35). Heaney et al. found that the minimal level of dietary calcium intake required to prevent negative calcium balance was 1000 milligrams per day in premenopausal women and 1500 milligrams per day in postmenopausal women (36). Although the current Recommended Dietary Allowance for calcium is 800 milligrams per day (37), many investigators recommend between 1000 to 1500 milligrams per day as prophylaxis against bone loss (23,25,26,28).

Calcium is found almost exclusively in a single class of foods, milk and dairy products. An eight ounce glass of milk contains almost 300 milligrams of calcium (38). A normal diet

excluding dairy products contains about 300 milligrams of calcium per day (28). Essentially all published reports reveal that calcium supplementation in the elderly has produced some degree of slowing of age-related bone loss, calcium balance improvement, or decrease in fracture prevalence (25,28,32,39,40).

Calcium:Phosphorus ratio. Phosphorus intake has long been suggested to influence bone growth and calcium balance with excess phosphorus or a low calcium:phosphorus ratio being associated with various skeletal defects (25). This effect has been postulated to reflect transient suppression of plasma ionized calcium following excessive phosphorus ingestion, compensatory hypersecretion of parathyroid hormone (PTH), and PTH-dependent bone resorption (3,25,41,42). Failure of optimal or excessive dietary calcium to counterbalance the effect of excessive dietary phosphorus may be attributed to the fact that efficiency of calcium absorption decreases markedly at high calcium intake whereas the efficiency of phosphorus absorption is unchanged at high phosphorus intake (3,42).

To prevent bone loss, the ratio of calcium:phosphorus should approximate one (3). In adults, the dietary intake of phosphorus is probably at least two to three times that of calcium regardless of milk consumption (3,43). The most commonly used foods contain a great deal of phosphorus and almost no calcium (bread, cereal, meat, potatoes), while very few foods contain considerable calcium and no phosphorus (3). Milk, nonprocessed cheese, and ice cream

contain slightly more calcium than phosphorus, but the ratio is almost one (3). The widespread use of phosphate food additives further complicates assessment of the relationship between calcium and phosphorus intake and bone health (42). Although soft drinks are not a major contributor to the dietary phosphorus intake, if soft drinks are substituted for milk, the overall effect on calcium: phosphorus metabolism may be substantial (44).

Vitamin D. Vitamin D is necessary for calcium absorption; even if calcium intake is adequate, the calcium will not be absorbed if vitamin D intake is low (3,15). Vitamin D is needed for the release of calcium from the bone in the regulation of plasma calcium and acts in concert with parathyroid hormone (45). The total body store of vitamin D, or the state of vitamin D nutriture, clearly depends on cutaneous production as well as on dietary intake. 25-hydroxyvitamin D or 25-OH-D_3 undergoes hydroxylation at the 25-position in the liver and at the 1-position in the kidney to produce 1,25-dihydroxyvitamin D or 1,25-OH_2D_3, the physiologically important, active form of vitamin D (45,46). Vitamin D obtained from diet and from cutaneous production by sunlight exposure are both subjected to the same metabolic conversions (45). Vitamin D deficiency leads to malabsorption of calcium, hypocalcemia, increased secretion of parathyroid hormone, and increased resorption of bone (45).

Dietary deficiency of vitamin D is rare in the United States where foods are supplemented with the vitamin; however, elderly

persons, particularly those living alone and eating poorly, may be deficient (3). There is relatively little data available in the United States regarding vitamin D intake in the elderly although a few studies have demonstrated intakes substantially lower than the Recommended Dietary Allowance of 400 I.U. per day (9,10,47,48,49, 50). For example, Omdahl et al. found that 60 percent of elderly subjects who were not taking supplemental vitamin D had median intakes less than 100 I.U. daily (48).

The effects of reduced intake may be enhanced by a significant decline with age in the intestinal absorption of vitamin D (45). Advancing age may be associated with an impairment of hepatic hydroxylation of vitamin D and/or reduced renal production of 1,25-dihydroxyvitamin D (4,45,51). When synthetic human parathyroid hormone was infused intravenously for 24 hours, serum levels of $1,25\text{-}OH_2D_3$ increased in healthy, young individuals, but increased little in elderly osteoporotic patients (51).

The extent to which vitamin D deficiency contributes to hip fractures in the United States is unknown and is an important area for future research (45). Until more data is available, Parfitt et al. recommend the vitamin D requirement of elderly people be 600 to 800 I.U. per day or 50 to 100 percent greater than the current Recommended Dietary Allowance for adults (45).

Protein. The American diet, rich in protein and excessive meat intake, has been implicated in the genesis of osteoporosis (28). When intake of dietary protein as an isolated nutrient is

increased, urinary calcium increases proportionately resulting in a negative balance shift (52,53). Marsh et al. reported a more rapid postmenopausal bone loss among elderly Caucasian omnivorous women compared to age-matched elderly Caucasian lacto-ova-vegetarian women (54). While more research needs to be done, there appears to be evidence that protein intake in excess of need results in reduced retention of absorbed calcium, and hence a relative increase in calcium requirement (25,53,55).

Other dietary factors. Other factors which may play a role in the development of osteoporosis are inadequate intake of vitamin C, which is essential for the biosynthesis of collagen, (23); and fluoride, which acts to stimulate new bone formation and to inhibit resorption (4). Both factors have received limited attention and require further investigation. Inadequate intake of calories and protein may result in decreased formation of collagen and tissue maintenance needed to maintain skeletal strength.

Environmental Factors

Environmental factors have been identified as major etiologic factors involved in the onset of osteoporosis. Various studies have indicated that amount of sunlight exposure and amount of physical activity may affect the rate of bone loss in the elderly (16,60).

Sunlight exposure. The major source of vitamin D is cutaneous production from 7-dehydrocholesterol by ultraviolet

photolysis in the skin, a process that is independent of any known nutritional factors (45,56,57). Sunlight alone is capable of supplying the total requirement of vitamin D (45).

Production of vitamin D by the skin depends on geographic location, season of the year, atmospheric conditions, indoor or outdoor work, extent of outdoor leisure activities, customary extent of protective clothing, degree of skin pigmentation, and probably other factors (45,48,56). Photochemical transformation of 7-dehydrocholesterol to previtamin D requires ultraviolet rays in the wavelength range of 280 to 320 nanometers, occurring in northern parts of America from March through October (3,56). Body stores of previtamin D build up during the summer to levels that permit continued production of 25-OH-D_3 with gradual depletion of body stores during the winter (45).

Elderly persons who are bedridden or totally housebound are especially subject to a further degree of sunlight deprivation and decrease in skin vitamin D synthesis (56,58,59). Diminished sunlight exposure is the principal cause of decline in plasma 25-OH-D_3 with age (45,58). Maintenance of adequate vitamin nutriture in older people who are unable or unwilling to obtain adequate sunlight exposure may need to depend on ingestion of a vitamin D supplement (45).

Physical activity. Bone mass decreases whenever physical activity is reduced and that loss begins immediately or at least soon after onset of immobilization (25,60). There is both increased

urinary loss and decreased absorption of calcium from the diet (25). Various studies have supported the hypothesis that exercise can modify involutional bone loss (61,62,63), although Tiemann found a marginal significance between increased physical activity and decreased bone density in a population of elderly women (9). While immobilization may contribute to bone loss, further investigation may be required to determine the amount of activity necessary to prevent involutional bone loss. Little doubt exists that the level of expended energy decreases with age (15).

Biochemical Factors

Alterations in biochemical or hormonal levels involved with bone and mineral metabolism have been identified as factors associated with bone loss. These factors include: serum calcium, 25-hydroxyvitamin D, and serum parathyroid hormone.

Serum calcium. The importance of a proper level of calcium ion in the extracellular fluid for biochemical regulatory actions can be seen in the extent to which the control mechanisms regulating plasma calcium concentration permit demineralization of bone to keep the plasma calcium level within fairly normal limits (19). Various studies have reported no significant differences in serum calcium between young and aged subjects, suggesting that the normal plasma calcium level of 10 milligrams/deciliter is vigorously defended (19,64,65).

Serum 25-hydroxyvitamin D. The most sensitive and generally

useful index of vitamin D status is the plasma level of 25-hydroxy-vitamin D or 25-OH-D_3 (45). Being present at about 30 nanograms per milliliter in normal human blood, 25-OH-D_3 is the most abundant form of vitamin D in the blood (18,45) and reflects the summated contributions of both dietary intake and skin synthesis (45).

Most investigators have found a significantly lower mean value of plasma 25-OH-D_3 in the elderly (24,48,57,58,59,66). Possible causes for the low plasma 25-OH-D_3 values in these elderly people could include low dietary intake of vitamin D, limited sunlight exposure, lesion in dermal synthesis of vitamin D from 7-dehydrocholesterol, impaired intestinal absorption of ingested vitamin D, diminished activity and/or concentration of the plasma vitamin D binding protein, suppressed hepatic synthesis of 25-OH-D_3, and increased metabolic requirement (45,48). Hodkinson et al. found that plasma 25-OH-D_3 levels in the elderly correlated more strongly with sun exposure than with dietary vitamin D intake (57,58), and seasonal fluctuation in plasma 25-OH-D_3 was significantly less in the elderly than in the young (57,66).

Serum parathyroid hormone. Parathyroid hormone (PTH) maintains serum calcium concentration by stimulating bone resorption, by increasing reabsorption of filtered calcium in the kidney and, indirectly, by increasing vitamin D activation which in turn increases calcium absorption in the intestine (14). Since the underlying pathology of clinically recognized osteoporosis of aging is increased bone resorption relative to bone formation, PTH has

been implicated as an endogenous hormone which might be partly responsible although the exact role of PTH in the pathogenesis of osteoporosis is controversial (65). Arnaud et al. found that serum PTH increased with age from 20 to 90 years in normal Caucasian women, suggesting that the primary defect is in the adaptive mechanism involved in increasing intestinal absorption of calcium in response to increased PTH secretion (65). The effect of estrogen withdrawal at menopause on the ensuing accelerated bone loss has been related to PTH. Estrogen withdrawal at menopause causes a transient increase in serum calcium and a suppression of PTH secretion resulting in a reduction in the hydroxylation of vitamin D to its active metabolite and to a subsequent decrease in calcium absorption (25,60).

Although the etiology of osteoporosis is unknown, many factors have been implicated. The purpose of this study was to investigate whether the relationship of dietary, environmental, and biochemical factors to bone density changed over a four year period in the elderly men and women who participated in the earlier studies by Tiemann and Goodnight. A follow-up study of these individuals may provide more insight into factors affecting bone loss associated with the development of osteoporosis.

CHAPTER 2

Methods and Procedures

The methods and procedures described in this chapter were utilized to determine dietary intake of specific nutrients (calcium, phosphorus, vitamins D and C, protein, and calories), amount of sunlight exposure and physical activity, serum levels of calcium, vitamin D, and parathyroid hormone, and bone density in the elderly subjects who participated in studies by Tiemann and Goodnight between October 1978 and November 1979.

Setting

Participants in the studies by Tiemann and Goodnight from ten cities in rural southeast Kansas (Altamont, Chanute, Coffeyville, Erie, Fort Scott, Fredonia, Girard, McCune, Oswego, and Parsons) were contacted by letter and telephone to determine availability for participation and to establish a meeting time for the follow-up study (Appendix A). This region was selected in the earlier two studies for the relatively high percentage of elderly residents and geographically stable population (9,10). Data collection was conducted either at the home of the subjects or at the University of Kansas Health Education Center in Chanute, Kansas, during August and September 1983.

Subjects

Fifteen male and sixteen female volunteers between the ages

67 and 88 were available for participation in the follow-up study. The average age was 79 for the men and 75 for the women. One man was black; the remaining subjects were Caucasian. An introduction to the study and a consent form were read aloud by the investigator and signed by each participant (Appendix B). A criteria for participation questionnaire was completed by interview with each subject to assure that they continued to be free of bone, liver, or kidney disease; malabsorption, chronic diarrhea, vomiting, convulsions, steroid treatment, or acute illness (Appendix C).

Dietary Data

A comprehensive dietary analysis including calcium, phosphorus, vitamin C, protein, and calories was calculated for each subject using the Missouri Nutrition Survey (developed at Iowa State University and modified for use by the Human Nutrition Research Program at Lincoln University, Jefferson City, Missouri) (Appendix D). The survey was selected because: 1) the survey provided the necessary nutrient data needed in this study and was precoded for computer analysis; and 2) the method was used in the earlier studies by Tiemann and Goodnight. The reliability of the survey for use with elderly was tested with 58 subjects who were interviewed twice, less than one month apart and no significant differences were found between the results obtained the first time and second time (67). Dietary histories were also cross-checked with one-day food records to assess accuracy (67).

The survey was designed to measure daily intake of a wide

variety of foods over a one year period of time. One interview was conducted with each subject. The first part of the interview consisted of a series of questions which asked the number of times per day, week, month, or year 100 food items were consumed. During the second part of the interview, the subject was questioned about portion sizes that he or she consumed using polyurethane food models to show volume and size. Computer analysis of the surveys was performed at the Human Nutrition Laboratory, Lincoln University in Jefferson City, Missouri, using the computer data base for calcium, phosphorus, vitamin C, protein, and calories from the United States Department of Agriculture Handbook Number 456 (68). Because the computer had not been programmed to calculate the vitamin D content of foods, calculations were based on values contained in food composition tables (69). The ratio of calcium to phosphorus consumed was manually calculated by dividing the phosphorus intake of each subject by his or her calcium intake. Supplementary vitamins and minerals were manually calculated and included in the individual daily dietary intake.

Environmental Data

Sunlight Exposure

Sunlight exposure was estimated using a questionnaire developed by Hodkinson and associates (58) (Appendix E). Each subject was interviewed regarding the frequency and length of time spent outside, the season of the year time was spent out of doors, and the clothing that was worn outside during the year prior to the

interview. Points were scored for frequency and length of sunlight exposure and deducted for articles of clothing that were worn outside. Values ranged from a minimum of zero points (no sunlight exposure) to a maximum of 14 points (extensive sunlight exposure). In a study of vitamin D intake, sunlight exposure, and osteomalacia in the elderly, Hodkinson was able to cross-check the results obtained from the questionnaire with a friend or relative who knew the home situation of the patient (58). The reliability of the questionnaire was tested in the present study by repeating the questionnaire with ten subjects within one month. Test-retest reliability was highly significant with a correlation coefficient of .88 and a p-value of .00069.

Physical Activity

General level of daily physical activity was estimated by interview for each subject using a questionnaire developed by Tiemann (9) (Appendix F). Numerical values were assigned to determine a quantitative value for physical activity of each subject. Values ranged from one (subjects with restricted activity) to four (subjects who engaged in active sports and/or did manual chores in addition to regular daily activity). The reliability of the questionnaire was tested in the present study by repeating the questionnaire with ten subjects within one month. Test-retest reliability was highly significant with a correlation coefficient of .82 and p-value of .0038.

Biochemical Data

The serum level of calcium, 25-hydroxyvitamin D, and parathyroid hormone was measured for each subject. Thirty milliliters of blood was drawn from the arm of each subject. Serum calcium values were quantitatively determined using a colorimetric method based on the cresolphthalein complexone reaction as modified by Baginski et al. (70). Serum 25-hydroxyvitamin D levels were quantitatively determined by competitive protein-binding radioimmunoassay (71). Parathyroid hormone levels were quantitatively determined by radioimmunoassay (72).

Bone Density

The bone density of each subject was measured by a physician using the Norland-Cameron bone mineral analyzer (73) (Appendix G). This procedure was used to measure the bone mineral of the distal one-third of the radius of the nondominant forearm by single beam photon absorption which consists of passing a collimated beam of monoenergetic photons through the combination of soft tissue and bone in a limb and monitoring the resulting attenuation with a photon detector. Four bone mineral scans were taken for each subject and the average bone mineral content was divided by the bone width to determine the bone density. Bone density values were reported in mass (grams) of mineral ash residue per unit length (centimeters) of bone. This method was selected because: 1) the same method was used in the earlier studies by Tiemann and Goodnight; 2) the method provided easily available quantitative data

about bone density; 3) the radiation produced is highly localized and of low energy; 4) the results are reproducible to within about three percent (73,74); and 5) scanning the radius gives good results which are well correlated with axial skeleton parameters (73,75).

Analysis of Data

The analysis of data was performed with assistance from the Department of Biometry at the University of Kansas College of Health Sciences and Hospital.

The mean, standard deviation, range, and percentage change were determined for dietary, environmental and biochemical factors and for bone density. The dietary factors were calcium, phosphorus, calcium:phosphorus ratio, vitamins D and C, animal and vegetable protein, and calories. The environmental factors were sunlight exposure and physical activity. Biochemical factors were serum calcium, serum 25-hydroxyvitamin D and serum parathyroid hormone.

Correlation matrices were generated to show the correlation among variables. Stepwise multiple regression was used to measure the predictor ability of the numerous independent variables on the dependent variable (76). A regression was performed to determine the relationship of dietary, environmental, and biochemical factors (independent variables) to bone density (dependent variable) for the data collected in 1983. Another regression was performed to determine the relationship of the change in dietary, environmental, and biochemical factors (independent variables) to the change in bone density (dependent variable) from 1978 to 1983.

Limitations of the Study

The limitations of the study are: 1) the subjects were not randomly selected; 2) the sample was relatively small and not representative of all elderly; 3) the information regarding dietary intake, sunlight exposure, and physical activity is difficult to assess from one interview; and 4) other factors that may affect bone density in this population of elderly men and women were not investigated.

CHAPTER 3

Results and Discussion

This study determined whether the relationship of dietary, environmental, and biochemical factors to bone density changed over a four year period in elderly men and women who participated in studies by Tiemann in 1978 (9) and Goodnight in 1979 (10). Data were collected during August and September 1983 and are reported in Appendix H. Data from the previous studies are reported in Appendix I.

Results

Subjects

Fifteen of thirty-five male and sixteen of twenty-nine female volunteers who participated in the two earlier studies were able to complete the follow-up study. The mean age for the men was 79, ranging from 73 to 88 years. The mean age for the women was 75, ranging from 67 to 86 years. One man was black; the other men and all the women were Caucasian.

All of the subjects continued to be free of bone, liver, or kidney disease; malabsorption, chronic diarrhea, vomiting, convulsions, steroid treatment, or acute illness. Two women reported having a fractured hip between 1978 and 1983.

Dietary Data

The mean, standard deviation, and range for dietary intake

of calcium, phosphorus, vitamins D and C, animal and vegetable protein, and calories and calculated calcium:phosphorus ratio are summarized in Table 1. Mean percentage change for dietary intake is presented in Figure 2. The number of subjects consuming less than two-thirds of the Recommended Dietary Allowances (RDA) for these nutrients is presented in Table 2.

The greatest changes in dietary intake were found in the elderly men. Mean dietary intake of calcium, vitamin D, animal and vegetable protein decreased by 27, 32, 27 and 17 percent, respectively. The calcium:phosphorus ratio increased by 52 percent. However, when the calcium:phosphorus ratio of 1:11.08 for one male subject who reported an excessive consumption of soft drinks was removed from the data, the mean calcium:phosphorus ratio became 1:1.39. The high calcium:phosphorus ratio of this subject was especially interesting since his bone density did not change. Less change in dietary intake was found in the elderly women except for a sizeable increase in intake of vitamins D (40 percent) and C (14 percent) which appeared to be related to increased ingestion of multiple vitamin supplements. Men and women decreased caloric intake by 15 and 14 percent, respectively. Only one man consumed less than the RDA for calories.

Environmental Data

The mean, standard deviation, and range for sunlight exposure and physical activity scores are summarized in Table 3. Mean percentage change for environmental data is presented in

Table 1

MEAN, STANDARD DEVIATION, AND RANGE VALUES
FOR DAILY DIETARY INTAKE

Dietary Factor	MEN		WOMEN	
	1979	1983	1978	1983
	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)
Ca (mg)	1341 ± 455 (581-2142)	985 ± 433 (324-1817)	1144 ± 427 (601-2094)	1085 ± 461 (403-1946)
P (mg)	1751 ± 501 (927-2334)	1802 ± 2091 (728-9233)	1447 ± 395 (899-2500)	1250 ± 388 (643-1869)
Ca:P	1:1.34 ± .12 (1:1.09-1:1.60)	1:2.04 ± 2.5 (1:0.97-1:11.08)	1:1.32 ± .23 (1:0.96 - 1:1.72)	1:1.26 ± .35 (1:0.63 - 1:1.75)
Vit D (I.U.)	379 ± 190 (151-769)	258 ± 163 (22-535)	211 ± 108 (35-394)	295 ± 193 (52-619)
Vit C (mg)	127 ± 74 (28-286)	133 ± 85 (38-381)	190.5 ± 65 (73-287)	218 ± 110 (78.5-5-463)
Animal Pro (gm)	64 ± 26 (16-103)	47 ± 17 (24-80)	53 ± 22 (24-118)	47 ± 17 (19-79)
Veg Pro (gm)	29 ± 11.5 (16-51)	24 ± 6 (15-36)	29 ± 7 (20-47)	25.5 ± 8 (13-42)
Kcals	2533 ± 708 (1402-3442)	2172 ± 599 (1125-3385)	2333 ± 431 (1696-3296)	1993 ± 553 (1149-3026)

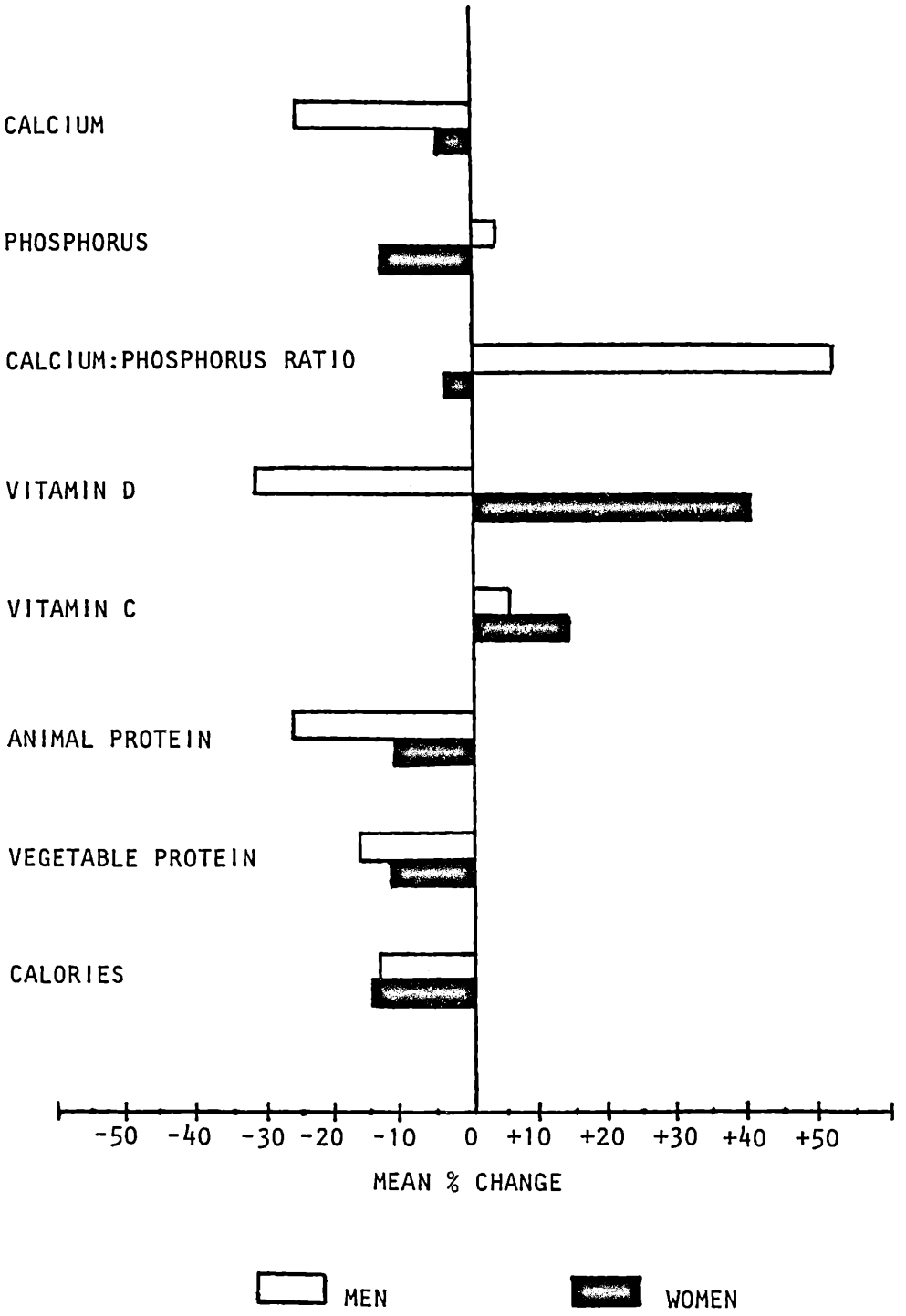

Figure 2. Mean Percentage Change in Dietary Intake.

Table 2

SUBJECTS CONSUMING LESS THAN TWO-THIRDS
THE RECOMMENDED DIETARY ALLOWANCE

Dietary Factor	MEN		WOMEN	
	1978	1983	1978	1983
CALCIUM	0	1	0	1
VITAMIN D	4	8	12	8
VITAMIN C	1	2	4	0
CALORIES	0	1	0	0

Table 3

MEAN, STANDARD DEVIATION, AND RANGE VALUES
FOR SUNLIGHT EXPOSURE AND PHYSICAL ACTIVITY

Environmental Factor	MEN		WOMEN	
	1979	1983	1978	1983
	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)
Sunlight Exposure (points)	9.0 ± 1.85 (5.5–13.0)	7.5 ± 1.00 (5.5–9.5)	8.0 ± 1.89 (3.0–10.0)	7.25 ± 1.33 (5.0–10.0)
Physical Activity (points)	---	3.0 ± 0.79 (2.0–4.0)	3.0 ± 0.86 (1.0–4.0)	2.37 ± 0.62 (1.0–4.0)

Figure 3. The amount of sunlight exposure decreased for both men and women.

The mean score for physical activity in men was 3.0 or a moderate activity level. The level of physical activity was not measured in the men in the previous study. The mean score of physical activity in the women was 2.36 points or light activity, a decrease from 3.0 points or moderate activity in the previous study.

Biochemical Data

The mean, standard deviation, and range for serum calcium, serum 25-hydroxyvitamin D (25-OH-D_3), and serum parathyroid hormone (PTH) are summarized in Table 4. Mean percentage for biochemical factors is presented in Figure 3.

The change in mean serum calcium was minimal for both men and women. Mean serum 25-OH-D_3 increased by 54 percent in the men and by ten percent in the women. The increase in serum 25-OH-D_3 in both men and women may be due to a seasonal difference in time of data collection. Blood samples for the serum 25-OH-D_3 analysis for the previous studies were collected between October and November 1979 for the men and October 1978 through January 1979 for the women. Blood samples were collected for the follow-up study during early September 1983.

Bone Density Data

The mean, standard deviation, and range for bone density are summarized in Table 5. Mean percentage change for bone density

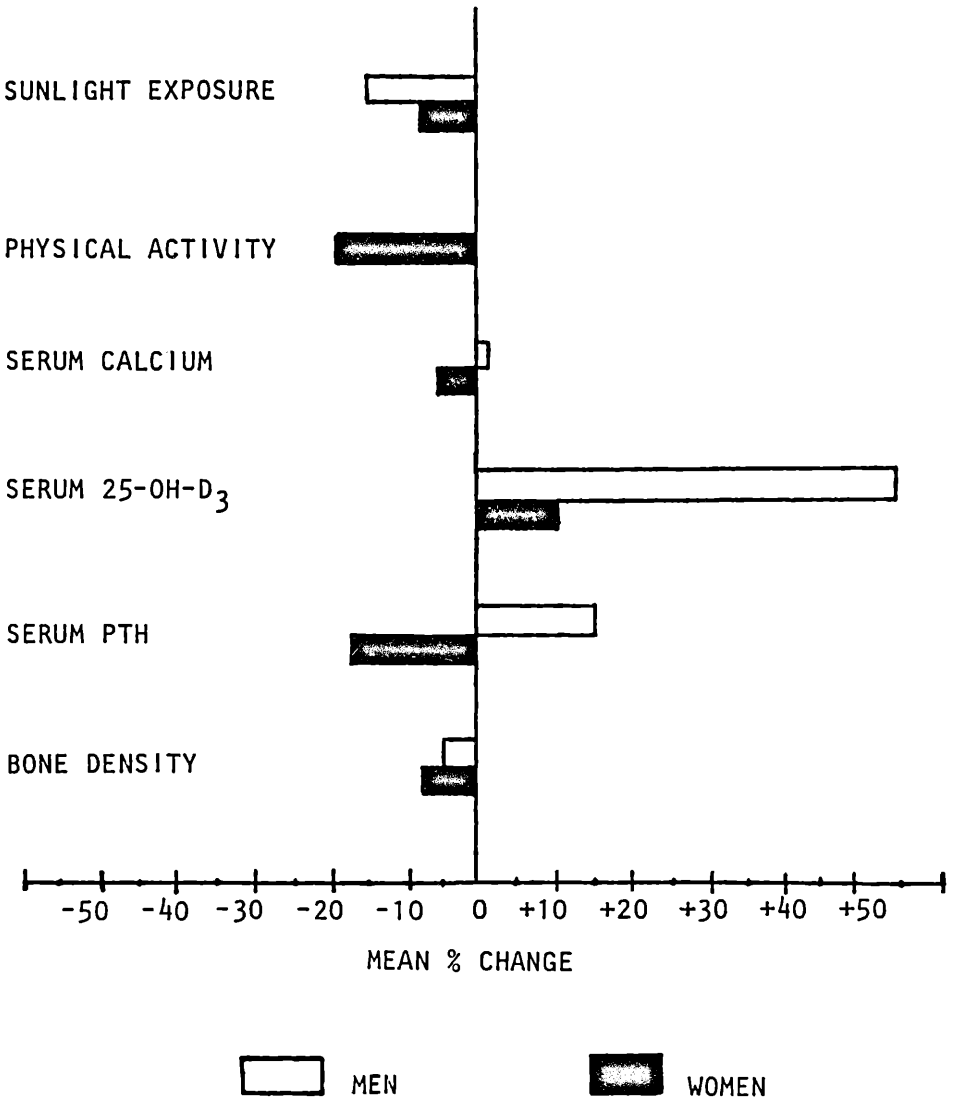

Figure 3. Mean Percentage Change in Environmental, Biochemical, and Bone Density Values.

Table 4

MEAN, STANDARD DEVIATION, AND RANGE VALUES
FOR BIOCHEMICAL DATA

Biochemical Factor	MEN		WOMEN	
	1979	1983	1978	1983
	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)
Serum Ca (mg/dl)	9.06 ± 0.35 (8.30–9.50)	9.17 ± 0.44 (8.50–9.90)	9.62 ± 0.32 (8.90–10.20)	9.06 ± 0.44 (8.50–10.0)
Serum 25-OH-D_3 (ng/ml)	23.23 ± 11.21 (6.30–48.20)	35.71 ± 13.68 (20.50–75.10)	30.23 ± 8.77 (15.20–45.60)	33.30 ± 11.60 (11.70–61.50)
Serum PTH (ng/ml)	0.58 ± 0.23 (0.30–1.00)	0.67 ± 0.12 (0.36–0.81)	0.92 ± 0.32 (0.23–1.35)	0.75 ± 0.26 (0.49–1.37)

Table 5

MEAN, STANDARD DEVIATION, AND RANGE VALUES FOR BONE DENSITY

	MEN		WOMEN	
	1979	1983	1978	1983
	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)
Bone Density (gm/cm^2)	0.78 ± 0.12 (0.65-1.05)	0.739 ± 0.108 (0.449-0.889)	0.594 ± 0.079 (0.455-0.735)	0.548 ± 0.060 (0.459-0.674)

data is presented in Figure 3. Mean bone density decreased by five percent in the men and by eight percent in the women.

Relationship Between Bone Density and Dietary, Environmental, and Biochemical Factors

The correlation matrices of bone density with dietary, environmental, and biochemical data for men and women are presented in Appendix J. Although the correlation matrices indicated possible relationships between the factors studied and bone density, many were not significant when analyzed through a stepwise multiple regression.

Stepwise multiple regression was utilized to measure the predictor ability of the dietary, environmental, and biochemical factors (independent variables) on bone density (dependent variable) for the data collected in 1983.

In the men, the resulting equation, with the dependent or y-variable of bone density, was:

$$y = -0.0143 - 0.0615X_1 + 0.122X_2 + 0.9975X_3$$

where X_1 = sunlight exposure; X_2 = serum calcium; and X_3 = calcium intake. Sunlight exposure was negatively associated with a significant p-value of .013; serum calcium was positively associated with a significant p-value of 0.36; and calcium intake was positively associated with a marginally significant p-value of .076. The results of this analysis predicted that the men with less sunlight exposure, higher serum calcium levels, and higher dietary calcium intake had higher bone density values.

In the women, the resulting equation, with the dependent

or y-variable of bone density, was:

$$y = 0.975 - 0.0065X_1 + 0.295X_2$$

where X_1 = age and X_2 = vitamin C intake. Age was negatively associated with a significant p-value of .013 and vitamin C intake was positively associated with a significant p-value of .016. The results of this analysis predicted that the women who were younger and had higher dietary vitamin C intakes had higher bone density values.

Relationship Between Change in Bone Density and Change in Dietary, Environmental, and Biochemical Factors

The correlation matrices of change in bone density with change in dietary, environmental, and biochemical data for men and women are presented in Appendix K. Data was further analyzed through a stepwise multiple regression.

Stepwise multiple regression was utilized to measure the predictor ability of the change in dietary, environmental, and biochemical factors (independent variables) on the change in bone density (dependent variable) from 1978 to 1983. No significant associations were found between change in dietary, environmental, and biochemical factors with change in bone density in the elderly men.

In the women, the resulting equation, with the dependent or y-variable of change in bone density, was:

$$y = -0.153 - 0.003X_1 - 0.040X_2$$

where X_1 = change in vegetable protein and X_2 = change in physical activity. Change in vegetable protein was negatively associated

with a significant p-value of 0.03 and change in physical activity was negatively associated with a significant p-value of 0.02. The results of this analysis predicted that the women who consumed less vegetable protein and engaged in less physical activity had less of a decrease in bone density.

Discussion

Various dietary, environmental, and biochemical factors have been implicated as contributors to the loss of bone associated with the development of osteoporosis (25,45). For the data collected in 1983, significant relationships were found between a lower sunlight exposure, higher serum calcium levels, and higher calcium intake with a higher bone density in the men; and between a younger age and higher vitamin C intake with a higher bone density in the women. Results of the change in values of the factors studied predicted that women who consumed less vegetable protein and engaged in less physical activity had less of a decrease in bone density.

Dietary Relationships

Although dietary calcium intake decreased in this population of elderly men and women, the mean daily intake exceeded the RDA for calcium (800 milligrams) by 23 percent in the men and 36 percent in the women. The mean daily calcium intakes in this study exceeded or equaled intakes reported in other studies of the elderly (33,47,49, 50,67,77,78,79,80).

The decrease in calcium intake in the men was attributed

to a substantial decrease in consumption of milk as a beverage. Six of the men consumed less than half as much milk as they consumed in 1978 and three of these men had a greater percentage decrease in bone density than the mean. Although consumption of milk as a beverage decreased in the women, there was an increase in the consumption of dairy products (ice cream, pudding, and cottage cheese) and an increase in the number of women taking calcium supplements. Three of four female subjects who decreased calcium intake by half had a greater percentage decrease in bone density than the mean. Three female subjects who increased their calcium intake by almost half had a smaller percentage decrease in bone density than the mean. The oldest female subject (86 years) reported having a hip fracture between 1978 and 1983. This subject consumed the least amount of calcium in the previous study (601 milligrams) and had the lowest bone density value in this study (.459 gm/cm^2). The other women who reported having a hip fracture was one of those subjects who increased calcium intake by half and had a smaller percentage decrease in bone density than the mean.

No other studies have examined whether the relationship of calcium intake to bone density changes over a period of time in an elderly population without diagnosed osteoporosis. A number of investigators have, however, suggested that patients with clinical osteoporosis have a lower calcium intake than patients without osteoporosis (25,26,32), and calcium supplementation in the elderly has produced some degree of slowing of age-related bone loss,

improved calcium balance, or decrease in prevalence of fractures (25,28,32,39,40).

To prevent bone loss, Jowsey has suggested that calcium: phosphorus ratio should approximate one (3). Although the mean calcium:phosphorus ratio increased for the men and decreased for the women, the majority of men and women in this study had a decrease in calcium:phosphorus ratio which may be attributed to a decreased consumption of milk and meat. The results in this study do not support or contradict the theory that a high calcium: phosphorus ratio may cause bone loss in the elderly (3,25,41,42).

The relationship of vitamin D to the nutritional health of the elderly has largely been ignored. No data was reported for vitamin D intake in various studies of nutritional status in the elderly (67,77,78,79,80). In this study, the increased number of men consuming less than two-thirds of the RDA for vitamin D may be attributed to a decrease in milk consumption. The decreased number of women consuming less than two-thirds of the RDA for vitamin D may be attributed to an increase in the number of subjects taking multiple vitamin supplements.

Mean dietary intake of animal and vegetable protein decreased for both the men and women although none of the subjects consumed less than the RDA for total protein intake. The decrease in protein consumption could be attributed to a decrease in milk and meat intake. The mean total protein intake in the women was similar to, and in the men less than, intakes reported in studies of nutritional status in the elderly (67,77,78,79,80). The reason that no signif-

icant relationship was found between animal protein intake and bone density could be attributed to the fact that these subjects did not have intakes high enough to cause an increase in calcium excretion. These results do not support or refute the theory that diets high in animal protein cause an increase in calcium excretion that may lead to a loss of bone density (28). The relationship of a higher vegetable protein intake with a lower bone density in the women in this study does not support the finding of Marsh et al. who reported a less rapid postmenopausal bone loss among Caucasian lacto-ova-vegetarian women compared to age-matched Caucasian omnivorous women (54).

The mean intake of vitamin C exceeded the RDA (60 milligrams) by more than 300 percent in women and by more than 200 percent in men. The mean intake of vitamin C was similar to or exceeded the intake reported by other studies (67,77,78,80). The increase in mean vitamin C intake in the women could be attributed to an increase in the number of subjects taking multiple vitamin supplements.

Environmental Relationships

Although the mean amount of sunlight exposure decreased for the men and women, these subjects scored at least 7.0 points, half the maximum score of 14.0 points. Forty-eight male and sixty-one female inpatient subjects studied by Hodkinson and associates (58) had a lower mean sunlight exposure, 6.4 and 5.2, respectively, than the subjects in this study. The significant negative associa-

tion between sunlight exposure and bone density in the men in this study is contradictory to the positive association found in the elderly women by Tiemann in 1978 (9).

Although age-related bone loss has been implicated as a result of decreased muscle stress (15,62,63), limited physical activity did not appear to be associated with lower bone densities in this population of elderly women. The significant negative relationship between physical activity and bone density in the women in this study was especially interesting since it supports the marginally significant relationship found in the elderly women by Tiemann in 1978 (9).

Biochemical Relationships

Although many subjects consumed less than two-thirds of the RDA for vitamin D, none had abnormally low plasma 25-OH-D_3 levels which may be attributed to an adequate amount of sunlight exposure. Devgun et al. found seasonal fluctuations in serum 25-OH-D_3 levels (56) which were similar to levels found by Tiemann and Goodnight and by this study.

Bone Density

The mean bone density decreased by five percent in the men since 1979 and eight percent in the women since 1978, although none of the subjects were below the standard bone density value for their age (74). Skillman estimated the rate of bone loss to be four percent per decade in men and eight percent per decade in women (4). The mean percentage bone loss in this study exceeded

that which has been estimated by Skillman, although the sample size in this study was relatively small. This study does, however, support the hypothesis that loss of bone with increasing age is a universal phenomenon in humans (4,6,7).

Conclusions

Although overall dietary intake decreased in this population of elderly men and women, few subjects consumed less than two-thirds of the Recommended Daily Allowance and the bone density for all subjects was within normal range. The most significant factors associated with a higher bone density in the men were higher serum calcium level and higher calcium intake without excessive sunlight exposure; and the most significant factors associated with a higher bone density in the women were a younger age and higher vitamin C intake. Results of the change in values of the factors studied predicted that women who consumed less vegetable protein and engaged in less physical activity had less of a decrease in bone density. However, the number of subjects in this study was relatively small and does not account for the dietary intake, sunlight exposure, or physical activity over the lifetime of these subjects.

The results of this study indicate a need for future research into the relationship of various factors to bone density. These factors include: calcium:phosphorus ratio, protein, vitamin C, and amount of sunlight exposure and physical activity.

CHAPTER 4

Summary

The purpose of this study was to investigate whether the relationship of dietary, environmental, and biochemical factors to bone density changed over a four year period in a group of normal healthy elderly men and women in southeast Kansas.

Fifteen of thirty-five male and sixteen of twenty-nine female volunteers who participated in earlier studies by Tiemann in 1978 and Goodnight in 1979 completed the follow-up study. The mean age for the men and women was 79 and 75, respectively. One man was black; the other men and all the women were Caucasian. Data was collected either at the home of the subjects or at the University of Kansas Health Education Center in Chanute, Kansas, during August and September 1983.

Dietary intake of calcium, phosphorus, vitamin C, animal and vegetable protein, and calories were analyzed using the Missouri Nutrition Survey. Vitamin D was calculated based on food composition tables. The ratio of calcium:phosphorus consumed was calculated by dividing the phosphorus intake of each subject by his or her calcium intake. Supplementary vitamins and minerals were included in the individual daily dietary intake.

The amount of sunlight exposure and physical activity were determined by questionnaire. Test-retest reliability of these questionnaires was found to be highly significant. Serum calcium

values were quantitatively determined using a colorimetric method. Serum 25-hydroxyvitamin D and parathyroid hormone levels were quantitatively determined by radioimmunoassay. Bone density was measured by single beam photon absorption using a Norland-Cameron bone mineral analyzer.

The mean, standard deviation, range, and percentage change was determined for dietary, environmental, and biochemical factors and bone density. Correlation matrices were generated to show the correlation among variables. A stepwise multiple regression was performed to determine the relationship of dietary, environmental, and biochemical factors (independent variables) to bone density (dependent variable) for the data collected in 1983. Another regression was performed to determine the relationship of the change in dietary, environmental, and biochemical factors (independent variables) to the change in bone density (dependent variable) from 1978 to 1983.

In the men, mean dietary intake of calcium, vitamin D, and animal and vegetable protein decreased considerably due to a decreased consumption of milk and meat. Less change in dietary intake was found in the women except for a sizeable increase in intake of vitamins D and C due to increased ingestion of multiple vitamin supplements. The mean sunlight exposure and bone density decreased for both men and women. Physical activity decreased in the women and was not measured in the men in the previous study. Serum 25-hydroxyvitamin D levels increased which may be due to a seasonal difference in time of data collection.

Stepwise multiple regression analysis demonstrated a significant relationship between a lower sunlight exposure, higher serum calcium level, and higher calcium intake with a higher bone density in the men; and between a younger age and higher vitamin C intake with a higher bone density in the women. Results of the change in values of the factors studied predicted that women who consumed less vegetable protein and engaged in less physical activity had less of a decrease in bone density; no significant associations were found in the men.

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APPENDIX A

INITIAL CONTACT LETTER

THE UNIVERSITY OF KANSAS

Department of Dietetics and Nutrition
College of Health Sciences and Hospital
39th and Rainbow Blvd., Kansas City, Kansas 66103
(913) 588-7681

Dear

Staff at the University of Kansas College of Health Sciences and Hospital in Kansas City, Kansas will be conducting a follow-up of the study you participated in approximately 4-5 years ago. You probably remember participating by answering questions about your dietary habits, sunlight exposure, and physical activity. You also had an x-ray taken of your arm and a blood sample drawn.

Would you consider participating as a volunteer in this follow-up study? Your participation would involve the same procedures as previously. Linda Doolan will be conducting the dietary, sunlight exposure, and physical activity interview; and a trained technician will take an x-ray of your arm and draw a blood sample. The interview can be done in your home or at the Nutrition Center. You will be provided transportation to the University of Kansas Education Center in Chanute, Kansas where the x-ray and blood will be drawn on the same day. The procedures will be scheduled sometime between August and October, 1983.

Linda will be contacting you by phone within the month of August to obtain your decision, answer any questions you have, and schedule your participation.

We hope you will be able to participate and provide this important service to the University of Kansas.

Sincerely,

Linda S. Doolan
Graduate Student
Department of Dietetics and Nutrition

Barbara P. Lukert, M.D.
Department of Medicine

Elizabeth M. Frakes, R.D.
Department of Dietetics and Nutrition

APPENDIX B

INTRODUCTION TO STUDY AND CONSENT FORM

Introduction to Study

Staff at the University of Kansas College of Health Sciences and Hospital will be conducting a follow-up of the study you participated in approximately 4-5 years ago. We will try to find out if your diet, activity, sunlight exposure, and bone density have changed over a period in time. This study may help to increase our understanding of osteoporosis, a condition resulting from loss of bone which occurs with aging.

With your consent, you will again participate in the following activities:

1) Be interviewed by a dietitian to determine your dietary intake, activity level, and the amount of time you spend in the sun.
2) Have one (1) ounce of blood drawn from your arm.
3) Have the bone density of your forearm measured by a method which quantitates the amount of radiation which will pass through the bone.

The possible adverse effects again include:

1) Your arm may become black and blue around the area where the blood is drawn.
2) Development of infection or phlebitis on the site of the needle puncture. This is very rare.
3) The dose of radiation received with a set of four measurements is about 1/100 of the dose delivered to a patient during one x-ray of the forearm.

CONSENT FORM

I authorize Dr. Barbara Lukert or her associates and assistants to take a dietary history, measure bone density of my forearm, and to draw one (1) ounce of blood from my arm. I understand that this is a follow-up to a study I participated in approximately 4-5 years ago and will involve the same procedures as previously. I understand that the possible risks involved in the drawing of blood include discoloration, and possible development of infection or phlebitis at the site of the needle puncture. I have been informed of the nature, duration, and means by which the study is to be administered, and I understand and agree with the contents of the study.

I understand that the University of Kansas College of Health Sciences and Hospital does not maintain a policy of medical treatment or compensation for physical injuries incurred as a result of participating in biomedical or behavioral research.

In giving my consent, I acknowledge that my participation in this study is voluntary and that I may withdraw at any time.

_____
Subject's Signature

_____
Date

The above named information
has been explained to

_____
and it appears that

_____
understands it.

_____
Investigator

_____
Date

_____
Witness

APPENDIX C

CRITERIA FOR PARTICIPATION QUESTIONNAIRE

CRITERIA FOR PARTICIPATION QUESTIONNAIRE

1. Name_____

2. Address_____

_____

3. Age_____

4. Do you have a history of bone disease? yes no

5. Do you have any history of malabsorption or chronic diarrhea and vomiting? yes no

6. Do you have any history of liver or kidney disease? yes no

7. Do you have any history of convulsions? yes no

8. Are you receiving any steroid treatment? yes no

9. Are you presently suffering from any acute illness? yes no

10. Have you, or are you currently taking any other medications? yes no

If so, please list_____

_____

_____

_____

_____

_____

_____

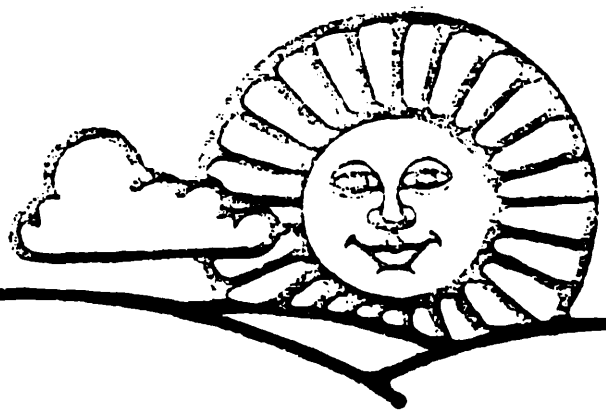
_____

_____

_____

APPENDIX D

MISSOURI NUTRITION SURVEY



DIETARY

History Form

Department of Agriculture,
Natural Resources and Home Economics

Human Nutrition Laboratory, Cooperative Research,
Lincoln University, Jefferson City, Missouri 65101

INSTRUCTIONS

WRITE IN THE INTAKE FREQUENCY AND FOOD CODE WHERE APPLICABLE IN THE BOXES ABOVE THE GRIDS WITH A YELLOW FELT TIP PEN, THEN USING A NO.2 SOFT LEAD PENCIL ONLY FILL IN THE CIRCLE COMPLETELY WHICH CORRESPONDS TO THE BOX ABOVE.

Revised 1982

ITEM	NO.	F	CODE
Whole milk			0 1 3 2 0

ITEM	NO.	F	CODE
5. Evaporated milk Circle response full strength or reconstituted			0 1 3 2 4

2. 2% milk			0 3 6 0 2	

6. Sweetened condensed milk			0 1 3 2 5	

3. Skim milk, reconstituted dry and buttermilk			0 3 6 0 3	

7. Instant Breakfast *1 pkg dry*			0 3 6 0 7	

4. Chocolate milk and cocoa			0 3 6 0 4	

8. Slender and Metracal *1 pkg dry*			0 3 6 0 8	

ITEM	NO.	F	CODE
9. Sego *1 can*			0 3 6 0 9

ITEM	NO.	F	CODE
13. Soft ice cream, ice milk and sherbet			0 3 6 1 3

ITEM	NO.	F	CODE
10. Pudding, custard and tapioca *Child = ¼ C.* *Adult = ½ C.*			0 3 6 1 0

ITEM	NO.	F	CODE
14. Malted milk and milk shake			0 3 6 1 4

ITEM	NO.	F	CODE
11. Yoghurt *partially skimmed* *Child = ½C.* *Adult = 1C.*			0 3 6 1 1

ITEM	NO.	F	CODE
15. Cottage Cheese			0 3 6 1 5

ITEM	NO.	F	CODE
12. Ice cream *12% fat*			0 3 6 1 2

ITEM	NO.	F	CODE
16. Other cheeses and cheese dishes			0 3 6 1 6

Read meat categories 21-23 before asking number of servings in each group.

ITEM	NO.	F	CODE
17. Light, table and sour cream			03617
18. Whipped topping			03618
19. Whipped cream			03619
20. How many times in a week do you eat meat and fish? This is meat of any kind, plain, in mixtures, or in sandwiches. Do not include bacon or liver.			0

ITEM	NO.	F	CODE
21. How many of these _____ servings would usually be ham and pork? Do not include bacon or sausage			03620
22. ...sausage, cold cuts and hot dogs?			03621
23. ...beef, hamburger and lamb?			03622
24. ...venison?			03623

Both the sum of the number of times in question 21-29 and the sum of the number of times in question 30-32 must equal the total number of times in question 33.

ITEM	NO.	F	CODE
25. ...raccoon?			0 3 6 2 4
26. ...chicken, turkey, quail, duck, squirrel, rabbit and opossum?			0 3 6 2 5
27. ...variety meats, such as, pork stomach, pig's feet, pig's ear, ham hocks, oxtail, tripe, brains, sweetbreads, tongue and kidney?' Circle Response			0
28. ...fresh or frozen fish and frog?			0 3 6 2 6
29. ...canned fish, such as, tuna, salmon, sardines, etc.?			0 3 6 2 7
30. Of the _____ meat servings how many would be mixtures, such as, casseroles, stew, meaty soups, spaghetti with meat sauce, chili, etc. Serving = 1 oz. per the casserole			0
31. Of the _____ meat servings, how many would be sandwiches, hot dogs or small hamburgers? Child = 28g. Adult = 45g.			0
32. Of the _____ meat servings how many would be plain meat, not mixed with anything?			0

ITEM	NO.	F	CODE
33. How often do you eat bacon? *1 slice*			0 3 6 2 8

ITEM	NO.	F	CODE
37. Soybeans *mature*			0 3 6 3 1

34. Liver *List Kind* _____ *serving = 2oz.*			0

38. Peanut butter and nuts			0 3 6 3 2

35. Eggs, such as, scrambled, fried, poached, deviled, etc. *Do not include those used in baking (1 egg)*			0 3 6 2 9

39. Carrots, cooked and raw			0 3 6 3 3

36. Cooked dried beans and peas, such as, navy, kidney and pinto beans, lentils and blackeye peas (*not* in pork and beans, lima and beans, soup, refried beans)			0 3 6 3 0

40. Squash, all kinds except zucchini			0 3 6 3 4

ITEM	NO.	F	CODE
41. Sweet potatoes, yams and pumpkin			0 3 6 3 5

ITEM	NO.	F	CODE
45. Cabbage, cooked and raw			0 3 6 3 9

42. Broccoli			0 3 6 3 6

46. Tomatoes, in season: include raw, canned, sauce and juice *Do not include catsup*			0 3 6 4 0

43. Green beans, peas and corn			0 3 6 3 7

47. Tomatoes, out of season			0 3 6 4 1

44. Brussels sprouts			0 3 6 3 8

48. Dark leafy greens in season: include chard, spinach, beet and turnip greens and wild greens, such as, silk, lambsquarter, rock and dandelion greens			0 3 6 4 2

ITEM	NO.	F	CODE
49. Dark leafy greens, out of season			0 3 6 4 3

ITEM	NO.	F	CODE
53. Noodles, macaroni and spaghetti			0 3 6 4 4

50. Seasonal vegetables frequently eaten *Specify* _____			0

54. Grits			0 3 6 4 5

51. Other vegetables frequently eaten *Specify* _____			0

55. Potatoes, instant			0 3 6 4 6

52. Rice, such as, instant, regular, long-cooking, converted, brown, wild and rice mix *Specify* _____			0

56. Potatoes, frozen: French fries, tater tots and hash browns			0 3 6 4 7

ITEM	NO.	F	CODE
57. Potatoes, fresh *prepared by any method*			0 3 5 4 8

ITEM	NO.	F	CODE
58. Pizza			0 3 6 4 9

ITEM	NO.	F	CODE
59. Lettuce Salad			0 3 6 5 0

ITEM	NO.	F	CODE
60. Fortified fruit flavored drinks: include Hi-C, Tang, Start, Awake, Orange Plus, Wagner's, Kool-Aid. Circle response If R. is unable to read a usual brand, but does know it is one of these kinds, circle the one mentioned			0

ITEM	NO.	F	CODE
61. Orange and grapefruit juice; and oranges, grapefruit and tangerines			0 3 6 5 1

ITEM	NO.	F	CODE
62. Other fruit juice *Do not include tomato, fortified fruit-flavored drinks and orange and grapefruit juice* *Specify* _____			0

ITEM	NO.	F	CODE
63. Other fruit juice *Specify* _____			0

ITEM	NO.	F	CODE
64. Apples and bananas, fresh *Child = 75g.* *Adult = 150g.*			0 3 6 5 2

ITEM	NO.	F	CODE
65. Canned fruit: include peaches, applesauce, fruit cocktail, pears, apricots, pine-apple, plums, etc.			03653

ITEM	NO.	F	CODE
69. Other fresh, frozen or canned fruit or juice not mentioned *Specify* _____			0

ITEM	NO.	F	CODE
66. Fresh fruit, such as, peaches, pears, pineapple, etc. *Specify* _____ *Child = 75g.* *Adult = 150g.*			0

ITEM	NO.	F	CODE
70. Hot or cold cereal *Specify brand* _____ _____ _____			0

ITEM	NO.	F	CODE
67. Dried fruit, such as, apricots, prunes, raisins, figs, etc. *Specify* _____			0

ITEM	NO.	F	CODE
71. Milk on cereal *Serving = 3/4 quantity of cereal*			0

ITEM	NO.	F	CODE
68. Muskmelons: canteloupe, honeydew and casaba *In season* *Child = 50g.* *Adult = 100g.*			03654

ITEM	NO.	F	CODE
72. Bread: include bread, toast, French toast, rolls, biscuits and muffins *Count 1 piece as 1 serving* *Serving size = 25g.*			03655

QUESTION 77, 78, 79 ASK:
Do you fry or season any vegetables or fruits with....

ITEM	NO.	F	CODE
73. Hamburger and hot dog buns, bagels and English muffins Count each bun as 2 servings Serving size =23g.			03655

ITEM	NO.	F	CODE
77. Vegetable oil, such as, corn, cottonseed, peanut, soybean and sunflower Circle response			0

ITEM	NO.	F	CODE
74. Sweet rolls and donuts 1 piece Child = 22g. Adult = 45g.			03658

ITEM	NO.	F	CODE
78. Hydrogenated vegetable shortening, such as, Crisco, etc.			03660

ITEM	NO.	F	CODE
75. Pancakes and waffles 1-4" diameter 45g.			03659

ITEM	NO.	F	CODE
79. Bacon grease, suet, lard and fatback Circle response			01241

ITEM	NO.	F	CODE
76. Butter or margarine, used on potatoes, vegetables, bread, etc. Do not include butter used in cooking Regular or Diet Circle response			0

ITEM	NO.	F	CODE
80. Gravy, drippings only			03662

ITEM	NO.	F	CODE
31. Gravy, drippings thickened with flour and water			03663

ITEM	NO.	F	CODE
35. Cake			03667

ITEM	NO.	F	CODE
82. White sauce or gravy made with milk			03664

ITEM	NO.	F	CODE
86. Cookies			03668

ITEM	NO.	F	CODE
83. Salad dressing of any kind, including mayonnaise			03665

ITEM	NO.	F	CODE
87. Candy and candy bars Specify kind and amount Child = 15g. Adult = 35g.			0

ITEM	NO.	F	CODE
34. Pie and pastry			03665

ITEM	NO.	F	CODE
88. Crispy munchy foods, such as, chips, (potato chips, corn chips and fritos) pretzels, popcorn, etc. Circle response			0

ITEM	NO.	F	CODE
89. Crackers			03669

ITEM	NO.	F	CODE
93. Low calorie soda Specify brand _____			0

90. Honey, ice cream toppings and syrups, including molasses, sorghum and table blends			03670

94. Regular soda			00404

91. Jams, jellies and preserves			03671

95. Tea and coffee			00800

92. Sugar, brown, white and confectioners			02230

96. Milk or cream in coffee Specify kind _____			0

ITEM	NO.	F	CODE
97. Beer, wine, whiskey or other alcohol *Specify kind* _____			0

ITEM	NO.	F	CODE
101.			0

98. Salt: iodized, plain or unknown *Circle response*			0

102. Fruit drink *ONLY*			0

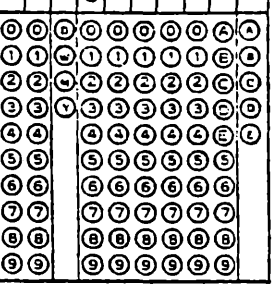
99. Vitamin or mineral supplements *Give brand, quantity and frequency*			0

103. Cereal *ONLY*			0

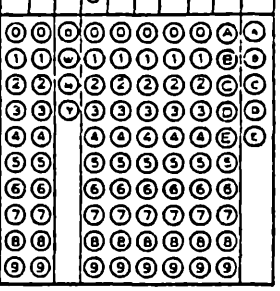
100.			0

104.			0

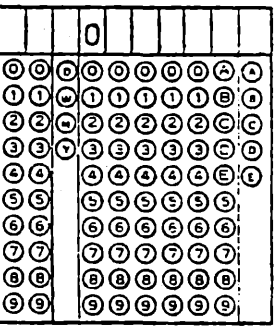
ITEM	NO.	F	CODE
104a.			0



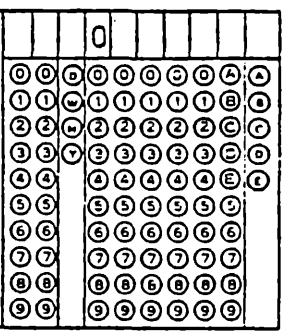
ITEM	NO.	F	CODE
104e.			0



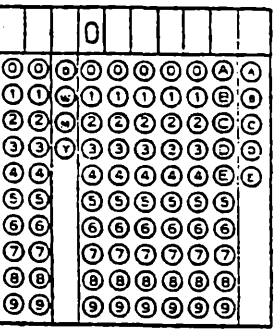
104b.			0



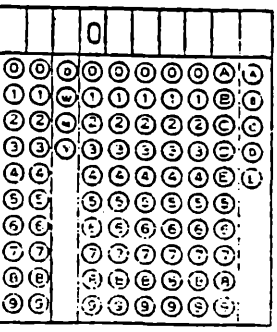
104f.			0



104c.			0



104d.			0



GRAY

09. Ice ream and herbet

GRAY

110. Cottage Cheese

GRAY

111. Whipped cream and whipped topping

GRAY

12. Rice

GRAY

113. Grits

GRAY

114. Potatoes

GRAY

5. Popcorn

GRAY

116. Canned fruit

GRAY

117. Dried fruit

GRAY

18. Casseroles

GRAY

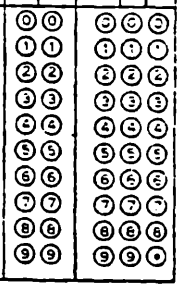
119. Cooked vegetables

GRAY

120. Cooked dried beans and peas

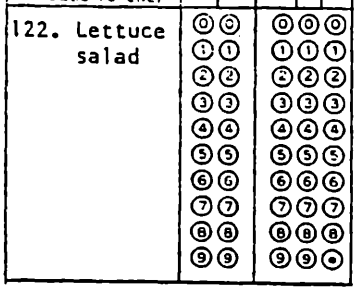
BOWL
Model 15 ONLY

121. Cereal



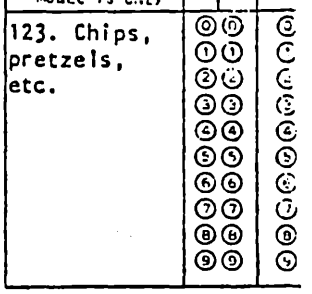
BOWL
Model 15 ONLY

122. Lettuce salad



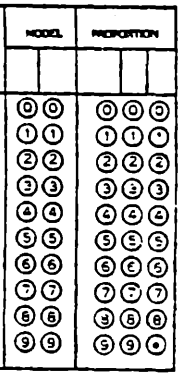
BOWL
Model 15 ONLY

123. Chips, pretzels, etc.



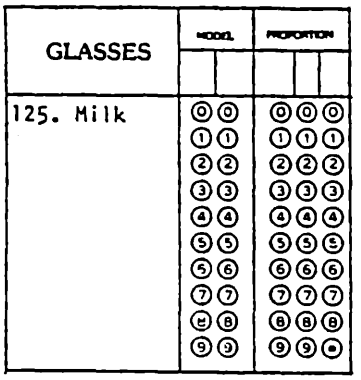
BROWN

124. Plain meat, no fat or bone



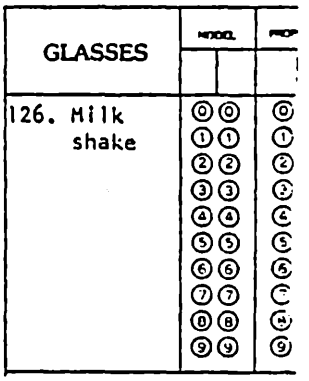
GLASSES

125. Milk



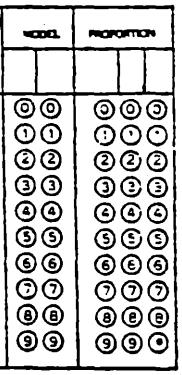
GLASSES

126. Milk shake



GLASSES

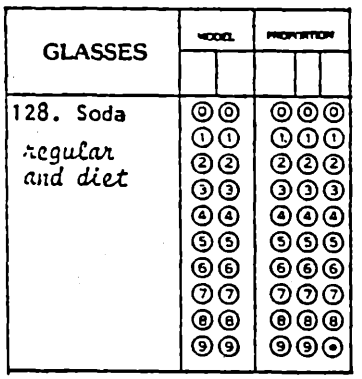
127. Fruit juices and fruit drinks



GLASSES

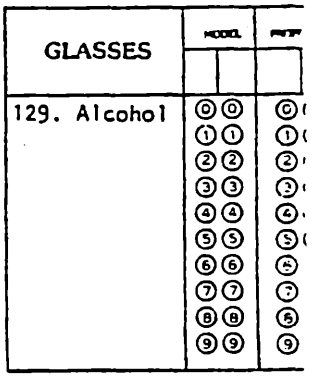
128. Soda

regular and diet



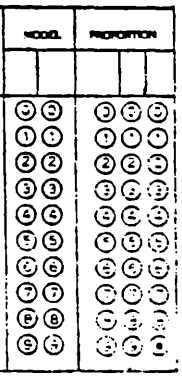
GLASSES

129. Alcohol



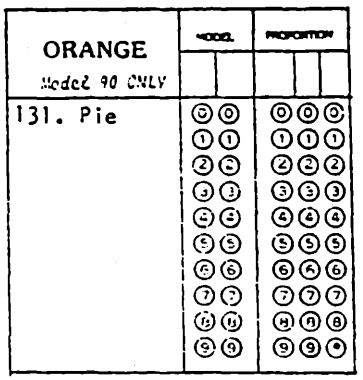
ORANGE

130. Cheese



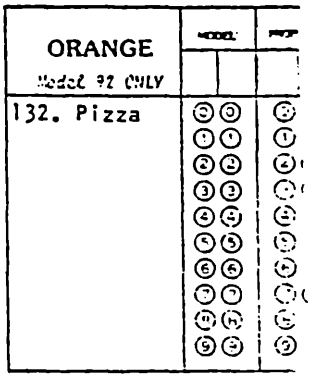
ORANGE
Model 90 ONLY

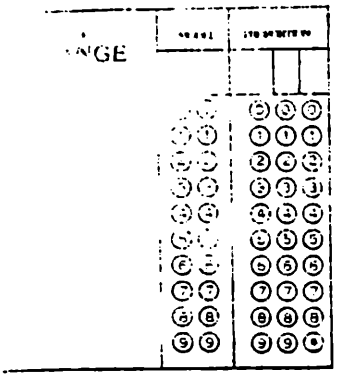
131. Pie

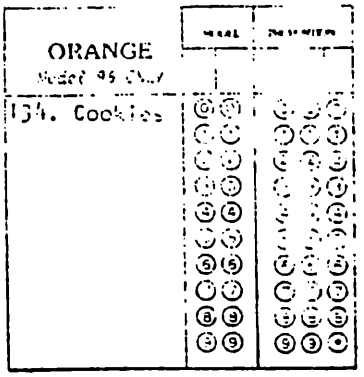


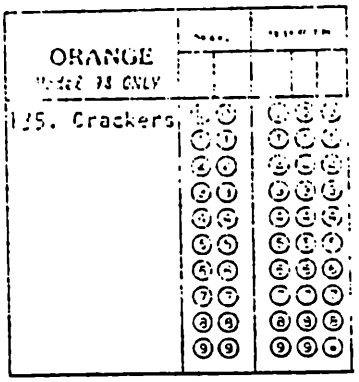
ORANGE
Model 92 ONLY

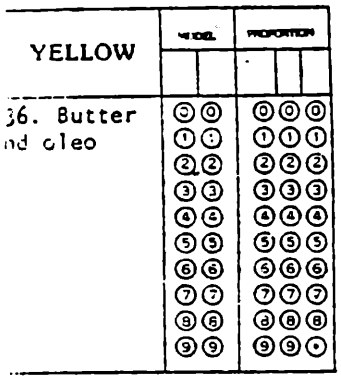
132. Pizza

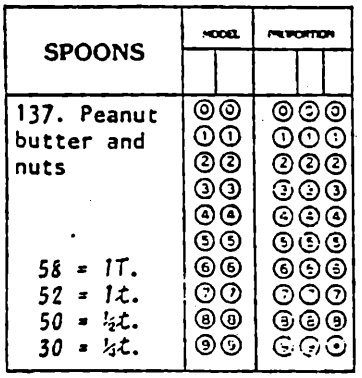


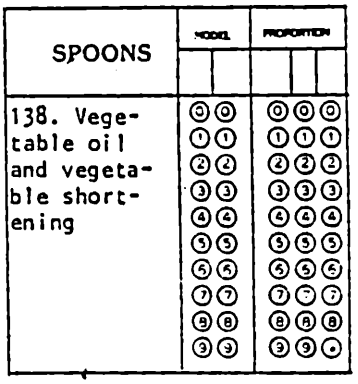

NGE
MODEL
PROPORTION

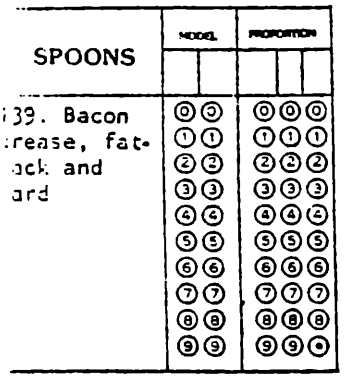

ORANGE
MODEL
PROPORTION
134. Cookies

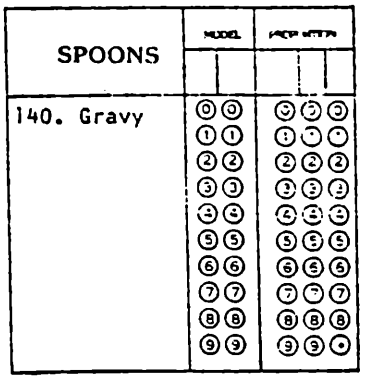

ORANGE
MODEL
PROPORTION
135. Crackers

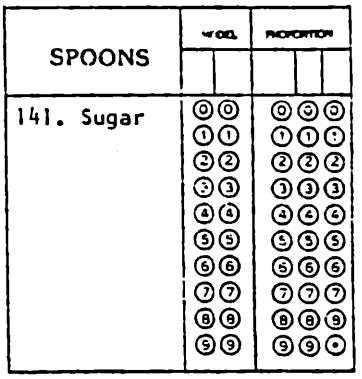

YELLOW
MODEL
PROPORTION
36. Butter
nd oleo

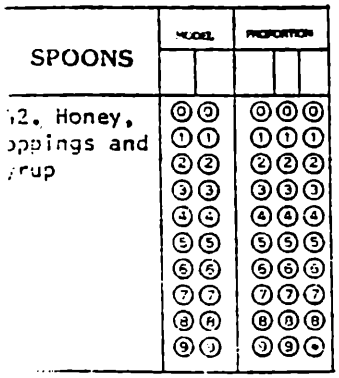

SPOONS
MODEL
PROPORTION
137. Peanut butter and nuts
58 = 1T.
52 = 1t.
50 = ½t.
30 = ¼t.

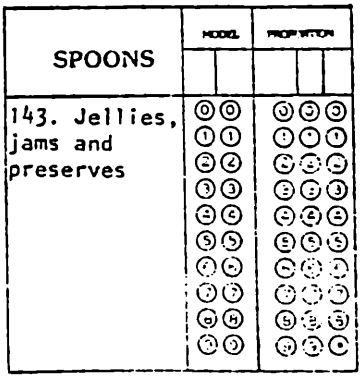

SPOONS
MODEL
PROPORTION
138. Vegetable oil and vegetable shortening

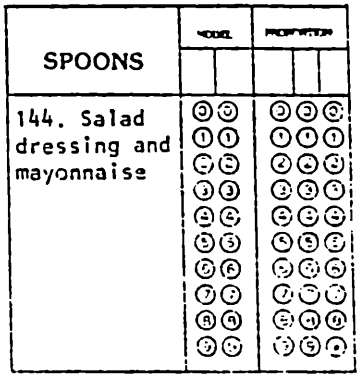

SPOONS
MODEL
PROPORTION
39. Bacon
rease, fat-
ck and
ard


SPOONS
MODEL
PROPORTION
140. Gravy


SPOONS
MODEL
PROPORTION
141. Sugar


SPOONS
MODEL
PROPORTION
42. Honey,
oppings and
rup


SPOONS
MODEL
PROPORTION
143. Jellies, jams and preserves


SPOONS
MODEL
PROPORTION
144. Salad dressing and mayonnaise

SPOONS		
145. Sour cream and light table cream	00 11 22 33 44 55 66 77 88 99	000 111 222 333 444 555 666 777 888 999

IDENTIFICATION NO.

0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	9

RACE

- Caucasian ①
- Am. Indian ②
- Black ③
- Oriental ④
- Spanish ⑤
- Other ⑥

HEIGHT (in.)

0	0	0
1	1	
2	2	
3	3	
4	4	
5	5	5
6	6	
7	7	
8	8	
9	9	

WEIGHT (lb.)

0	0	0	0
1	1	1	
2	2	2	
3	3	3	
4	4	4	
5	5	5	5
6	6	6	
7	7	7	
8	8	8	
9	9	9	

SEX

- Male ①
- Female ②
- Pregnant ③
- Lactating ④

DATE OF BIRTH

Mo.	Day	Yr.
00 11 22 33 44 55 66 77 88 99	00 11 22 33 44 55 66 77 88 99	00 11 22 33 44 55 66 77 88 99

EXAM DATE

Mo.	Day	Yr.
00 11 22 33 44 55 66 77 88 99	00 11 22 33 44 55 66 77 88 99	00 11 22 33 44 55 66 77 88 99

RELIABILITY

- Poor ○
- Average ○
- Good ○

APPENDIX E

SUNLIGHT EXPOSURE QUESTIONNAIRE AND SCORING INSTRUCTIONS FOR SUNLIGHT EXPOSURE QUESTIONNAIRE

SUNLIGHT EXPOSURE QUESTIONNAIRE

1. General

   Age:
   Weight:
   Living:
   Shopping:
   Cooking:

2. Do you ever go out of doors (within the last year)

	Yes	No

   If no - probe: taken out by car, etc.
   or gone on holiday within last year

   If yes - continue with questions.

3. How many times a week do you go out?

	Summer	Winter
Daily		
3 times per week or more		
2 times per week or less		

4. Reason — Approximate time out of doors

	Summer	Winter
Shopping		
Walking		
Gardening		
Other		

5. When you go out, what do you wear?

	Summer	Winter
Hat		
Scarf		
Gloves		
Overcoat		

6. Have you been on holiday this last year?

   When (time of year)
   Where
   For how long

7. When you go out, do you sit or walk in the sun?
   Or in the shade?

SCORING INSTRUCTIONS FOR SUNLIGHT EXPOSURE QUESTIONNAIRE

Score for exposure to sunlight		Score
Question 2	Answer "Yes"	1
Question 3	Daily - Summer and Winter	4
	Summer only	3
	3 days or more - Summer and Winter	3
	Summer only	2
	2 days or less - Summer and Winter	2
	Summer only	1
	Out only occasionally - 1-2 a month or less	0
Question 4	For each reason (maximum score 4)	1
Question 5	For each item clothing worn in summer, deduct 1/2 point, maximum minus	2
Question 6	May-September - for each week of holiday, score (Score only if subject goes out)	1
Question 7	Sun	1
	Shade	0

APPENDIX F

PHYSICAL ACTIVITY QUESTIONNAIRE

PHYSICAL ACTIVITY QUESTIONNAIRE

GUIDELINES FOR EXERCISE LEVEL

GUIDELINES	SCORE (POINTS)
I. Limited Activity:	
Walks only as necessary	1
II. Light Activity:	
Engages in light housework only Walks less than two blocks per day	2
III. Moderate Activity:	
Engages in light and heavy housework Walks two to four blocks per day Engages in outdoor activity, such as gardening	3
IV. Heavy Activity:	
Engages in light and heavy housework Walks more than four blocks per day Performs yardwork and home repairs Engages in activity for the sake of exercise, such as sports and calisthenics	4

APPENDIX G

BONE MINERAL ANALYZER TECHNIQUE AND ANALYSIS SHEET

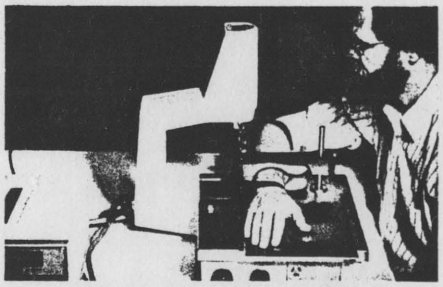
Bone Mineral Analyzer Technique

The Norland-Cameron Bone Mineral Analyzer measures the mineral content in a patient's radius by measuring the attenuation of gamma rays from a radioactive source. Research has shown a good correlation between the bone mineral content of the radius and that of the entire skeleton.

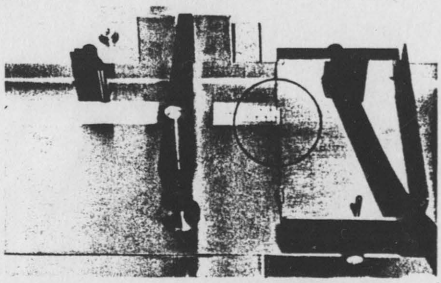
PATIENT SCANNING

1. Seat the patient comfortably at a desk or table on a normal height chair with a back. Do not use a hospital cart or bench for the machine, nor a stool for the patient.

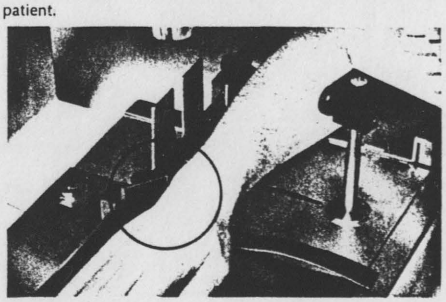
2. Position the scanner directly in front of and directly facing the patient.



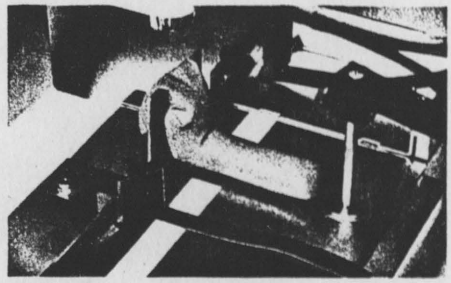
3. Measure the distance from the ulnar styloid process to the olecranon (call this length L). Set the limb positioning carriage so that the desired olecranon-to-scan-site distance appears at the marker window on the lower scale. (The usual setting is 2/3 L.)



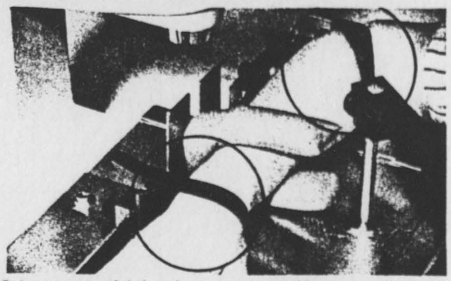
4. Place the patient's arm in position on the deck and move the wrist positioning stop to the gap between the ulnar styloid process and the triquetrum. Note the values on both indicator scales and record them for future use when measuring this patient.



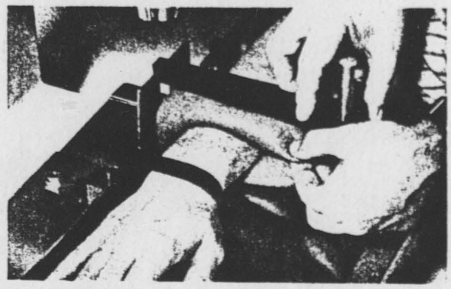
5. Remove the patient's arm and position the tissue equivalent bag as shown.



6. Carefully place the patient's arm across the bag, with the elbow flexed at an acute angle and pushed firmly into the carriage corner. Ask the patient to push his elbow into the carriage corner and to maintain this pressure throughout the examination. Attach the elbow strap and the wrist strap by **first** stretching, **then** wrapping around the arm and attaching to the velcro anchor point.



7. Being very careful that there are no wrinkles or gaps between the bag and the patient's arm or the metal limb holder, fold the bag over the patient's arm and push down the black clamping arm firmly.



8. Select the proper bone and a speed of 2mm/sec., and run 4 scans. Take the average of the 4 scans. Remind the patient again to maintain pressure with his elbow, and caution him not to move during the measuerments.

BONE MINERAL ANALYSIS

Patient_____ Hospital No._____

Physician_____ Date_____ Procedure No._____

Patient's Age_____ Sex_____ Height_____ Weight_____
Previously Scanned: Yes___ No___ Date of Last Measurement_____
Plotting Data: Yes___ No___ Scanning Other Site: Yes___ No___
Procedure Number for Other Sites_____

Instrument Calibrated Today: Yes__ No__ Calibration Data Sheet No.___
SCAN SITE: Forearm_____ Other_____
Scanner Deck Index No._____
Right____ Left____ First Bone____ Second Bone____

PATIENT SCANS: Note - Take fifth scan only if one of the first four values of bone mineral or bone width looks suspiciously out of line with the others. See reverse side for calculating sum or average.

	Bone Mineral	Bone Width
1st Scan	_____	_____
2nd Scan	_____	_____
3rd Scan	_____	_____
4th Scan	_____	_____
*(5th Scan)	(_____)	(_____)
SUM	_____	_____
Divide by 4 =	_____gm/cm	_____cm

$\frac{BM}{BW}$_____g/cm

INTERPRETATION:

Patient is: Above__ Below__ absolute limit for serious osteopenia.

Patient is_____ relative to norms for age group.

Other Comments_____

Norland Instruments Form N-51 By_____M.D.

APPENDIX H

INDIVIDUAL DIETARY, ENVIRONMENTAL, BIOCHEMICAL, AND BONE DENSITY VALUES - FOLLOW-UP STUDY

INDIVIDUAL DIETARY VALUES - FOLLOW-UP STUDY

	Subject	Age	Ca(mg)	P(mg)	Ca:P	Vit D (I.U.)	Vit C (mg)	Animal Pro (gm)	Vegetable Pro (gm)	Kcal
Male:	2	74	324.0	728	1:2.25	22.0	39.0	24.0	23.0	2141.0
	7	82	1387.0	1963	1:1.42	244.0	199.0	72.0	35.5	3125.0
	9	81	1475.0	1662	1:1.13	405.0	149.0	55.0	26.0	1660.0
	10	82	767.0	1054	1:1.37	535.0	114.0	31.0	23.0	2315.0
	11	82	1155.0	1338	1:1.16	279.0	191.0	42.0	26.0	1842.5
	16	75	568.5	1112	1:1.96	429.0	381.0	47.0	24.0	2483.0
	20	82	820.0	1175	1:1.43	202.0	86.0	49.0	21.0	1973.0
	22	73	788.0	1216	1:1.54	34.0	123.0	49.0	30.0	2578.0
	24	82	833.0	9233	1:11.08	198.0	37.5	42.0	30.0	3384.5
	27	74	1817.0	1863	1:1.03	434.0	123.0	74.5	19.0	2515.5
	28	82	629.0	941	1:1.50	35.0	159.0	25.0	30.0	2306.0
	29	78	1199.0	1164	1:0.97	354.0	41.0	40.0	15.0	1556.5
	30	75	534.0	741	1:1.39	113.0	131.0	33.0	17.0	1125.0
	31	77	1590.0	1754	1:1.10	390.0	90.0	80.0	18.0	1955.0
	34	88	888.0	1083	1:1.22	204.0	133.0	39.0	22.0	1624.0
Female:	1	77	1314.0	1751	1:1.33	192.0	151.0	74.0	32.5	2588.5
	2	73	1078.0	1204	1:1.12	149.0	166.0	36.0	35.0	2001.5
	3	67	1657.0	1762	1:1.06	429.0	78.5	71.0	19.0	2229.0
	5	77	1637.0	1031	1:0.63	474.0	264.0	21.0	34.5	2003.0
	6	77	1946.0	1572	1:0.81	569.0	309.0	68.0	28.0	2476.0
	10	70	1159.0	1715	1:1.48	296.0	351.0	49.0	42.0	2833.0
	11	80	1674.0	1869	1:1.12	218.0	266.0	55.0	33.0	3026.0
	12	79	403.0	643	1:1.60	72.0	124.0	19.0	19.0	1403.0
	13	81	985.0	1362	1:1.38	619.0	371.0	59.0	20.0	1621.0
	22	69	743.0	901	1:1.21	120.0	216.0	31.0	27.0	1654.0
	23	68	588.0	1003	1:1.71	52.0	100.0	45.5	21.0	1740.0
	24	75	652.0	1139	1:1.75	589.0	203.0	65.0	18.0	1699.0
	25	73	1051.0	1159	1:1.10	340.0	463.0	32.0	21.0	1378.0
	26	72	1148.0	824	1:0.72	354.0	117.0	36.0	18.0	1641.0
	27	76	567.5	782	1:1.38	139.5	172.0	39.0	13.0	1149.0
	28	86	760.0	1286	1:1.69	105.0	143.0	53.0	27.0	2441.0

INDIVIDUAL ENVIRONMENTAL, BIOCHEMICAL, AND BONE DENSITY VALUES - FOLLOW-UP STUDY

	Subject	Age	Sunlight Exposure (pts)	Physical Activity (pts)	Serum Ca(mg/dl)	Serum 25-OH-D3(ng/ml)	Serum PTH(ng/ml)	Bone Density (gm/cm2)
Male:	2	74	7.00	2	9.4	23.0	0.57	0.659
	7	82	9.00	4	9.6	32.9	0.71	0.775
	9	81	7.50	3	9.7	29.0	0.68	0.830
	10	82	7.00	4	8.9	45.2	0.59	0.708
	11	82	5.50	2	8.8	31.1	0.58	0.889
	16	75	8.50	4	9.4	40.0	0.70	0.767
	20	82	8.75	3	9.1	38.2	0.63	0.449
	22	73	7.00	3	9.0	34.4	0.74	0.725
	24	82	7.50	2	9.9	75.1	0.80	0.855
	27	74	8.00	4	9.3	20.5	0.81	0.748
	28	82	7.00	3	9.6	21.6	0.36	0.797
	29	78	7.00	3	9.2	28.5	0.75	0.844
	30	75	6.00	3	8.5	33.4	0.80	0.691
	31	77	7.50	2	8.5	50.5	0.62	0.718
	34	88	9.50	4	8.7	32.3	0.78	0.642
Female:	1	77	7.00	2	9.3	61.5	0.91	0.546
	2	73	7.00	3	8.6	45.4	0.68	0.503
	3	67	7.50	3	9.5	33.3	0.66	0.584
	5	77	6.00	2	8.7	45.2	0.81	0.467
	6	77	9.00	2	9.2	41.5	0.66	0.487
	10	70	8.50	3	9.2	31.4	0.49	0.605
	11	80	7.50	2	8.5	27.0	0.69	0.571
	12	79	7.00	2	9.1	23.7	0.64	0.480
	13	81	5.00	3	9.3	27.3	0.64	0.614
	22	69	5.00	1	10.0	11.7	1.37	0.573
	23	68	8.50	3	9.4	42.3	0.77	0.598
	24	75	6.50	2	8.8	28.7	1.34	0.510
	25	73	7.50	3	8.6	24.5	0.65	0.674
	26	72	10.00	3	9.5	32.4	0.56	0.549
	27	76	7.00	2	8.5	27.0	0.50	0.551
	28	86	7.00	2	8.7	29.9	0.68	0.459

APPENDIX I

INDIVIDUAL DIETARY, ENVIRONMENTAL, BIOCHEMICAL, AND BONE DENSITY VALUES - PREVIOUS STUDIES

INDIVIDUAL DIETARY VALUES - PREVIOUS STUDIES

	Subject	Age	Ca(mg)	P(mg)	Ca:P	Vit D (I.U.)	Vit C (mg)	Animal Pro (gm)	Vegetable Pro (gm)	Kcal
Male:	2	70	1615.0	1875	1:1.16	445.0	71	76.0	17	2553.0
	7	78	1829.0	2268	1:1.24	489.0	158	89.0	37	3382.0
	9	77	1320.0	1852	1:1.40	282.0	28	103.0	16	1722.0
	10	78	744.0	1099	1:1.48	211.0	44	16.0	27	2022.0
	11	78	1163.0	1549	1:1.33	289.0	66	54.0	31	2327.0
	16	71	875.0	1149	1:1.31	206.0	156	45.0	20	1687.0
	20	78	1451.0	2073	1:1.43	313.0	172	74.0	48	2941.0
	22	70	1653.0	2003	1:1.21	334.0	248	54.0	44	3371.0
	24	78	1610.0	2211	1:1.37	608.0	71	69.0	25	2957.0
	27	70	1603.0	2133	1:1.33	407.0	173	91.0	26	3294.0
	28	77	1644.0	2224	1:1.35	698.0	286	78.0	51	3442.0
	29	74	1163.0	1584	1:1.36	327.0	64	33.0	31	2497.0
	30	77	581.0	927	1:1.60	161.0	129	33.0	18	1402.0
	31	73	2142.0	2334	1:1.09	769.0	124	94.0	26	2771.0
	34	84	716.0	978	1:1.37	151.0	112	47.0	17	1624.0
Female:	1	73	1078.5	1372	1:1.27	220.0	181	66.0	20	2078.0
	2	68	1087.0	1292	1:1.19	147.0	126	47.0	28	2160.0
	3	62	1464.0	1694	1:1.16	366.0	157	56.0	29	2440.0
	5	72	884.0	1338	1:1.51	91.0	232	39.0	47	3070.0
	6	72	2094.0	2500	1:1.19	330.5	175	118.0	28	2813.0
	10	64	990.0	1537	1:1.55	151.0	252	51.5	38	2609.0
	11	75	903.0	1101	1:1.22	173.0	287	32.5	28	2127.0
	12	74	617.0	899	1:1.46	173.0	155	24.0	23	1970.0
	13	76	1035.0	1406	1:1.36	264.0	257	53.0	29	1998.0
	22	65	1541.5	1837	1:1.19	234.0	98	71.0	34	3296.0
	23	64	909.5	1176	1:1.29	123.0	73	52.0	20	1696.0
	24	70	1246.0	1406	1:1.13	234.0	194	51.0	26	2156.0
	25	69	1222.0	1625	1:1.33	354.0	129	60.0	28	2221.0
	26	68	723.0	1103	1:1.53	83.0	203	43.0	29	2103.0
	27	71	1903.0	1831	1:0.96	394.0	284	64.0	22	2051.5
	28	86	601.0	1033	1:1.72	35.0	245	27.0	38	2534.0

INDIVIDUAL ENVIRONMENTAL, BIOCHEMICAL, AND BONE DENSITY VALUES - PREVIOUS STUDIES

	Subject	Age	Sunlight Exposure (pts)	Physical Activity (pts)	Serum Ca(mg/dl)	Serum 25-OH-D3(ng/ml)	Serum PTH(ng/ml)	Bone Density (gm/cm2)
Male:	2	70	9.0	-	-	15.3	-	0.65
	7	78	10.0	-	9.2	22.0	0.90	0.83
	9	77	9.0	-	-	6.3	-	1.05
	10	78	8.5	-	9.1	14.2	0.70	0.75
	11	78	7.5	-	9.0	26.6	0.80	0.88
	16	71	13.0	-	9.5	38.9	0.30	0.83
	20	78	9.5	-	-	12.9	0.60	0.54
	22	70	9.0	-	9.0	19.4	1.00	0.85
	24	78	6.5	-	9.5	48.2	0.30	0.85
	27	70	8.5	-	-	15.6	-	0.68
	28	77	7.5	-	-	38.3	0.30	0.80
	29	74	10.0	-	9.2	22.5	0.50	0.79
	30	77	5.5	-	8.8	27.6	0.50	0.69
	31	73	11.0	-	9.0	19.1	0.60	0.76
	34	84	10.5	-	8.3	21.5	0.50	0.78
Female:	1	73	7.0	1.0	9.9	29.8	0.80	0.735
	2	68	8.0	3.0	-	-	-	0.572
	3	62	10.0	3.0	9.9	37.1	0.92	0.590
	5	72	7.0	3.0	9.4	29.6	0.80	0.475
	6	72	5.0	3.0	9.6	30.3	1.25	0.553
	10	64	9.5	4.0	9.7	25.7	1.10	0.645
	11	75	9.0	4.0	9.6	16.3	1.35	0.574
	12	74	7.5	3.0	9.4	24.0	1.05	0.455
	13	76	9.5	4.0	9.8	33.1	0.70	0.622
	22	65	3.0	1.5	9.7	45.6	-	0.631
	23	64	9.5	4.0	10.2	36.3	0.67	0.677
	24	70	8.5	3.0	9.8	42.3	1.32	0.583
	25	69	9.5	4.0	9.2	33.7	0.83	0.735
	26	68	9.5	3.0	9.8	15.2	-	0.587
	27	71	9.0	3.0	9.4	34.1	-	0.556
	28	86	8.0	3.0	8.9	20.4	0.23	0.523

APPENDIX J

CORRELATION MATRICES OF BONE DENSITY WITH DIETARY, ENVIRONMENTAL, AND BIOCHEMICAL DATA

CORRELATION MATRIX OF BONE DENSITY WITH DIETARY, ENVIRONMENTAL, AND BIOCHEMICAL DATA IN THE MEN

	BONEDEN2	AGE	CA	P	CAPRAT	VITD	VITC	APROT	VPROT	KCAL
BONEDEN2	1.0000									
AGE	-0.0182	1.0000								
CA	0.2941	0.0581	1.0000							
P	0.3373	0.1916	0.0716	1.0000						
CAPRAT	0.2579	0.1380	-0.1956	0.9619	1.0000					
VITD	0.2088	0.0833	0.5607	-0.0117	-0.1587	1.0000				
VITC	0.1785	-0.0444	-0.0600	-0.2849	-0.2749	0.2618	1.0000			
APROT	0.0389	-0.1495	0.8428	0.0919	-0.1359	0.4405	0.1326	1.0000		
VPROT	0.2895	0.2714	-0.0950	0.3289	0.3295	-0.3194	0.2705	0.0130	1.0000	
KCAL	0.2109	-0.0061	0.0565	0.6155	0.5864	-0.0173	0.0988	0.2544	0.7077	1.0000
SUN	-0.4344	0.3451	0.1742	0.0576	0.0022	0.1573	0.2045	0.3874	0.1500	0.3045
EXER	-0.1821	0.1420	0.1117	-0.3327	-0.3774	0.3831	0.4832	0.1485	0.0398	0.0846
CASERUM	0.3516	0.0663	0.0071	0.4875	0.4955	-0.0637	0.0548	-0.0314	0.5797	0.6289
OH25	0.0830	0.1963	-0.0681	0.7942	0.7822	0.1715	-0.1553	0.1014	0.1464	0.4397
PTH	0.0089	-0.1765	0.2657	0.3266	0.2473	0.1975	-0.0713	0.3531	-0.2399	0.0290

	SUN	EXER	CASERUM	OH25	PTH
SUN	1.0000				
EXER	0.5742	1.0000			
CASERUM	0.2304	0.0259	1.0000		
OH25	0.0690	-0.2909	0.0744	1.0000	
PTH	0.2634	0.2560	-0.0964	0.2621	1.0000

CORRELATION MATRIX OF BONE DENSITY WITH DIETARY, ENVIRONMENTAL, AND BIOCHEMICAL DATA IN THE WOMEN

	BONEDEN2	AGE	CA	P	CAPRAT	VITD	VITC	APROT	VPROT	KCAL
BONEDEN2	1.0000									
AGE	-0.4824	1.0000								
CA	-0.0312	-0.0199	1.0000							
P	0.2097	0.0475	0.6957	1.0000						
CAPRAT	0.0572	0.1598	-0.7346	-0.0789	1.0000					
VITD	0.0554	0.0547	0.4952	0.2871	-0.3727	1.0000				
VITC	0.4543	0.1472	0.2569	0.2400	-0.1893	0.4615	1.0000			
APROT	0.0952	0.0675	0.3890	0.7588	0.1755	0.3936	-0.0507	1.0000		
VPROT	-0.1381	0.0382	0.4681	0.5343	-0.1933	-0.0826	0.2746	0.0296	1.0000	
KCAL	-0.1324	0.1589	0.6445	0.8556	-0.0872	0.0422	0.0615	0.5293	0.7591	1.0000
SUN	0.0426	-0.2664	0.2600	0.1244	-0.2148	-0.0546	-0.1660	0.1232	0.0093	0.2430
EXER	0.4721	-0.3120	0.0963	0.1742	-0.0577	0.1951	0.1176	0.0922	-0.0532	-0.0325
CASERUM	0.2205	-0.5079	-0.0219	-0.0265	-0.0572	0.0192	-0.2406	0.1396	-0.0905	-0.0426
OH25	-0.2665	0.0213	0.4092	0.3530	-0.2031	0.0536	-0.2228	0.3473	0.3819	0.3813
PTH	-0.1244	-0.1547	-0.2033	-0.1276	0.1970	0.0773	-0.1059	0.0903	-0.0242	-0.1054

	SUN	EXER	CASERUM	OH25	PTH
SUN	1.0000				
EXER	0.4456	1.0000			
CASERUM	0.0199	-0.0580	1.0000		
OH25	0.2697	0.2329	-0.1060	1.0000	
PTH	-0.5115	-0.6024	0.3098	-0.1526	1.0000

APPENDIX K

CORRELATION MATRICES OF CHANGE IN BONE DENSITY WITH CHANGE IN DIETARY, ENVIRONMENTAL, AND BIOCHEMICAL DATA

CORRELATION MATRIX OF CHANGE IN BONE DENSITY WITH CHANGE IN DIETARY, ENVIRONMENTAL, AND BIOCHEMICAL DATA IN THE MEN

	AGEDIFF	CADIFF	PDIFF	CAPDIFF	VITDDIFF	VITCDIFF	APROTDIF	VPROTDIF	KCALDIFF	SUNDIFF
AGEDIFF	1.0000									
CADIFF	-0.1862	1.0000								
PDIFF	0.0211	-0.0572	1.0000							
CAPDIFF	0.0939	-0.3528	0.9515	1.0000						
VITDDIFF	-0.1308	0.8212	-0.1148	-0.3437	1.0000					
VITCDIFF	0.0109	0.5106	0.0396	-0.1045	0.6917	1.0000				
APROTDIF	-0.3192	0.5293	0.0040	-0.1892	0.6240	0.2847	1.0000			
VPROTDIFF	-0.1286	0.2284	0.3641	0.2915	0.2796	0.6211	-0.0696	1.0000		
KCALDIFF	-0.0666	0.2245	0.5089	0.4140	0.4835	0.7338	0.2620	0.7667	1.0000	
SUNDIFF	-0.3220	-0.0313	0.4459	0.4223	-0.2688	-0.4300	-0.2779	-0.0012	-0.0578	1.0000
OH25DIFF	0.0288	0.0944	0.3466	0.2774	0.2222	0.0394	0.2054	0.1545	0.2432	0.0191
BONEDIFF	-0.0799	-0.0918	0.1274	0.1572	-0.2109	-0.2250	0.1477	-0.2368	-0.2418	0.1096

	OH25DIFF	BONEDIFF
OH25DIFF	1.0000	
BONEDIFF	-0.3608	1.0000

CORRELATION MATRIX OF CHANGE IN BONE DENSITY WITH CHANGE IN DIETARY, ENVIRONMENTAL, AND BIOCHEMICAL DATA IN THE WOMEN

	AGEDIFF	CADIFF	PDIFF	CAPDIFF	VITDDIFF	VITCDIFF	APROTDIF	VPROTDIF	KCALDIFF	SUNDIFF
AGEDIFF	1.0000									
CADIFF	-0.0519	1.0000								
PDIFF	-0.1507	0.7180	1.0000							
CAPDIFF	0.0005	-0.6657	0.0026	1.0000						
VITDDIFF	0.1505	0.5056	0.1722	-0.5160	1.0000					
VITCDIFF	0.2188	-0.0645	-0.2631	-0.1255	0.1296	1.0000				
APROTDIF	-0.2655	0.4279	0.8618	0.2371	0.1615	-0.5223	1.0000			
VPROTDIFF	0.2236	0.1774	0.4225	0.2670	-0.2663	0.0872	0.0950	1.0000		
KCALDIFF	-0.0072	0.5154	0.8248	0.1634	0.0483	-0.2703	0.6537	0.6911	1.0000	
SUNDIFF	-0.0801	-0.0395	-0.4022	-0.3014	-0.0907	0.0854	-0.6015	0.2571	-0.1592	1.0000
EXERDIFF	-0.1091	0.0254	0.0051	-0.1375	-0.0741	-0.2132	0.0122	0.1573	-0.0231	0.1819
OH25DIFF	-0.1130	0.6680	0.5135	-0.3981	0.2265	-0.3329	0.2939	0.4436	0.6440	0.1313
BONEDIFF	0.3116	0.0207	-0.1255	-0.1496	0.0624	-0.1178	-0.0070	-0.5484	-0.2629	-0.3166

	EXERDIFF	OH25DIFF	BONEDIFF
EXERDIFF	1.0000		
OH25DIFF	0.2795	1.0000	
BONEDIFF	-0.6005	-0.3341	1.0000