# THE MULTIFACTORIAL ASPECTS OF BONE DENSITY IN HUMANS

A THESIS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# CHAPTER I

## INTRODUCTION

Development of skeletal bone is a dynamic process in which the bones of the human skeleton undergo continuous change throughout the life cycle (10). During fetal life the first formation of bone develops as a cartilaginous matrix. Development, including a limited amount of mineralization, continues throughout pregnancy, however, the matrix remains flexible until after birth. The hardening process, mineralization, continues rapidly from infancy until full height is achieved in late adolescence.

Factors which contribute to changes in matrix composition and properties include physical forces and hormonal changes which occur during growth. Throughout the entire growth process lengthening of the bone is constant. There is formation of new collagen matrix followed by calcification. A porous crystalline structure, trabecula, appears at the ends of long bones while compact bone of the shafts consist of a solid inorganic matrix with no visible pores. All changes in the shape of normal bones occur by resorption and deposition at bone surfaces. The active surface is situated both inside and outside the medullary cavity, the surface of each trabecula and inside every Haversian and Volkmann's canal.

In healthy individuals there is a constancy of calcium turnover in which calcium deposition and resorption are approximately equal. However, numerous studies have shown that many elderly adults lose bone mass with advancing age. In studies of age-related changes in bone the major concern has been with the amount of bone present, with the age at which bone loss begins, and whether bone loss is a physical process of aging or a disease peculiar to old age.

When measured by densitometric procedures, the values for mean radius density in both males and females increase for each five year increment in age from 10-14 through 30-34 years (61). In females, combined cortical thickness of the metacarpal reaches a maximum during the third decade of life (58). This amount is either sustained until the menopausal years (42,49) or gradually lost in the years just prior to menopause (26). After menopause, bone mass generally decreases rapidly for two or three years, less rapidly for an additional two or three years, and then continues a steady decline throughout the remaining years of life (33).

In the male, bones reach and then maintain maximum mass a decade longer than females, but by the age of sixty, loss in metacarpal cortical score is equivalent for men and women. The cortical scores of males over 70 years of age are definitely reduced. Diminished bone mass may lead to bone fragility and the syndrome of bone rarefaction known as

osteoporosis (49). Increased fractures caused by decrease in bone mass constitute a health problem for the aged.

Barnett and Nordin (12) have reviewed the pathogenesis of osteoporosis and osteomalacia. Their investigation led them to conclude that osteoporosis was a condition caused by prolonged negative calcium balance, due to low intake, intestinal malabsorption, or excessive excretion into the urine of calcium.

Bone loss, which accompanies advancing age, has primarily been measured in two ways. First, radiographic procedures, relying on visual interpretation of x-rays of various bones of the anatomy, have been utilized for assessing bone status. Radiographic densitometry has been used extensively as a means of quantifying bone status. A second procedure involves analyzing films for morphometric changes. The morphometry is performed at a selected site such as mid-shaft of metacarpals. The thickness of two cortices is summed and frequently divided by periosteal diameter. Both procedures are indices of bone quality. In recent years, newer procedures have been implemented. These include computerized tomography, whole body neutron activation analysis and the scanning of bone sites using mono or dual energetic sources.

Continued research into factors affecting bone quality throughout the life cycle is imperative if the problem of

osteoporosis among the aging population is to be prevented. Among the symptoms associated with this disease are: fractures, bone pain, decrease in height in the spine, denture problems and, possibly, loss of hearing. In 1978 the Department of Nutrition and Food Sciences at the Texas Woman's University (TWU) began a three year study entitled "Bone Quality Changes in Humans: A Twenty-Year Follow-Up". The primary objective of the longitudinal study was to determine whether individuals followed the predicted "normal" bone mass profile with age, and if so, how environmental, dietary, and pathological factors affected bone rarefaction over a 15-30 year span of time.

## Statement of the Problem

In spite of the innumerable investigations dealing with the relationship between bone density and a variety of individual factors [age (28,29,31,32,34,35,53,58,61), sex (28,29,31-35,58,61), race (31,51,56), physical activity (7,10,35,53,60), hormonal function (42,49,50), and disease (49,61)] few have explored the combined effect of several variables. No studies have considered multifactorial effects over a long period of time. The degree to which these factors exert an effect is still open to question.

Goldsmith et al. (23) found that bone mineral content (BMC) at a site on the distal radius, determined by the photon absorption method, was sufficiently related to that

of the spine to be of diagnostic value in separating normal and osteoporotic individuals. The radio-densitometric procedure used at TWU (23,38), one which measures the relative density of the middle phalanx, has been recognized as a diagnostic index for osteoporosis of the spine. Both BMC and phalanx V-2 measurements can be used to indicate if osteoporosis exists at the time of analysis. However, a formula is needed which will predict future bone status. Predicted bone values might be used to identify high risk individuals. Such identification could then lead to implementation of intervention and prevention programs for osteoporosis prone persons. Since osteoporosis is not usually diagnosed prior to the occurrence of a spontaneous fracture of the wrist or hip, an estimate of the fracture risk is of pragmatic interest. Identification of high risk individuals at an early age would be important for the prevention of osteoporosis.

## Specific Objectives

The major objective of this study was to identify those factors (variables) which most adequately explain the decrease in bone density with age. Former bone measurements, age, sex, physical activity, typical dietary intake patterns, and use of vitamin and hormonal preparations will be explored to identify a subset which best provides a predictive equation.

# CHAPTER II

## REVIEW OF LITERATURE

The major objective of this study was to identify the factors or combination of factors responsible for the loss of bone density with age. A review of research literature regarding the relationship of age, sex, physical activity, vitamin, mineral, and hormone usage, and dietary factors with bone mineralization follows.

### Age

Helela (30) has stated that marked increases in bone size, volume, and density occur in the human long bones during childhood and adolescence. Between maturation and old age gradual and subtle changes that occur in bone quantity are less well defined and often unpredictable (53). Most of the available data which support this are based on one-time observations of moderately large numbers of subjects. Limited data, based on longitudinal studies of the same individuals, have provided normal curves relating bone mass and age. In a longitudinal study of 34 men and 53 women which included serial radiographs taken over a period of time which averaged 15 years for males and 23 years for females, Garn (22) measured cortical thickness and cortical area. Results indicated a slight but significant increase

in periosteal diameter and a marked loss in cortical diameter in both sexes with advancing age. In many of the subjects there was actually a gain in cross-sectional area despite reduction in cortical thickness. Decreased cortical loss with aging occurred in taller people, but periosteal apposition was increased.

Exton-Smith et al. (19) took repeat x-rays of 16 elderly women for 6-1/2 years, and observed continued loss of bone with age. They hypothesized that better skeletal development is a characteristic of those who survive to an extreme old age.

Adams, Davies, and Sweetman (1) concluded from an 11-year follow-up study of 60 men and 54 women aged 55-64 years that cortical bone loss of the metacarpal is not uniform with age. In contrast Newton-John and Morgan (47) analyzed data from thirty publications describing the status of bone density and cortical thickness in relation to age and sex and stated that people lose bone from all parts of the skeleton as they age.

Smith, Khairi, Johnston, and Norton (52) investigated the loss of bone mineral with aging. Since a quadratic function gave a significantly better fit to their data than a linear function, they hypothesized that rate of bone loss slows in later years. However, Trotter (57) has demonstrated that bone density of various bones of the skeleton decreases

at similar rates with age. The major factor responsible for this decline in skeletal density was the loss of bone mass resulting from cortical thinning and the increased rarefaction of the trabecular bone in the epiphyses. An imbalance occurs in the equilibrium between rate of bone formation and resorption. Bone resorption, which occurred mainly at the endosteal surface of the long bones, gradually caused the endosteal surface to have a ragged "moth-eaten" appearance characterized by large resorption spaces and indentations.

Meema (43) reported that adult cortical thickness of the radius does not change appreciably up to 50 years of age, and that decreased thickness within the 20-50 year age group usually signifies bone loss resulting from pathologic conditions. Bone loss, when it occurs beyond the age of 50, may be either "physiologic" or "pathologic." In other investigations (28,29,31,32,34,53,61) the age of subjects has been found to be related to both cortical thickness and bone density.

## Sex

Differences in cortical bone thickness of males and females have been noted for various bones in the body. Helela (28) stated that maximum thickness of femur, second metatarsal, humerus, radius, second metacarpal, and proximal phalanx of the second finger is reached between 20 and 50 years of age, irrespective of sex, but that after menopause,

females show a decrease in cortical thickness sooner than males of comparable age. Similarly, Nordin (49) has shown that after the age of 45-50 years, loss of metacarpal cortical thickness occurs in women at a faster rate than it does in men. Newton-John and Morgan (47) found that the decrease in the amount of bone as a percentage of the initial mean value was about 10 percent per decade in women and possibly less in men. Garn (21) measured the cortical thickness of the metacarpal of a group of men and women over a thirty year period. He found that the majority of both men and women exhibited a loss of five to ten percent per decade with men losing less than women. Studies of Mazess and Cameron (39) have shown similar results.

By cutting rectangular cubes from the mid-central area of the lumbar vertebra during autopsies, Arnold and Bartley (9) found that levels of ash per cubic centimeter of the vertebra and femurs from females were always lower than those of males. The results suggest that the differences in vertebral mineralization between men and women may be due, chiefly, to the difference in behavior of cortical bone. Female bones appear to atrophy while male bones do not.

## Physical Activity

Effects of disuse on bone have been reviewed by McDonald (40). Investigations were done as early as 1855, however, they were not controlled and often were not

formally published. In 1941 Albright et al. (4) studied one 14-year old boy and concluded that immobilization of a large part of the skeleton resulted in hypercalcemia and a rapidly developing osteoporosis.

By 1969 studies were being conducted at Texas Woman's University in cooperation with the National Aeronautics and Space Administration and the importance of regular activity and exercise to normal body function was confirmed. Nevertheless investigations into effects of activity factors on bone mass have been conflicting. Helela (31) noted that men engaged in manual work had thicker humeri and radii than a more sedentary group of men. However, Kumlin, Wiikeri, and Sumari (35) found no significant differences between lumberjacks and control subjects with respect to metacarpal cortical thickness. In a study of post-menopausal women who exercised for three hours per week for one year Aloia et al. (7) found that bone loss decreased with an increase in physical activity.

Watson (63) has studied bone growth and physical activity in 203 young, male, amateur baseball players. The pattern for bone mineral content of the dominant radius and ulna were inconsistent. The dominant humerus was significantly more mineralized, however, for all age groups and the degree of bone mineral content increased significantly with age.

Smith et al. (54) hypothesized that physical activity effectively retards the process of bone loss and causes bone mineral accretion in the aged. They divided 39 male and female subjects into a control group, a physical activity group and a physical therapy group. They concluded that physical activity slows the normal process of bone loss and causes bone accretion in the aged.

Chalmers and Ho (14) conducted an epidemiological study of hip fractures as an index of osteoporosis among the Chinese in Hong Kong. From a population of approximately 3.7 million, 1,040 cases of hip fracture were documented in a three-year period. In both sexes, incidence was low prior to the age of forty-five and then rose steeply with increasing age. Based on fracture incidence relative to 100,000 individuals in each age group, the incidence was the same in males and females in all but those over eighty years old. After eliminating hereditary, environmental, and dietary (particularly calcium and vitamin D) effects, these authors held that one obvious factor which did correlate with the observed racial differences in incidence of hip fracture (used as an index of osteoporosis) was the living standard and the degree of physical activity undertaken by the different populations and sexes.

Vitamins, Minerals and Hormone Supplementation

The use of vitamin and mineral supplements, oral

contraceptive agents, and cortisone preparations have been investigated as factors effecting changes in bone mineral status. Albanese (3) administered 700-800 mg calcium/day to 12 elderly females with a history of subnormal dietary calcium intake. In twenty-four to thirty-six months he noted deceleration or reversal of bone loss that was measurable, radiogrammetrically. Nordin et al. (50) reported wide variation in the estrogen status of post-menopausal women. This difference may contribute to the wide variation in rate of bone loss that is observed.

Horsman et al. (33) have investigated sequential bone loss in 37 women who have undergone bilateral oophorectomy. Bone loss was monitored by radiographic morphometry of the 2nd, 3rd, and 4th metacarpals of both hands. There was a significantly greater rate of decrease of mean cortical width in women observed within three years of the artificial menopause than in other women observed. These authors postulated that the diminishing rate of bone loss with time might occur as a consequence of a partial restoration of the plasma estrogen levels through either increased secretion or conversion of androstenedione from the adrenals.

In a 10 year, double blind study Nachtigall et al. (45) evaluated the effects of estrogen replacement therapy in 168 pair-matched, randomly chosen, postmenopausal patients. The estrogen-treated patients whose therapy started within

three years of menopause showed improvement (by photon absorptiometry at the mid-point of the third metacarpal) and no increase in degree of osteoporosis. Control subjects demonstrated an increased level of osteoporosis.

Crilly et al. (15) tested the effects of six types of therapy in 75 post-menopausal women who had wrist fractures. They found osteoporotic patients to have lower plasma estrone and androstenedione levels, lower calcium absorption, and higher urinary hydroxyproline levels than matched controls. The most successful treatment was the use of hormones with or without 1-alpha-dihydroxy-D3 and calcium supplements. When only calcium and vitamin D were given in combination less success was obtained in treating osteoporosis.

Lindsay et al. (37) conducted a study of bone response to termination of estrogen treatment. The histories of forty three oophorectomised patients showed that fifteen patients who had been treated with an estrogen did not lose a significant amount of bone (measured by photon absorptiometry at the mid-point of the third metacarpal of the right hand) during 8 years of therapy. A placebo group had an initial bone loss of 2.6% per annum which fell to an average of 0.75% per annum. Fourteen patients were treated with estrogen for four years and exhibited no bone loss, but on withdrawal of estrogen bone mineral content decreased 2.5% per annum during the next four years.

## Dietary Factors

Long term intake and utilization of nutrients affect bone density and bone quality. Although calcium and phosphorus are the principal elemental components of bone, adequate dietary intake of these minerals is not an assurance of good bone quality. Protein, calcium/phosphorus ratio, vitamins A, C and D, as well as other nutrients, are essential for optimum skeletal development and maintenance. The relationship between low calcium ingestion and reduction in bone mass remains controversial. Garn et al. (20) have induced osteoporosis in adult laboratory animals maintained upon a very low calcium intake. However, these authors could not relate intake of calories, calcium, and protein to metacarpal cortical thickness in a study involving 382 human subjects.

Allen, MacLeod and Young (6) studied 158 children between the ages of 1 to 16 years and found that one-half of the children showed adequate growth and calcification (as assessed by anthropometric measurements, hand x-rays, and laboratory tests) on substandard diets while 30% did not. Hegsted et al. (27) stated that there is no convincing evidence of harm with intakes as low as 300 mg calcium/day.

Imbalance in the ratio of dietary calcium and phosphorus is thought to be associated with bone disease in a variety of animal species (44,62). In a recent long-term

investigation, however, Anderson et al. (8) reported that low dietary calcium:phosphate ratios had no significant short term or long term effects on the skeletons of young growing monkeys. Clearly more data are needed in examining the effect of dietary calcium:phosphate ratio on the human skeleton. This could be particularly critical when diets are high in animal proteins which contain large amounts of phosphorus and little calcium (2).

Spencer et al. (5) have investigated the effect of a high meat protein intake (2g/kg) on calcium metabolism in man. The effect of this high protein intake on calcium excretion and retention and on intestinal absorption was measured. A low level of dietary calcium (200 mg/day) was provided in conjunction with the high protein diet during one phase of the study. Additional studies were carried out using a calcium intake of 1,100 mg/day and a 2,000 mg/day. The lack of a significant increase in urinary calcium during the studies was attributed to a higher phosphorus content in the high protein diets. For vegetarian diets, however, approximately 80% of the dietary phosphorus is considered unavailable. This could also have a significant effect on bone mineralization. Ellis, Holish, and Ellis (18) measured radiographic bone density and noted significantly greater density in human vegetarians than in omnivores.

Allen, Bartlett, and Block (5) concluded that in human subjects the consumption of dietary protein reduces the amount of filtered calcium reabsorbed by the kidney. The authors further stated that protein-induced calciuria occurs as the result of altered renal handling of calcium, rather than through an increase in intestinal calcium absorption or an initial effect on bone calcium turnover.

Licata et al. (36) studied five untreated osteoporotic patients in a metabolic unit for 30 days. The subjects received control diets containing normal amounts of protein (0.8g/kg) and then high protein diets (2.0g/kg) made with purified protein. The high protein diet increased calciuria and resulted in a negative calcium balance without causing significant change in calcium absorption, serum chemical values, or urinary sodium and potassium levels. Variable effects of calcium and/or estrogens were found with this negative balance.

Both vitamin A and C have been investigated because of their association with bone formation. A recent study by Harris and Navia (24) sought to examine the effect of vitamin A deficiency on the osteoblastic process in newly formed bones of guinea pigs. Their results have shown that the lack of dietary vitamin A results in a decrease in the calcium content of the newly formed bone. Vitamin C may affect the calcification of bone via its role in the

biosynthesis of collagen. DeClerch and Jones (17) have demonstrated that ascorbic acid is essential for the production of insoluble fully hydroxylated collagen.

McLennen, Caird, and MacLeod (41) measured metacarpal cortical thickness in 65 men and 160 women and found no relationship between bone thickness and the intake of calcium, protein, or ascorbic acid. Hayes, Bowser, and Trulson (25) concluded from their study of 47 males and females over 65 who had been hospitalized for femur fractures that eating habits or milk intake during childhood did not relate to fracture incidence. Conversely, studies by Dallas and Nordin (16), Hurxthal and Vose (34) and, Albanese, Lorenze and Wein (3) have related inadequate intake of calcium to bone loss in women.

# CHAPTER III

# PROCEDURE

## Methods and Materials

In spite of innumerable investigations dealing with the relationship between bone density and a variety of individual factors, few have explored the combined effect of several variables. None have considered multifactorial effects over a long period of time. The major objective of this study was to identify those variables which explain the universally observed change in bone density with age.

The plan of study includes compilation of anthropometric, dietary and biochemical data obtained from examination of participants during the period from 1953 to 1966 and again in 1979 to 1981. These data were entered into the TWU Dec-20 computer and analyzed using stepwise multiple linear regression to determine possible prediction factors relating to bone density and cortical score.

Subjects. The subjects were 167 men and women who were tested between 1953 and 1966 in the Nelda Childers Stark Laboratory for Human Nutrition Research. The age of the subjects ranged from 3 to 94 years. Informed consent was obtained from each subject (see Appendix A), and the participants were administered a questionnaire (see Appendix B).

Questionnaire. The questionnaire ascertained levels of occupational activity, athletic-recreational activities and other activities of daily living. Scoring of activity was by the "positive scale" technique developed by Smith et al. (53). A score of zero was accorded an individual who was unemployed, who did not participate in any athletic or recreational activity, and who did not do daily chores around the home. The maximum activity score of 24 could be attained by being employed full time, by participating at least once a week in athletic recreational activities, by being involved in at least five activities of daily living, and by performing heavy labor in a job. Questions relating to frequency of use of vitamin and mineral preparations, oral contraceptive agents, and cortisone or cortisone-like preparations were included in the questionnaire.

Diet Records. A 24-hour diet recall was included in the twenty-year follow-up study and three day diet records were requested from each participant. Seven day dietaries were available from the initial tests. In addition a food frequency check-list was used to secure an accurate and typical daily intake of nutrients. Each of the intakes were evaluated for protein, calcium, phosphorus, vitamin A and vitamin C. Protein intake was computed and reported both as total, and as that derived from animal and vegetable sources. The calcium/phosphorus ratio was computed without considera-

tion of source. The listed nutrients were summed to get the average daily intake for each subject. The method used to analyze the diets for nutrient content utilized a nutrient data base which originated from the United States Department of Agriculture Handbook 8 (64) and was expanded to include updated values from manufacturers and journal sources. The food items were coded numerically and the amounts eaten determined by household conversion codes. A nutrient profile for each subject was generated via a computerized system of dietary analysis.

Densitometric and Radiogrammetric Bone Measurements. The densitometric methods used in evaluating bone in vivo include measurement of bone density of the radius and phalanx by x-ray densitometry (23) and of the distal radius (3 cm site) by photon absorptiometry (13). A radiograph was taken of the subject's left hand in a postero-anterior position using Kodak fine grain AA, non screen, film. Calibration of exposed films was attained by placing an aluminum alloy reference wedge on each film adjacent to the hand. The wedge alloy was selected because it has an x-ray absorption coefficient similar to that of bone. The exposed film was processed according to an established standardized technique and evaluated densitometrically for optical density which, in turn, was converted to aluminum equivalency (23,38). A single densitometric tracing was made of the radius and

three transverse scans were made of the middle phalanx of the little finger (see Figure 1). In addition the left distal radius was scanned by a Norland-Cameron photon absorption instrument which utilizes the isotope, Iodine-125. The I-125 source passed below the bone, while a sodium iodide detector coupled to the source, passed above the bone. A tissue equivalent bag (filled with water) was placed around the subject's arm (see Figure 2). Bone mineral content and bone mineral divided by bone width was measured by the Cameron technique (13) at the distal radius site (three centimeters from its distal articular surface).

By radiogrammetrical scaling the cortical score was calculated from measurements made at the mid-shaft for total metacarpal width and medullary cavity width using the following formula: total width (TW) minus medullary cavity width (MC) divided by total width [(TW-MC)/TW]. The second metacarpal (II-4) and third metacarpal (III-4) values were calculated by the procedure of Barnett and Nordin (11).

## Statistical Treatment of the Data

Analysis of the data was by multiple regression analysis (46) to determine prediction factors relating to bone density and cortical score. The data was analyzed using stepwise multiple linear regression to find a subset of factors that appeared to give the best linear prediction equation. All subjects were included in the statistical

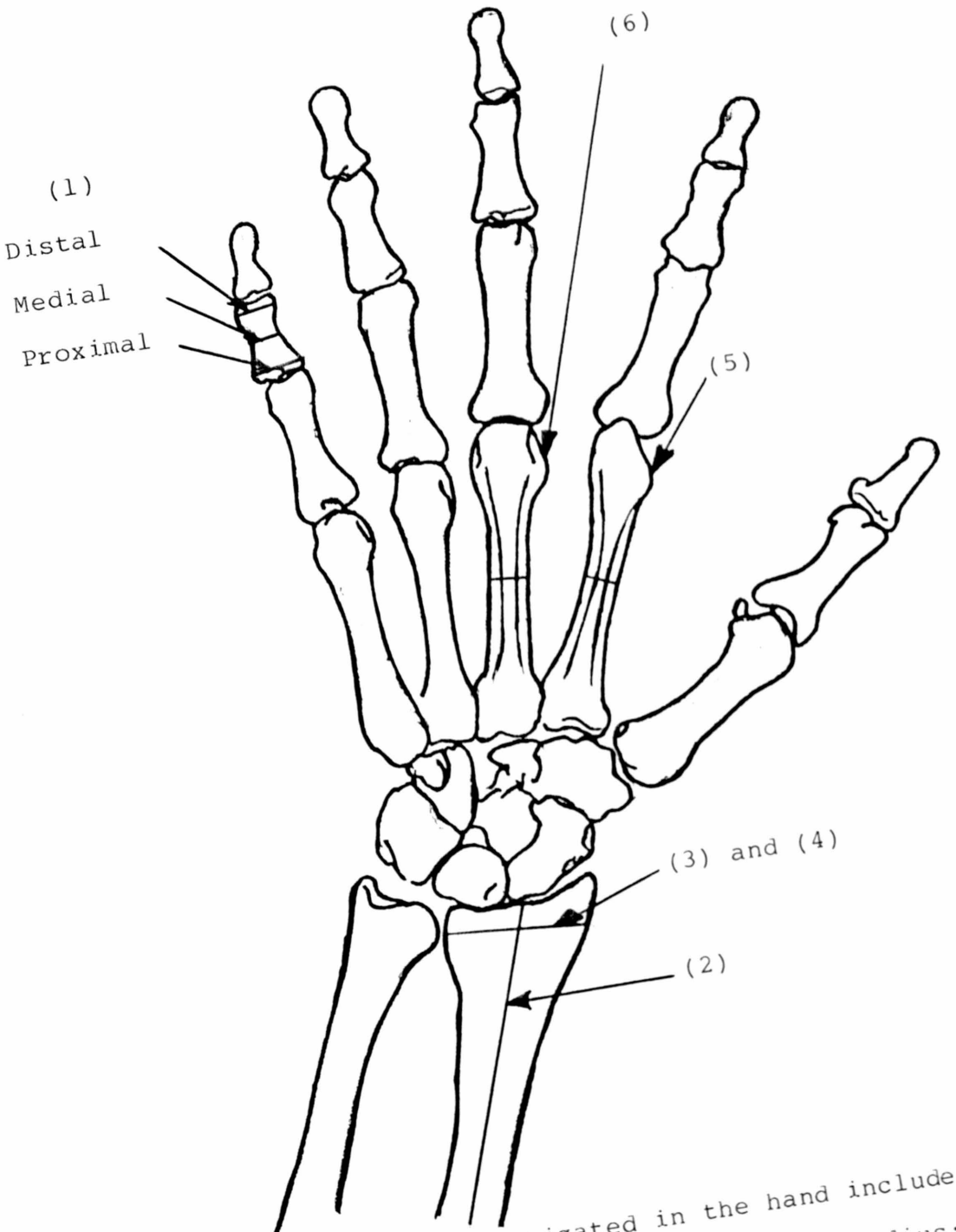


Figure 1. The sites investigated in the hand include: (1) Phalanx V-2; (2) Radius; (3) I-125 Distal Radius; (4) BMC/BW; (5) Metacarpal II-4; and (6) Metacarpal III-4

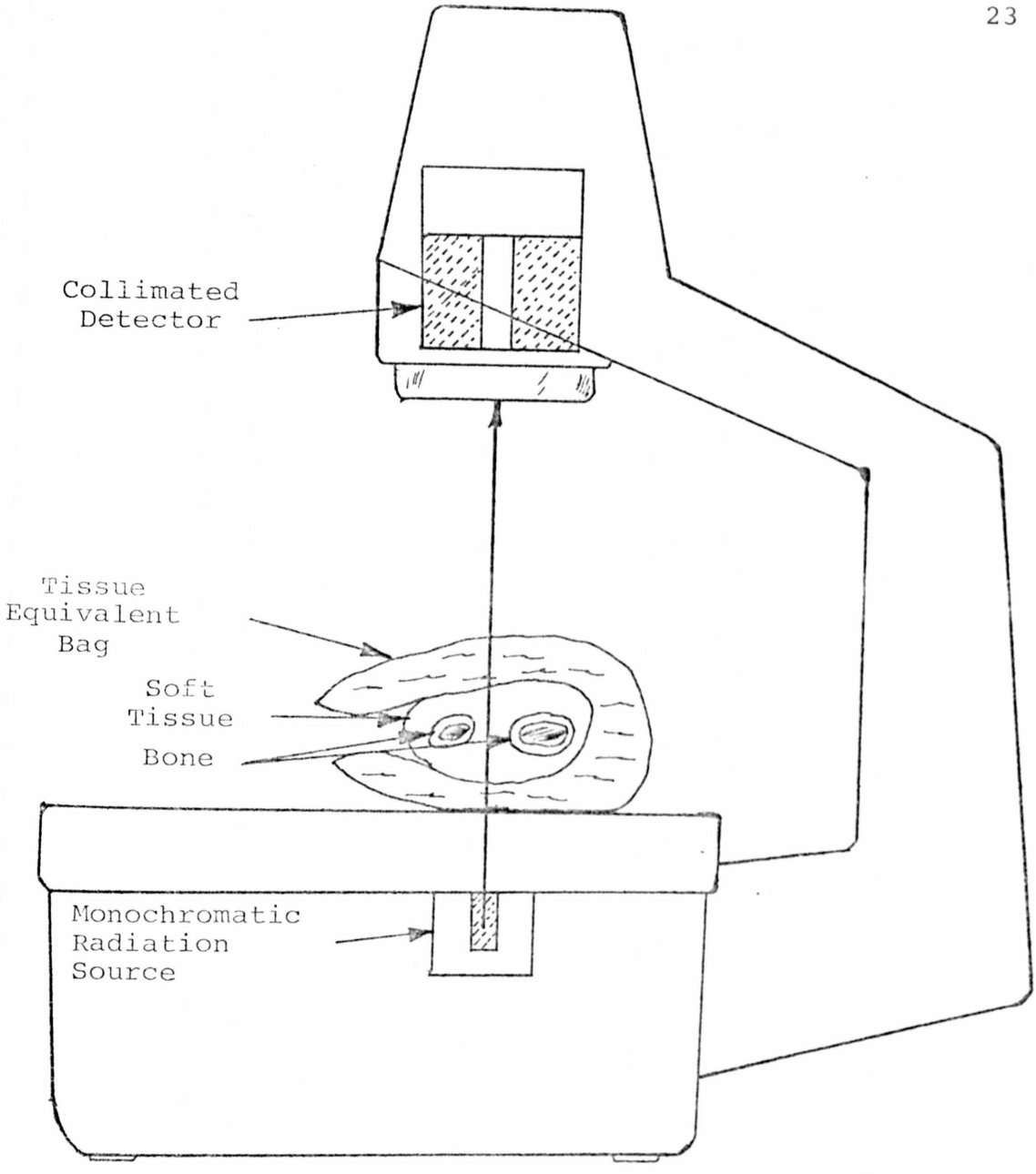


Figure 2. Bone mineral content (BMC) of the distal radius and BMC normalized for bone width (BW) are derived using Iodine-125.

analyses initially. Males and females were subsequently treated separately for statistical analysis. For some bone-related parameters, sub-sets of independent variables were analyzed further. In the stepwise multiple regression analysis, the variable added at each step was the variable that produced the largest multiple correlation and thus the largest partial correlation with the dependent variable. Stepwise additions to the multiple regression equation were continued until the analysis of variance, which tested significance for adding the independent variable to the equation, was not significant at the $p<0.05$ level.

# CHAPTER IV

## RESULTS AND DISCUSSION

The major objective of this study was to identify the factors (variables) or group of factors (subset) responsible for decrease in bone mineralization with aging and to derive, if possible, a predictive equation which might be useful as a diagnostic tool. A description of age and sex distribution among the 167 participants is given in Table 1. There were approximately twice as many females as males. The most representative ages in the initial study were less than 19 years old, therefore, in the follow-up study most of the subjects were 20-49 years old. This was true for both males and females.

Indices of bone mineralization (dependent variables) were: bone density of phalanx V-2, bone density of the distal radius, cortical score for metacarpals II-4 and III-4, bone mineral content at the distal radius, and the bone mineral content value normalized for bone width (Table 2). Each of the bone parameters measured at the final test period were analyzed as the dependent variable by multiple regression analysis (48). The independent variables considered for investigation were chosen because of their accepted relationships to normal bone integrity in humans.

TABLE 1

SEX-AGE DISTRIBUTION OF THE SUBJECTS PARTICIPATING IN THE TWENTY-YEAR FOLLOW-UP STUDY

| Age of Subjects (Years) | Number of Subjects | | | | | |
|---|---|---|---|---|---|---|
| | Males | | Females | | Total | |
| | Initial | Final | Initial | Final | Initial | Final |
| 1 - 9 | 19 | 0 | 23 | 0 | 42 | 0 |
| 10 - 19 | 29 | 3 | 60 | 1 | 89 | 4 |
| 20 - 29 | 0 | 10 | 4 | 5 | 4 | 15 |
| 30 - 39 | 0 | 32 | 0 | 62 | 0 | 94 |
| 40 - 49 | 0 | 3 | 3 | 19 | 3 | 22 |
| 50 - 59 | 2 | 0 | 11 | 0 | 13 | 0 |
| 60 - 69 | 2 | 0 | 11 | 5 | 13 | 5 |
| 70 - 79 | 2 | 1 | 1 | 14 | 3 | 15 |
| 80 - 89 | 0 | 4 | 0 | 6 | 0 | 10 |
| 90 - 99 | 0 | 1 | 0 | 1 | 0 | 2 |
| Total | 54 | 54 | 113 | 113 | 167 | 167 |
| Mean Age: | 16.2 | 37.7 | 23.3 | 44.9 | 21.1 | 42.6 |
| Standard Deviation: | 18.1 | 17.2 | 20.0 | 17.9 | 19.7 | 17.9 |

TABLE 2

BONE MEASUREMENTS AND CORRESPONDING ABBREVIATIONS USED IN COMPUTATIONAL ANALYSES

| Method and Site of Measurements | Test Period | Abbreviation of Variable |
|---|---|---|
| Photometric Evaluation (Aluminum Equivalency) | | |
| Phalanx V-2 | Initial | FIVIN |
| | Final | FIVFI |
| Distal Radius | Initial | R-IN |
| | Final | R-FI |
| Morphometric Evaluation (Cortical Score) | | |
| Metacarpal II-4 | Initial | TWOIN |
| | Final | TWOFI |
| Metacarpal III-4 | Initial | THRIN |
| | Final | THRFI |
| Absorptiometric Evaluation | | |
| Radius 3 cm. (g/cm) | Initial | * |
| | Final | BMC |
| Mineral/Width ($g/cm^2$) | Initial | * |
| | Final | BMC/BW |

*Not available at the initial test period

Tables 3 and 4 list the factors which were analyzed in a step-wise development of a prediction equation for each of six bone mineralization values. Each parameter was taken as a separate entity and analyzed three times (total subjects, males only and females only). When significance for adding a new variable changed the significance standings of variables drawn into the equation at an earlier step, subsets of the independent variables were resubmitted for analysis by controlling the order of drawing variables into the equation. The means for each of the six bone growth parameters were computed by the regression statistical program (Table 5). The trends for change in bone density with age followed expected patterns. The mean densities for phalanx V-2 were almost identical for males at the follow-up and initial test period. The phalanx V-2 values demonstrated a maintenance of bone integrity among the males whose mean ages at the respective test periods were 16 and 38 years. The group stability patterns for bone measurement factors are being reported in a separate study.

The means derived for total subjects were weighted toward the female results because there were twice as many females as males in the study. The mean density values of phalanx V-2 indicated that women, as a group, had lost

TABLE 3

LIST OF PHYSICAL ACTIVITY, VITAMIN, MINERAL AND HORMONE VARIABLES AND THE CORRESPONDING ABBREVIATIONS USED IN COMPUTATIONAL ANALYSES

| Variables | Test Period | Abbreviation of Variable |
|---|---|---|
| Physical Activity (Scores) | | |
| | Present | ACT1 |
| | During Past Year | ACT2 |
| | During Past 10 Years | ACT3 |
| Multiple Vitamins (Frequency of Use) | | |
| | Present | V1 |
| | During Past | V2 |
| Calcium Tablets (Frequency of Use) | | |
| | Present | CA1 |
| | During Past | CA2 |
| Female Hormones (Frequency of Use) | | |
| | Present | FH1 |
| | During Past | FH2 |
| Cortisone and Related Medications (Frequency of Use) | | |
| | Present | CO1 |
| | During Past | CO2 |

TABLE 4
LIST OF NUTRIENT INTAKE VARIABLES
AND THE CORRESPONDING ABBREVIATIONS
USED IN COMPUTATIONAL ANALYSES

| Nutrient Intake Variables | Test Period | Abbreviation of Variable |
|---|---|---|
| Protein Intake (g) | | |
| Animal Source | Initial | APIN |
| | Final | APFI |
| Vegetable Source | Initial | VPIN |
| | Final | VPFI |
| Total | Initial | TPIN |
| | Final | TPFI |
| Calcium Intake (mg) | Initial | CA-IN |
| | Final | CA-FI |
| Phosphorus Intake (mg) | Initial | P-IN |
| | Final | P-FI |
| Calcium/Phosphorus | Initial | CAPIN |
| | Final | CAPFI |
| Vitamin A Intake (I.U.) | Initial | VA-IN |
| | Final | VA-FI |
| Vitamin C Intake (mg) | Initial | VCIN |
| | Final | VCFI |

TABLE 5

SUMMARY OF BONE ASSESSMENTS TAKEN AT TWENTY-YEAR* INTERVAL

| Parameters | Males (N=54) Mean ± S.D. | | Females (N=113) Mean ± S.D. | | Total (N=167) Mean ± S.D. | |
|---|---|---|---|---|---|---|
| †Phalanx V-2 | | | | | | |
| Initial | 0.208 | 0.029 | 0.212 | 0.029 | 0.211 | 0.029 |
| Final | 0.205 | 0.024 | 0.193 | 0.031 | 0.197 | 0.029 |
| †Distal Radius | | | | | | |
| Initial | 0.282 | 0.033 | 0.278 | 0.040 | 0.279 | 0.038 |
| Final | 0.289 | 0.055 | 0.261 | 0.043 | 0.270 | 0.049 |
| ††Metacarpal II-4 | | | | | | |
| Initial | 0.492 | 0.100 | 0.568 | 0.088 | 0.498 | 0.099 |
| Final | 0.607 | 0.096 | 0.594 | 0.107 | 0.598 | 0.103 |
| ††Metacarpal III-4 | | | | | | |
| Initial | 0.476 | 0.095 | 0.509 | 0.082 | 0.498 | 0.088 |
| Final | 0.598 | 0.104 | 0.562 | 0.114 | 0.574 | 0.112 |
| §Radius 3 cm Site | | | | | | |
| Final | 1.266 | 0.209 | 0.817 | 0.140 | 0.962 | 0.021 |
| §Radius Mineral/Width | | | | | | |
| Final | 0.572 | 0.092 | 0.459 | 0.076 | 0.496 | 0.097 |

*Initial Interval 1953-1966, Follow-Up Interval 1979-1981
†Photometric evaluations - aluminum equivalency
††Morphometric evaluations - total cortical thickness/width
§Absorptiometric evaluation - bone mineral content (g/mm) and $g/mm^2$)

significant amounts of bone from the initial study to the final study (0.212 to 0.193 aluminum equivalents/mm bone). Mean ages of the females in the initial study were 23 years and in the final study were 45 years. Other trends such as the slight increase in cortical scores for men over the twenty year period and the slight gains in the metacarpals by women were in agreement with work done by Garn et al. (22). Regretably, the photon absorption technique was not available at the time of the initial tests.

The mean intakes of selected nutrients are listed in Table 6. The average protein intake changed little or none with time, the greatest being in that of men's changes to vegetable protein (27g decreased to 22g). Dietary vitamin A, calcium and phosphorus, as well as calcium/phosphorus ratio, decreased during the period from initial to final in all sex-age groups. Vitamin C intakes, however, increased in all groups during the intervening years.

## Multiple Regression Analyses for Predictive Equations

The multiple correlation coefficients for each of the six bone mineralization parameters are presented in Table 7. The resulting prediction equations generated for phalanx V-2, distal radius, metacarpal II-4, metacarpal III-4, bone mineral content of the radius (3 cm site), and bone mineral

TABLE 6

SUMMARY OF DIETARY INTAKES OF SELECTED NUTRIENTS AT TWENTY-YEAR* INTERVAL

| Nutrient Intake Variables | Males | | Females | | Total | |
|---|---|---|---|---|---|---|
| | Mean | ± S.D. | Mean | ± S.D. | Mean | ± S.D. |
| Protein (g) | | | | | | |
| Animal | | | | | | |
| Initial | 58.0 | 17.0 | 49.0 | 15.0 | 51.0 | 16.0 |
| Final | 63.0 | 32.0 | 45.0 | 21.0 | 51.0 | 26.0 |
| Vegetable | | | | | | |
| Initial | 27.0 | 9.0 | 21.0 | 6.0 | 22.0 | 7.0 |
| Final | 22.0 | 10.0 | 19.0 | 10.0 | 20.0 | 10.0 |
| Total | | | | | | |
| Initial | 83.0 | 19.0 | 70.0 | 21.0 | 73.0 | 21.0 |
| Final | 85.0 | 33.0 | 64.0 | 27.0 | 71.0 | 30.0 |
| Calcium ($mg \times 10^{-3}$) | | | | | | |
| Initial | 1.02 | 0.32 | 0.77 | 0.31 | 0.85 | 0.34 |
| Final | 0.67 | 0.40 | 0.55 | 0.35 | 0.59 | 0.37 |
| Phosphorus ($mg \times 10^{-3}$) | | | | | | |
| Initial | 1.34 | 0.32 | 1.07 | 0.34 | 1.16 | 0.35 |
| Final | 1.24 | 0.49 | 0.92 | 0.40 | 1.03 | 0.46 |
| Ca/P | | | | | | |
| Initial | 0.75 | 0.13 | 0.70 | 0.13 | 0.72 | 0.14 |
| Final | 0.54 | 0.23 | 0.58 | 0.21 | 0.57 | 0.22 |
| Vitamin A ($I.U. \times 10^{-3}$) | | | | | | |
| Initial | 6.99 | 4.48 | 6.61 | 3.05 | 6.73 | 3.56 |
| Final | 5.34 | 9.91 | 5.44 | 4.87 | 5.41 | 6.88 |
| Vitamin C (mg) | | | | | | |
| Initial | 77.0 | 37.0 | 76.0 | 38.0 | 76.0 | 38.0 |
| Final | 103.0 | 97.0 | 84.0 | 60.0 | 90.0 | 74.0 |

*Initial Interval 1953-1966, Follow-Up Interval 1979-1981

TABLE 7

LIST OF MULTIPLE CORRELATION COEFFICIENTS
FOR PREDICTION EQUATIONS

| Sites of Bone Measurements | r | $r^2$ |
|---|---|---|
| Phalanx V-2 (FIVFI) | | |
| Equation 4.1 All Subjects | 0.58 | 0.34 |
| Equation 4.2 Males | - | - |
| Equation 4.3 Females | 0.74 | 0.55 |
| Distal Radius (R-FI) | | |
| Equation 4.4 All Subjects | 0.51 | 0.26 |
| Equation 4.5 Males | 0.60 | 0.36 |
| Equation 4.6 Females | 0.49 | 0.24 |
| Metacarpal II-4 (TWOFI) | | |
| Equation 4.7 All Subjects | 0.65 | 0.42 |
| Equation 4.8 Males | 0.57 | 0.33 |
| Equation 4.9 Females | 0.72 | 0.52 |
| Metacarpal III-4 (THRFI) | | |
| Equation 4.10 All Subjects | 0.72 | 0.51 |
| Equation 4.11 Males | 0.74 | 0.55 |
| Equation 4.12 Females | 0.71 | 0.51 |
| Distal Radius 3 cm Site (BMC) | | |
| Equation 4.13 All Subjects | 0.86 | 0.73 |
| Equation 4.14 Males | 0.46 | 0.21 |
| Equation 4.15 Females | 0.71 | 0.50 |
| Distal Radius 3 cm Site Mineral/Width (BMC/BW) | | |
| Equation 4.16 All Subjects | 0.72 | 0.52 |
| Equation 4.17 Males | | |
| Step 1 | 0.35 | 0.12 |
| Step 7 | 0.61 | 0.37 |
| Equation 4.18 Females | 0.64 | 0.41 |

content/bone width (3 cm site) are discussed in the following sections.

Prediction Equations for Phalanx V-2. When data for all subjects for phalanx V-2 were submitted for multiple regression analysis the resulting equation was reported through step three. The variables which were found to explain the variance in bone density of phalanx V-2 (FIVFI) for total subjects were age, cortical score of metacarpal III-4 at the initial test period (THRIN) and the phosphorus intake at the initial test period (P-IN). However, only 33.7% of the variance in FIVFI was explained by these three variables. At step seven and eight when vitamin C intake at the initial test period (VCIN) and sex were introduced as variables the F values for the analyses of variance changed markedly. These analyses of variance indicate whether the inclusion of the several variables in the predictive equation are significant in explaining the variance of the criterion variable. Further examination of subsets of variables was performed, however, no better equation was found. The predictive equation developed from all subjects includes the three variables, age, cortical score for metacarpal III-4, and intake of phosphorus at the initial test period.

$$FIVFI = 0.195 - 0.0009\ (AGE) + 0.053\ (THRIN) + 0.00001\ (P\text{-}IN)$$

Equation (4.1)

When males were examined as a single group for all variables, phosphorus intake at the initial test period (P-IN) was the variable apparently responsible for the greatest variance in FIVFI. The variable, "physical activity score during the past ten years" (ACT3) was significant to the equation at the $p<0.10$ level. This is consistent with the studies of Smith et al. (54), however, based on the present data it could not be used as a predictor variable for bone density of phalanx V-2. The problem may well reside in the technique for measuring physical activity which may not be sufficiently sensitive for this purpose. No useful predictive equation was derived for this parameter based only on data from the male subjects.

In females, variance in bone density of the phalanx V-2 (FIVFI) measured at the follow-up test period was most closely related to age. The initial phalanx V-2 densities (FIVIN) and age accounted for 48% of the variance in FIVFI. Total protein intake at the initial test period (TPIN) was a significant predictor variable. An inverse relationship existed between FIVFI and vitamin A intake at the initial period (VA-IN). The analysis of variance applied to test significance of adding each new variable to the prediction equation remained significant at the $p<0.05$ level through the fifth step when radius density at the initial test

period (R-IN) was brought into the equation. The total variance that was explained by the five variables was 55%. The prediction equation follows:

$$FIVFI = 0.1432 - 0.001\ (AGE) + 0.278\ (FIVIN) + 0.0003\ (TPIN) - 0.0000022\ (VA\text{-}IN) + 0.105\ (R\text{-}IN)$$

Equation (4.3)

Prediction Equations for Distal Radius. Multiple regression analysis of data regarding the densities of the distal radii resulted in an equation with four significant predictor variables. In this set of analyses the initial bone density for the distal radius ranked second in importance in predicting radius density at the follow-up test period (R-FI).

The variable, physical activity score during the past 10 years (ACT3), as determined subjectively by the subjects, ranked third in explaining variance in R-FI. Sex was drawn into the equation at step four. With the introduction of calcium/phosphorus values of the initial intake period (CAPIN) the level of significance for other variables in the equation changed. The effect of calcium:phosphorus ratio and its interrelationships with activity, sex and bone density of the radius warrants further investigation. There is an equation for both males and females, although the equation was derived from data for all subjects, which differs at the constant term after adjustment was made for

the effect of sex.

$$R\text{-}FI = 0.151 - 0.00055\ (AGE) + 0.3478\ (R\text{-}IN) + 0.002\ (ACT3) + 0.052\ (CAPIN)$$

Equation (4.4A)
for males

$$R\text{-}FI = 0.1335 - 0.00055\ (AGE) + 0.3478\ (R\text{-}IN) + 0.002\ (ACT3) + 0.052\ (CAPIN)$$

Equation (4.4B)
for females

The males and females were divided into two groups and the regression analyses for bone density of the distal radius were repeated for both groups. Analyzing the males separately led to the combined effect of physical activity scores, calcium/phosphorus, age, and bone density of the phalanx V-2 at the initial test period predicting only 36% of the variance in R-FI. The derived prediction equation for males based on this analysis follows:

$$R\text{-}FI = 0.173 + 0.005\ (ACT3) + 0.112\ (CAPIN) - 0.0008\ (AGE) + 0.018\ (FIVIN)$$

Equation (4.5)

Analysis of data for females resulted in a prediction equation for bone density of the distal radius which explained only 24% of the variance in this parameter.

$$R\text{-}FI = 0.1725 + 0.3713\ (R\text{-}IN) - 0.000695\ (AGE) + 0.000896\ (VPFI)$$

Equation (4.6)

Prediction Equations for Cortical Scores of Metacarpal II-4. Multiple regression analysis of cortical score data for the 167 subjects yielded an equation for metacarpal II-4 (TWOFI) which included age and cortical scores for metacarpals III-4 (THRIN) and II-4 (TWOIN) at the initial test period. The variables, age, THRIN, and TWOIN, explained 42% of the variance in cortical score of metacarpal II-4 (TWOFI) and were significant variables in the prediction equation.

$$TWOFI = 0.41836 - 0.00347\ (AGE) + 0.333\ (THRIN) + 0.2977\ (TWOIN)$$

Equation (4.7)

The equation for cortical score of metacarpal II-4 (TWOFI), derived for males only, includes the same three variables but only 33% of the variance in TWOFI was explained by them.

$$TWOFI = 0.389 + 0.2948\ (THRIN) - 0.00336\ (AGE) + 0.4147\ (TWOIN)$$

Equation (4.8)

The prediction equation for females contains six of the independent variables. Fifty two percent of the variance in TWOFI is explained by them. The variables include age, cortical scores of metacarpals II-4 and III-4, protein intake from animal sources at the follow-up test period (APFI), vitamin A intake at the initial test period (VA-IN)

and total protein intake at the initial test period (TPIN).

$$TWOFI = 0.3548 - 0.0031\ (AGE) + 0.314\ (TWOIN) + 0.295\ (THRIN) + 0.00075\ (APFI) - 0.000006\ (VA\text{-}IN) + 0.000787\ (TPIN)$$

Equation (4.9)

For the calculation of a predicted value for TWOFI using the derived equation the value to use for protein intake from animal sources would present a problem. By using the value calculated from the individual's current diet the value for TWOFI could be found and compared to the values for cortical scores that are considered optimum for the individual's sex and age. If it were found that the individual's predicted value for TWOFI was predicted to be low, then a reasonable value for protein intake could be substituted into the equation to see whether changing the intake of animal protein would increase the value and to what extent.

Prediction Equations for Cortical Scores of Metacarpal III-4.

$$THRFI = 0.3011 - 0.00365\ (AGE) + 0.5639\ (THRIN) + 0.11\ (CAPIN) + 0.17\ (TWOIN)$$

Equation (4.10)

Sex, age, cortical scores of metacarpal III-4 from the initial test period, calcium/phosphorus values from the initial intake record, and cortical scores of metacarpals

II-4 from the initial test period are significant predictor variables of this parameter. Since sex is a variable, the equation for females has the constant term of 0.2656 instead of 0.3011.

Frequency of use of multiple vitamins (V1) is a predictor variable for cortical score when cortical score data for metacarpal III-4 was analyzed using only data from males. Calcium/phosphorus ratios from the initial diet records (CAPIN), the cortical scores for metacarpal III-4 from the initial test period, and age were other significant predictor variables for THRFI. The equation for males follows:

$$THRFI = 0.317 - 0.0289\ (V1) + 0.177\ (CAPIN) + 0.61\ (THRIN) - 0.003\ (AGE)$$

Equation (4.11)

The prediction equation for cortical scores of metacarpal III-4 follows:

$$THRFI = 0.4824 - 0.003656\ (AGE) + 0.693\ (THRIN) - 0.3899\ (R\text{-}IN)$$

Equation (4.12)

Prediction Equations for Bone Mineral Content of the Distal Radius - Photon Absorption. Sex, age, bone density of the distal radius as measured from initial radiographs (R-IN), physical activity during the past ten years (ACT3), cortical scores for metacarpal II-4 as measured on initial

films (TWOIN) and protein intake from vegetable sources, measured from follow-up diet histories (VPFI) gave the best prediction equation for bone mineral content of the distal radius (BMC). The multiple correlation coefficient (r=0.86) for equation 4.13 was the highest of the study. The constant term of the equation was adjusted to include the calculation for the variable, sex.

$$BMC = 0.888 - 0.00385\ (AGE) + 0.838\ (R\text{-}IN) + 0.0073\ (ACT3) + 0.3015\ (TWOIN) + 0.0026\ (VPFI)$$

Equation (4.13)

The equation for females has a constant term of 0.4641.

When data from males was analyzed separately, the bone mineral content of the distal radius (3 cm site) yielded only two significant predictor variables, physical activity during the past ten years (ACT3) and vitamin A intake at the initial test period.

$$BMC = 1.156 + 0.0177\ (ACT3) - 0.0000118\ (VA\text{-}IN)$$

Equation (4.14)

Data for bone mineral content for the females yielded four predictor variables, age, initial radius density (R-IN), initial phalanx V-2 density (FIVIN), and frequency of multiple vitamin useage during the past (V2). Since the BMC scan is from the same area of the radius as the densitometric scan, the expectation was that these two variables

would be related.

$$BMC = 0.523 - 0.00477\ (AGE) + 0.961\ (R\text{-}IN) + 1.0\ (FIVIN) + 0.027\ (V2)$$

Equation (4.15)

Prediction Equations for Bone Mineral Content/Bone Width - Photon Absorption. The bone width measurement is used to normalyze bone mineral content measurements in the photon absorption technique. The results of multiple regression analysis for BMC/BW produced an equation in which sex, age, initial cortical score of metacarpal II-4 (TWOIN) and initial radius density (R-IN) were significant predictor variables. The equation derived from data on all subjects follows:

$$BMC = 0.472 - 0.00256\ (AGE) + 0.2055\ (TWOIN) + 0.3396\ (R\text{-}IN)$$

Equation (4.16)

The effect of sex was added to the constant term, therefore, the equation for males shows a different constant term (0.472) than that for females (0.3633).

The regression program used in analyzing the data continued through twenty eight steps, but usually the significance for adding new variables to the regression equation was lost after only a few variables were entered. However, in the analysis of BMC/BW data for males only, two equations are reported because of the change in the results from the analysis of variance which tested

significance for adding new variables to the equation. Age was the only variable which was significant in explaining the variance in BMC/BW at step 1. However, at step 7 all variables in the equation became significant predictor variables. The equation is reported after step 1 and step 7.

$$BMC/BW = 0.643 - 0.00187 (AGE).$$

Equation (4.17A - Step 1)

The seven predictor variables are age, multiple vitamin useage during the past, physical activity scores (ACT3), bone density of the radius, present calcium tablet useage, present physical activity scores and frequency of calcium tablet useage during the past.

$$BMC/BW = 0.469 - 0.0022 (AGE) - 0.041 (V2) + 0.011 (ACT3) + 0.724 (R-IN) + 0.14 (CA-1) - 0.009 ACT1) - 0.1 (CA-2)$$

Equation (4.17B - Step 7)

The analysis of BMC/BW data for females yielded age, THRIN, and R-IN as significant variables to the prediction equation. The three variables explained 41% of the variance in BMC/BW.

$$BMC/BW = 0.375 - 0.0025 (AGE) + 0.23 (THRIN) + 0.29 (R-IN).$$

Equation (4.18)

# CHAPTER V

## SUMMARY

The main objective of this study was to idenfity the factors which may contribute to the decrease in bone mass with age and to derive, if possible, a predictive equation which would be useful as a diagnostic tool. To reach this objective, data were collected from over three hundred individuals who had participated in earlier studies at the Nelda Childers Stark Human Nutrition Research Laboratories from 1953 to 1966. The data were analyzed by multiple regression analyses to predict bone mass measurements from age, sex, physical activity scores, use of vitamin, mineral, and hormone preparations, and intake of protein from animal, vegetable and total sources, calcium, phosphorus, calcium/phosphorus ratios and vitamins A and C. From the total group, 167 individuals (Table 1) were selected for the present study.

Six factors gave the best prediction of bone mineral content of the left distal radius (3 cm site): (1) sex, (2) age, (3) radiographic bone density of the left radius, (4) physical activity participation during the past 10 years, (5) cortical score of the left metacarpal II-4, and (6) vegetable protein intake. The factors having the most significant role in predicting future status of bone

mineralization, as arbitrarily chosen on the basis of correlation coefficients above 0.70, are summarized below.

1. For prediction of radiographic density of the phalanx V-2 of females (r=0.74):
   a) Age
   b) Initial bone density of phalanx V-2
   c) Initial dietary vitamin A and total protein
   d) Initial density of the distal radius

2. For prediction of the cortical score of the metacarpal II-4 of females (r=0.72):
   a) Age
   b) Initial cortical score of metacarpal II-4
   c) Initial cortical score of metacarpal III-4
   d) Current dietary animal protein
   e) Initial dietary total protein and vitamin A

3. For prediction of the cortical score of the metacarpal III-4:
   A. Total subjects (r=0.72)
      a) Age
      b) Initial cortical score of metacarpal III-4
      c) Sex
      d) Initial dietary Ca/P ratio
      e) Initial cortical score of metacarpal II-4
   B. Males (r=0.74)
      a) Use of multiple vitamins

b) Initial dietary Ca/P ratio

c) Initial cortical score of metacarpal III-4

d) Age

C. Females (r=0.71)

a) Age

b) Initial cortical score of metacarpal III-4

c) Initial bone density of distal radius

4. For prediction of the mineralization of distal radius by photon absorptiometry:

A. Total subjects (r=0.86)

a) Sex

b) Age

c) Initial density of distal radius

d) Physical activity

e) Initial cortical score of metacarpal II-4

f) Current dietary vegetable protein

B. Females (r=0.71)

a) Age

b) Initial density of distal radius

c) Initial density of phalanx V-2

d) Use of multiple vitamins

5. For prediction of bone mineral of distal radius with correction to normalize for bone width (BMC/BW), all subjects (r=0.72):

a) Sex

b) Age

c) Initial cortical score of metacarpal II-4

d) Initial bone density of the radius

In summary, the data indicate the most consistent factors are age, initial bone status and, to a lesser extent, sex. Dietary factors and physical activity are more variable. This may be due to the inadequate techniques which do not permit collection of precise data.

# APPENDICES

## APPENDIX A

### CONSENT FORM

1. The purpose of this study is to make follow-up bone quality measurements after 20-25 years to determine how nutritional and environmental factors have affected skeletal development among persons tested previously in these laboratories.
2. I hereby authorize the research personnel of the TWU Research Institute to perform the following procedures:
   (a) An x-ray of the left hand will be taken for mineral analysis of the small finger and wrist.
   (b) The Norland-Cameron bone mineral analyzer will be used for mineral analysis of the wrist (distal radius).
   (c) A blood specimen will be taken by venipuncture and/or finger prick.
   (d) Nutritional and environmental questionnaires will be administered.
3. The procedures of the investigation have been explained to me by:

   _____
   (Name)
4. The radiation dose delivered to the hand (x-ray) and distal forearm (bone mineral analysis) will be approximately 168 milliroentgens (mR) and 1.68 mR, respec-

tively. The bone mineral analyzer exposes a section of tissue only 5.0 millimeters wide and 2.0 centimeters in length. The dose to the whole body will be 0.

According to the National Committee for Radiation Protection, Report No. 39, the average allowable dose is 75,000 mR/year to the hand and 30,000 mR/year to the forearm.

5. I understand that the procedures to be followed involve these possible risks or discomforts:
   (a) Excessive radiation exposure.
   (b) Infection in blood-drawing process.
   (c) Possiblity of public embarrassment.
   (d) Improper release of stored data.
6. I understand that the procedures will have the following potential benefits to myself and/or others:
   (a) I will be eligible to receive a detailed evaluation of my bone mineral status in comparison with the general U.S. population. In addition, all collected data will be available to my physician if I so desire, and the information gained may provide a future safeguard against bone fracture.
   (b) The value of the program to society is that the knowledge gained may be beneficial in prescribing those therapies and precautions that will best maintain the skeleton and prevent bone rarefaction.

7. An offer to answer all of my questions regarding the study has been made. If alternative procedures are more advantageous to me, they have been explained.
8. I understand that I may terminate my participation in the study at any time, and in this event all x-rays and records will be destroyed. My name will not be shown on the x-ray films or records, only a number for identification purposes by the principal investigators.
9. I understand that no medical treatment or other compensation is provided by the University for any injury suffered as a result of the research project.

_____ _____

Signature Date

# APPENDIX B

## QUESTIONNAIRE

Case Number_____ Sex (Circle) F M Age _____

Date of Test _____

ACTIVITY

| | Present | During Last 12 Months | During Last 10 Years |
|---|---|---|---|
| Occupational Activity | | | |
| 0. Not employed ... | ____ | ____ | ____ |
| 1. Part-time employed ..... | ____ | ____ | ____ |
| 2. Full-time employed ..... | ____ | ____ | ____ |
| Participation in athletic activities such as golf, tennis, cycling, skiing, bowling and dancing. | | | |
| 0. No participation | ____ | ____ | ____ |
| 1. Occasional participation--once a month ..... | ____ | ____ | ____ |
| 2. Seasonal participation-- twice a month.. | ____ | ____ | ____ |
| 3. Seasonal participation-- once a week ... | ____ | ____ | ____ |

| | Present | During Last 12 Months | During Last 10 Years |
|---|---|---|---|
| 4. Seasonal participation-- over once a week ..... | ____ | ____ | ____ |
| 5. Year-round participation twice a month.. | ____ | ____ | ____ |
| 6. Year-round participation once a week ... | ____ | ____ | ____ |
| 7. Year-round participation over once a week ..... | ____ | ____ | ____ |
| Activities of daily living | | | |
| 1. Makes shopping trips of any type at least three times ..... | ____ | ____ | ____ |
| 2. Daily involved in either preparing meals or cleaning up afterwards . | ____ | ____ | ____ |
| 3. Does some yard work of any degree at least once a week in season . | ____ | ____ | ____ |
| 4. Some involvement in cleaning, mopping, sweeping, or vacuuming floors at least once a week ..... | ____ | ____ | ____ |

| | Present | During Last 12 Months | During Last 10 Years |
|---|---|---|---|
| 5. Some involvement in washing or ironing clothes at least once a week ..... | ____ | ____ | ____ |
| 6. Total score (Subject, do not fill in).. | ____ | ____ | ____ |

Present Employment:

| Type of Position | How long employed | Between what ages | Physical activity required 1. Light | 2. Med. | 3. Heavy |
|---|---|---|---|---|---|
| _____ | _____ | _____ | _____ | _____ | _____ |

Location of Work

0. Indoors 1. Outdoors

_____ _____

Past Employment:

| Type of Position | How long employed | Between what ages | Physical activity required 1. Light | 2. Med. | 3. Heavy |
|---|---|---|---|---|---|
| _____ | _____ | _____ | _____ | _____ | _____ |

Location of Work

0. Indoors 1. Outdoors

_____ _____

| Use of Medications | 0. Never | 1. Occasionally | 2. Regularly | 9. Do not know |
|---|---|---|---|---|
| How often do you use vitamins at present | ____ | ____ | ____ | ____ |
| If you do, what type, if known<br>_____ | | | | |
| How often have you used vitamins over the past 20 years? | ____ | ____ | ____ | ____ |
| If regularly, what type, if known<br>_____ | | | | |
| How often do you use calcium tablets or calcium wafers at present? | ____ | ____ | ____ | ____ |
| If regularly, what type, if known<br>_____ | | | | |
| How often have you used calcium tablets or wafers in the past 20 years? | ____ | ____ | ____ | ____ |
| If regularly, how many per day<br>_____ | | | | |
| How often do you use female hormones at present? | ____ | ____ | ____ | ____ |
| If regularly, what type, if known<br>_____ | | | | |

| Use of Medication (continued) | 0. Never | 1. Occasionally | 2. Regularly | 9. Do not know |
|---|---|---|---|---|
| For how long _____ | | | | |
| For what condition _____ | | | | |
| How many daily _____ | | | | |
| How often have you used female hormones in the past? | ____ | ____ | ____ | ____ |
| If regularly, what type, if known _____ | | | | |
| For how long _____ | | | | |
| For what condition _____ | | | | |
| How many daily _____ | | | | |
| At what age _____ | | | | |
| How often do you use cortisone-like hormones at present? | ____ | ____ | ____ | ____ |
| If regularly, what type, if known _____ | | | | |
| For how long _____ | | | | |
| For what condition _____ | | | | |
| How many daily _____ | | | | |
| How often did you use cortisone-like hormones in the past? | ____ | ____ | ____ | ____ |

If regularly, what type if known

_____

For how long _____

For what condition

_____

How many daily ____

At what age _____

THIS IS A SURVEY OF YOUR PRESENT EATING HABITS

Do you eat about the same foods as you have during the past two or three years?

1. Yes ____ 0. No ____

Are you on a special diet?

1. Yes ____ 0. No ____

If yes, what type of diet is it? _____

PLEASE COMPLETE THE REMAINDER OF THE QUESTIONNAIRE BY

(1) Circling the appropriate number
(2) Or, if necessary, by writing a number in the blank space.

---

DAIRY PRODUCTS

How many CUPS of milk do you use in food preparation each WEEK? ... 0 1/2 1 2 3 4 5 or __

How many CUPS of Ice Cream or other ready-to-eat milk foods do you eat each WEEK? ..... 0 1/2 1 2 3 4 5 or __

How many CUPS of milk (as such) do you drink each DAY? ..... 0 1/2 1 2 3 4 5 or __

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CHEESE

How many CUPS of Cottage Cheese do you eat each WEEK? ..... 0 1 2 3 or __

How many OUNCES of Swiss, Cheddar, American or other kinds of cheese do you each each WEEK? ..... 0 1 2 3 4 or __

1 ounce = 1 slice or 2 inch cubes

EGGS

How many eggs (as such) do you eat each WEEK? ..... 0 1 2 3 4 5 or __

How many eggs do you use each WEEK in food preparation ..... 0 1 2 3 4 5 or __

---

POULTRY - FISH - MEAT

How many MEALS each WEEK do you eat Poultry? (Chicken, Turkey, etc.).. 0 1 2 3 or __

What is the average size of each serving in POUNDS? ..... 0 1/4 1/3 1/2 1 or __

How many MEALS a WEEK do you eat canned Salmon, Sardines, Mackerel? 0 1 2 3 or __

What is the average size of each serving in OUNCES? ..... 0 2 3 4 5 6 or __

How many MEALS a WEEK do you eat other kinds of fish or seafood?... 0 1 2 3 or __

What is the average size of each serving in POUNDS? ..... 0 1/4 1/3 1/2 1 or __

How many MEALS a WEEK do you eat meat? (not including poultry) .... 0 2 4 6 8 10 or __

What is the average size of each serving in POUNDS? ..... 0 1/4 1/3 1/2 1 or __

---

CEREALS

How many TIMES a WEEK do you eat cooked or dry cereal? ..... 0 1 2 3 4 5 or __

What is the average size of each serving in CUPS? ..... 0 1/4 1/3 3/4 1 or __

STARCHES

How many pieces of bread, rolls, or muffins do you eat each day? ..... 0 1 2 3 4 5 or __

How many MEALS a WEEK do you eat potatoes, noodles, rice, macaroni or other starches? ..... 0 1 2 3 4 5 or __

What is the average size of each serving in CUPS? ..... 0 1/4 1/2 3/4 1 or __

---

VEGETABLES

How many MEALS a WEEK do you eat Spinach, Broccoli or other leafy greens? ..... 0 1 2 3 4 5 or __

What is the average size of each serving in CUPS? ..... 0 1/4 1/3 1/2 1 or __

How many MEALS a WEEK do you eat dried Peas, Beans or other legumes? ..... 0 1 2 3 4 or __

What is the average size of each serving in CUPS? ..... 0 1/4 1/3 1/2 1 or __

How many MEALS a WEEK do you eat Beets, Peas, Lettuce or other vegetables? ..... 0 1 2 3 4 5 or __

What is the average size of each serving in CUPS? ..... 0 1/4 1/3 1/2 1 or __

---

FRUITS

How many CUPS a DAY do you eat or drink Orange or Grapefruit juice or fruits? ..... 0 1/4 1/3 1/2 1 or __

How many CUPS a DAY do you eat
Peaches, Pears, Bananas or other
fruits or juice? ..... 0 1/4 1/3 1/2 1 or __

---

(For study of 24-hour recall) Was yesterday a typical day for you? _____

Yesterday was (day) _____. List all foods eaten yesterday, including amounts:

| Breakfast | Lunch | Evening | Snacks |
|---|---|---|---|
| | | | |

Body Measurements

Weight _____ (kg) Height _____(cm)

Sitting Height _____ Ratio: Sit./Stand._____

Bone Mineral Analysis

Radiological Assessment:

Al. Equiv. Phalanx 5-2 _____/mm bone

Radius Mineral _____/mm bone

Cortical Index Metacarpal II-4 _____

Cortical Index Metacarpal

III-4 ____

Bone Mineral Analyzer Assessment:

_____gm/cm $\frac{BM}{BW}$ _____$g/cm^2$

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