RELATIONSHIP OF TOTAL BODY BONE MINERAL DENSITY TO FAT MASS, LEAN TISSUE MASS, BODY WEIGHT, AND CALCIUM INTAKE IN NORMAL-WEIGHT, PREPUBERTAL CHILDREN

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The relationship of total body bone mineral density (TBMD) to fat mass (FM), lean tissue mass (LTM), body weight, and calcium intake was examined in 40 normal-weight (10th - 90th percentile for weight) children, aged 6-10.5 years. Dual energy X-ray absorptiometry (DXA) was utilized to measure TBMD, FM, LTM, and abdominal FM. Extended research analysis was performed for body fat analysis. Parents completed a seven-day food diary for each child. Food diaries were analyzed for nutrient content utilizing Nutritionist IV. Spearman rank order was used to determine the relationship between the tested variables. TBMD was strongly and positively associated with LTM (.81) and fat-free (FFM) (.79). TBMD was moderately and positively associated with weight (.75), FM (.54), and abdominal FM (.47). TBMD was weakly and positively associated with calcium intake (.28). These results indicate that LTM and FFM are strong determinants of peak TBMD in prepubertal children.

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CHAPTER I

INTRODUCTION

Rationale for the Study

The most effective strategy in the prevention of osteoporosis is the development of peak bone mineral density (BMD) (1-12). If skeletal mineral accretion can be optimized during childhood and adolescence, the most critical periods of skeletal mineralization, individuals will begin adulthood with improved mineral stores (5). Several sources suggested that the single best predictor for bone status in later life may be bone density during childhood and adolescence (4,6,13,14).

Bone mineralization is a complex process, and much controversy revolves around conditions for peak mineral attainment. The list of factors relating to peak bone mass includes race (15), sex (16), genetics (17-30), hormonal factors (31-33), weight (34-38), body composition (39-46), and environmental factors such as calcium intake (47-49) and physical activity (2,7,10,50-55).

Several modifiable factors can have substantial effects on rates of skeletal mineralization during childhood (40). Optimization of peak bone mass by life-long adequate calcium intake is recognized as one of the most effective prophylactic techniques to reduce the risk of developing osteoporotic fractures later in life (47-49,56,57). Some studies in children and adolescents have shown a positive (1,3,4,8-10,14,34,47,49,56-66)

and other studies found no (35,67-68) correlation between bone density and calcium intake. Johnston et al. (3) demonstrated that rates of bone mineralization were increased by 3-5% at most sites in prepubertal twins given calcium supplements which increased total calcium intake from 900 mg/day to 1600 mg/day.

Another factor associated with BMD is body composition (41-46). However, findings relating peak bone mass to body composition have varied. While some researchers have found a significant correlation between fat mass and BMD in adults (36,41,43-45,69,70), other studies have demonstrated a strong positive correlation between fat-free mass and BMD (6,40,47,71-74). Several investigators have found weight alone to be the best predictor of BMD (7,12,33-37,39,41,70). These factors vary based on the gender and age group studied.

Fat-free mass has been shown to be positively correlated with BMD in both women and children (6,40,44,68,73,74). One explanation for such a relationship is that fat-free mass could exert increased mechanical stress on bone due to increased loading, stimulating osteogenesis (12,46,71). Sandler (71) reported that muscle mass and/or muscle strength reflect the forces that the muscle exerts on the bone to which it is attached. Therefore, muscle is an important determinant of bone strength (71). Doyle et al. (46) demonstrated there exists a basic quantitative relationship between weights of bones and their attached muscles.

Studies in children aged 1-19 (7,75) and young women aged 14-18 (38) showed weight to be the most important determinant of bone mass. Even though weight is

strongly associated with BMD in children, adolescents, pre- and postmenopausal women, the mechanism of this effect is not well understood (36). The increased weight of a larger skeletal frame, as well as the greater mechanical loading effects on skeletal density are possible explanations for the relationship seen between BMD and weight (70). No research indicating a strong positive relationship between fat mass and BMD in children was found.

Because there have been limited studies conducted in prepubertal children examining the contributing factors to peak BMD, and because children are of an age when prophylactic measures need to be applied and encouraged, there exists a need for further study of the relationship of BMD to body composition and calcium intake in this age group. Clarification of factors affecting peak BMD is important for prevention of osteoporosis.

Purpose of the Study

The purpose of this study is to determine the relationship of total body bone mineral density (TBMD) to body composition and calcium intake in normal-weight, prepubertal children aged 6 to 10.5 years, and to determine the relationship between body weight and TBMD. Specific objectives of the study include:

1. To determine the relationship between TBMD and estimated fat mass in normal-weight, prepubertal children.

2. To determine the relationship between TBMD and estimated lean tissue mass in normal-weight, prepubertal children.

3. To determine the relationship between TBMD and calcium intake in normal-weight, prepubertal children.

4. To determine the relationship between TBMD and body weight in normal-weight, prepubertal children.

5. To determine the relationship of TBMD and abdominal body fat mass in normal-weight, prepubertal children.

CHAPTER II

REVIEW OF LITERATURE

Bone Structure

Bone is a metabolically active tissue. It is perpetually being resorbed and rebuilt through the coordinated activity of its cells. Physiologically, mechanically, and chemically, this active tissue has many purposes. First, it is a major organ for calcium homeostasis, and it functions as a significant store of calcium, phosphate, magnesium, potassium, and bicarbonate (76). Second, bone provides mechanical support for the soft tissues and is the lever for muscle action (76). Third, bone is the major site of hematopoiesis in the human adult (76).

Bone tissue contains three major types of cells -- osteoblasts, osteocytes, and osteoclasts. Osteoblasts are the bone-forming cells that secrete a bone-specific extracellular organic matrix (osteoid) which subsequently becomes calcified into bone (77). These cells originate from mesenchymal stem cells, and they are very rich in alkaline phosphatase, which participates in the mineralization process (76). Osteoblasts deposit approximately five μm of matrix per day, and their forming period lasts approximately 100 days (76).

Some osteoblasts are buried within the matrix they formed and become osteocytes. Others become flattened cells on the surface of bone and are called lining cells (76).

These lining cells are the gatekeepers of the bone (78). These gatekeepers communicate with each other via projections, which join in gap junctions (76). The lining cells respond to many hormonal, nutritional, and mechanical stimuli (79). As the gatekeepers, they are informed of the need for remodeling (78). They then get this cycle started by mediating the activities for remodeling: preparation of site, recruitment of mononuclear preosteoclasts, building of capillaries, and attraction of preosteoclasts to the site where they will become multinucleated osteoclasts (78).

Osteoclasts, which originate from the stem cells of the bone marrow, are responsible for bone resorption. These cells possess a unique, specialized surface characterized as the ruffled border. This convoluted membrane adheres to the bone and seals the resorption space. The content of the enclosed space is very acidic (pH 4), and lysosomal enzymes degrade the matrix (76). The process is continual. Osteoclasts resorb, and osteoblasts form new bone. The coupling of the actions between osteoblasts and osteoclasts is the basis for bone turnover or remodeling. This continuous skeletal activity is also related to the maintenance of mineral homeostasis (76).

There exist two main types of bone tissue -- cortical (compact) and trabecular (spongy) (79). Cortical and trabecular bone are constituted of the same cells and the same matrix elements, but there are structural and functional differences (77). Structurally, 80% to 90% of the volume of compact bone is calcified vs. 15% to 25% of the volume of trabecular bone (77). The remaining 75% to 85% of the volume of trabecular bone is occupied by bone marrow, blood vessels, and connective tissue (77). Functionally, the

cortical bone fulfills mainly the mechanical and protective duties and the trabecular bone the metabolic tasks (77).

Cortical bone is found in the outer dense shell of long bones and is covered by periosteum (76). It is compact in nature, making up approximately 75- 80% of bone tissue (79). This compact bone forms a cylinder that surrounds a central, medullary cavity which is lined with the endosteum. The matrix is laid down around a central (Haversian) canal containing minute nutrient vessels and a nerve (80). This process may be important in maintaining the mechanical strength of bone (80). Trabecular bone is the spongy bone characteristically found at the ends of long bones and in vertebral bodies. Making up 20-25% of bone tissue, this tissue is metabolically more active, covers greater surface area, and responds more rapidly to external change and bone turnover (79).

Bone Metabolism

The amount and shape of bone alters continually during growth and in adult life (79). Modeling occurs during growth. Bone is removed at this time because it is needed in a different location, as opposed to correcting a default within the matrix (78). Remodeling occurs when the bone is replaced because it is unable to carry out its function, which is mainly mechanical in cortical bone and mainly support for homeostasis and hematopoiesis in cancellous bone (78). Bone continuously remodels by resorbing old material and forming new replacement material (81). The remodeling process functions to preserve the organization of bone from the point of view of chemical structure, form, and biological properties (81).

Osteoblasts and osteoclasts work in teams of bone multicellular units (BMUs) (79). These units are comprised of osteoclasts leading the way, with osteoblasts following (78). Each BMU burrows through bone at a rate of approximately 20-40 μm/day for a distance of approximately 2-6 mm (78). Each BMU lifespan is approximately 6-12 months (78). The remainder of the cavity is filled with connective tissue, blood vessels, and nerves (76).

Each BMU cycle begins with the recruitment of bone-resorbing osteoclasts to a specific site. These cells then work to digest bone surface to form an erosion cavity (76). Osteoblasts then migrate onto this surface and start producing osteoid to fill the deficit generated by the resorbing osteoclasts. The osteoid is then mineralized. It may take approximately three to four months to replenish bone lost during a two-week resorption period (76). When bone resorption equals formation, no loss in bone mass occurs. Loss of bone, as evidenced in osteoporosis, is regulated by an increased turnover rate of BMUs, causing greater resorption versus formation. Conversely, when bone formation exceeds resorption, net gain of bone mass occurs.

Although the process of bone growth and shaping is made possible through the coordinated action of these osteoblasts and osteoclasts, the action is mediated by several different stimuli. The factors that regulate bone growth can be divided into four main categories: calcium regulating hormones, systemic hormones, growth and local factors, and ions (80).

Bone resorption is activated by calcium-regulating hormones, thyroid hormones, prostaglandins, some growth factors, glucocorticoids, and some ions. 99% of body calcium is stored in the bones. Because bones serve as a reserve for this calcium, resorption occurs when serum calcium levels fall. The calcium-regulating hormones - parathyroid hormone (PTH) and 1,25 dihydroxyvitamin D (calcitriol) - stimulate resorption and have a direct inhibitory effect on osteoblasts (80).

Parathyroid hormone regulates the levels of calcium and phosphate in the blood by modulating the activity of specific cells in the bone and kidney. When serum calcium concentrations fall, PTH restores extracellular calcium concentrations by stimulating the mobilization of bone calcium and phosphorus, the renal tubular reabsorption of calcium, and the synthesis of calcitriol (82). It accomplishes this by: 1) stimulating the release of calcium and phosphate from bone; 2) stimulating reabsorption of calcium and inhibiting reabsorption of phosphate from glomerular filtrate; and 3) stimulating the renal synthesis of 1,25 dihydroxyvitamin D [$1,25(OH)_2D_3$], thereby increasing intestinal absorption of calcium and phosphate (82). The net result of these actions is to raise the level of blood calcium and lower the level of blood phosphate (82).

Calcitriol works to stimulate calcium and phosphate absorption in the intestine, and it can also stimulate bone resorption. When intake of calcium and phosphorus are adequate, the major function of calcitriol is to stimulate intestinal absorption of calcium and phosphate. When the mineral supply is reduced, the synthesis of calcitriol increases. This increase in

calcitriol concentration can mobilize calcium and phosphate from the skeleton by direct stimulation of osteoclastic bone resorption (80).

Thyroid hormones stimulate bone resorption by accelerating osteoblastic differentiation (83) Prostaglandins, especially prostaglandin E_2, are potent stimulators of bone resorption (82). They act by increasing both the replication and differentiation of osteoclast precursors (84). Other growth factors that directly lead to a decrease in bone formation include epidermal growth factor and fibroblast growth factor. In vivo, growth hormone increases bone turnover, the intestinal absorption of calcium, and vitamin D-dependent intestinal calcium-binding protein (80).

Glucocorticoids also have complex effects on calcium regulation and bone metabolism (85). An excess of glucocorticoid decreases the intestinal absorption of calcium and phosphate and increases their renal excretion (85). Ions may also have a regulatory role. Calcium, phosphate, and magnesium ions all have been shown to exert direct effects on the function of the bone-forming, bone-resorbing cells (85).

Other factors enhance bone formation or inhibition of resorption. These factors include calcitonin, insulin, estrogen, growth hormone, PTH, and local factors such as prostaglandin E_2, calcium, and phosphate. Calcitonin inhibits resorption by directly inhibiting the action of the osteoclast (80). Insulin enhances bone formation through the direct stimulation of osteoblasts. It also can work indirectly via insulin-like growth factor (IGF-I), interleukins, tissue-derived growth factor, or prostaglandins (86).

Estrogen may prevent osteoporosis indirectly by increasing intestinal calcium absorption, serum calcitriol synthesis, and calcitonin secretion, which inhibits bone resorption (85). One possible theory for the effect of estrogen on bone mass is that this hormone changes the set point for mechanical feedback (87). Alteration of set point theoretically occurs by adjusting the sensitivity of bone cells to mechanical stimuli (87). PGE_2 also enhances bone formation (87). Increased mechanical strain causes PGE_2 production which results in osteoblastic proliferation. Like estrogen, PGE_2 may be a set-point altering agent, through its ability to override or augment the effects of mechanical feedback signals (87). Growth hormone and PTH have also been characterized as set-point altering agents (87).

Growth hormone demonstrates its anabolic effects of bone by increasing Haversian remodeling, increasing bone formation, and increasing the efficiency of calcium absorption and decreasing calcium excretion (87). PTH has also been shown to have anabolic effects on bone (87).

The Mechanostat Theory

Ideas about the regulation of bone formation and resorption are constantly evolving. More is being learned both at the cellular level as well as the tissue level. There exists a mechanism that ensures the bone's material and structural properties can adapt to meet the requirements to which it is exposed (88). Frost's Mechanostat theory seeks to explain the coupling between osteoblastic bone formation and osteoclastic resorption and the relationship between bone turnover and skeletal growth (89). It is proposed that cellular activities are controlled and orchestrated by the mechanostat, a controller of bone

biology (88). This mechanism controls and orchestrates cellular activities of osteoblasts, osteoclasts, osteocytes, hemopoiesis, and progenitor cell line activities (88).

This mechanostat is a control circuit with a sensor for sensing bone strain (88). It also has some type of activator that compares an applied bone strain to thresholds and activates a response through changing bone cell dynamics (88). In this feedback control system bone structure is maintained in a way that ordinary mechanical strains do not exceed a minimum effective strain (MES). Two minimum strain thresholds have been proposed. There exists a minimum strain threshold controlling modeling drifts, and another minimum strain threshold controlling basic-multicellular-unit (BMU)-based remodeling (88). Bone will undergo modeling or sculpting if local strains within bone surpass the MES (88). For example, exercise beyond the MES can stimulate bone formation. Bone will change its structure to reduce the local strains to below the MES. Conversely, if local strains fall below the MES, bone tissue will be resorbed until the local strains are increased (88).

Background of Osteoporosis

Osteoporosis is generally associated with the aging segment of our society (90). This disease, however, concerns more than postmenopausal women because it slowly robs the skeleton of its mass and strength, well in advance of the obvious fractures and maladies evident in those who suffer its consequences (71,90). The National Institutes of Health Consensus Conference on Osteoporosis in 1984 formulated the following definition for osteoporosis: primary osteoporosis is an age-related disorder characterized by

decreased bone mass and by increased susceptibility to fracture in the absence of other recognizable causes of bone loss (90). Medically, osteoporosis is defined as a decrease in qualitatively normal bone, which renders the individual more susceptible to fracture (90).

Millions of people are daily suffering from the effects of osteoporosis. Statistics from the National Osteoporosis Foundation estimate that 25 million Americans are afflicted (90). This condition results in greater than 1.2 million bone fractures each year, qualifying it as a major health concern in the United States (91). Treating this disease is costing Americans approximately $10 billion annually (90). By the year 2020, the costs are expected to reach $30 billion or more, as the aged are the fastest growing segment of our population (90). Currently, most available treatments of established osteoporosis prevent further progression of disease, but do not significantly restore previously lost bone (1,20). The failure to cure osteoporosis once it has developed emphasizes the need to focus on prevention of its occurrence (35).

Development of Peak Bone Mass

The most effective strategy against potential osteoporosis is optimal development of maximal bone mass at skeletal maturity (2). Childhood and adolescence are the most critical periods of skeletal mineralization. If skeletal mineral accretion can be optimized during this time, individuals will begin adulthood with improved mineral stores (5,6,13). Bone mineralization, however, is a complex process, and much controversy revolves around conditions for peak bone mass attainment (7,92). The list of factors relating to peak bone mass include race (15), sex (16), genetics (17-30), hormonal factors (31-33),

weight (34-38), body composition (39-46), and environmental factors such as calcium intake (47-49) and physical activity (2,7,10,50-55). As one ages, bone mass is a reflection of the interaction between these factors, which certainly do not operate independently of each other (92).

There is little agreement about the age at which peak bone mass is attained. Variations exist based upon the method of evaluation used to measure bone density (93). Gilsanz et al. (94), using quantitative computed tomography (QCT), found that the acquisition of peak bone mass takes place up to the age of 20. Halioua and Anderson (95) used single photon absorptiometry (SPA) on the forearm of 181 females and found that cortical bone mass was acquired up to age 20, and trabecular bone mass up to age 30. Others have speculated that peak BMD could be attained as early as the mid-teens (1,90,92) to as late as the mid-thirties (2). Bonjour et al. (96) found significant increases in BMD in adolescent females between the ages of 13 and 15 years, but skeletal mass growth dramatically slowed at the levels of the lumbar spine and femoral neck at ages 15-16. It was suggested that the apparent early peak in BMD needs further confirmation by longitudinal studies (96).

Peak bone mass, however, is usually reached by age 30, with some variations according to the particular type of bone tissue considered (2,8,9,92). The shafts of the long bones and hips (cortical bone) appear to increase in density until about the age of 35

(49,63,90,97). Women can gain 12.5% of total bone mass during the third decade of life (97). The spine (trabecular bone), however, increases very little in density after the age of 20 (49,90).

Genetics and Race

Both hereditary factors and race make important contributions to peak bone development. Johnston and Slemenda (98) reported that approximately 80% of an individual's bone mass is genetically controlled. Twin studies provided the best data about the influence of genetics on bone mass. Pocock et al. (17) showed that BMD was significantly correlated in monozygotic compared to dizygotic twins for the spine, proximal femur, and forearm, which is consistent with significant genetic contributions to bone mass at all these sites. Smith et al. (19) found similar results in 71 juvenile and 80 adult twin pairs when measuring the right radius with photon absorption. When BMD is more highly correlated in identical twins versus fraternal twins, genetic rather than environmental factors are suggested (19). Differences between monozygotic twins are assumed to arise exclusively from environmental factors (19).

Several other family and twin studies supported a strong influence of genetic factors in determination of BMD (18-24). Dequeker et al. (18) found a significant genetic determinant in 30 pairs of twins aged 10-72 for the bone mass of the radius in adults, and for spinal bone mass measured via SPA and dual photon absorptiometry (DPA) in the age group younger than 25 years. Seeman et al. (20), utilizing DPA, reported that premenopausal daughters of women with postmenopausal osteoporosis had lower bone

mass of the lumbar spine, femoral neck, and femoral midshaft than the daughters of nonosteoporotic women. Similarly, other studies of mother/daughter pairs reported significant correlations for radius bone mineral content (BMC) (21) and lumbar spine and femur (24). Tylavsky (22) examined the familial resemblance of mid- and distal-radial bone mass with SPA between premenopausal mothers and their college-age daughters and found that a strong genetic contribution came from the mothers.

Some researchers did not find significant associations between genetics and BMD, based on the bone type considered. Christian et al. (25) examined the genetic effects on changes in bone density with time in the midshaft radial bone mass of male twins using photon absorptiometry, finding no genetic effect in a 15-year increment. Although this research was conducted on male subjects, it demonstrated that genetic influences may not play as important a role as the influence of environmental factors on midshaft radial bone densities. Sowers et al. (26) found little or no genetic influence but strong environmental influences in a study of mother-daughter and sibling sets when measuring the distal radius via SPA. However, they noted their small number of mother-daughter sets (n=70) and sibling sets (n=59) might have limited their ability to detect familial resemblance (26).

Although the genetic effects on peak bone mass have been demonstrated (18-24), whether these genetic effects operate on changes in bone mass with age is still questioned (28). Tylavsky (22) suggested that genetic contribution plays a very critical role in the accrual of bone mass by the biological daughters of premenopausal mothers by the ages of 18-22, but that environmental influences on bone consolidation during premenopausal

decades may be more important in optimizing peak bone mass. However, after examining changes in lumbar spine and femoral neck bone density determined by DPA, Kelly et al. (27) found that genetic effects are operative on rates of change in bone density following attainment of peak bone density, and concluded that subsequent changes in bone mass are genetically linked. Genetic markers have now also been identified in osteoporosis (27,29). Common allelic variants in the gene encoding the Vitamin D receptor can be used to predict differences in bone density (27,29).

In addition to genetics, some races have both larger muscles and denser bones. For example, blacks have a higher bone mass than whites (15,30), and Chinese and Japanese have significantly less cortical mass than whites (30). The underlying mechanism(s) for the race-related differences is/are unknown (30).

Calcium

Dietary calcium serves an important factor in the development of peak bone mass and prevention of osteoporosis because it can be modified (92). Many researchers have taken different approaches to determine whether or not, and/or to what degree bone mass and bone loss are related to dietary calcium intake. Several hypothesized that better calcium nutrition during childhood and adolescence may optimize, within genetic boundaries, peak bone mass (47-49,56,59). Optimization of peak bone mass by life-long adequate calcium intake is recognized as the most effective prophylaxis to reduce the risk of developing osteoporotic fractures later in life (48,57).

Slemenda et al. (60) found that among young monozygotic twins treated with either calcium supplements or placebo for three years, the calcium-treated children had greater increases in bone density. These findings are similar to the findings of a randomized, double-blind, controlled calcium supplementation trial conducted over an 18-month period in 162 seven-year-old Chinese children, which confirmed a positive effect of calcium intake on bone acquisition but no effect on height increment (57). Johnston et al. (3) showed that oral calcium supplementations, for up to three years, increased the BMD of the radius and lumbar spine by three percent in 22 pairs of identical, prepubertal twins consuming a diet containing approximately 900 mg/day of calcium. The subjects given supplements received on average 719 mg more calcium/day than their twins. Among the 23 pubertal and postpubertal pairs, the twins given supplements received no benefit (3). In a randomized, double-blind, placebo-controlled trial, Lloyd et al. (48) found that increasing daily calcium intake from 80% of the RDA to 110% via supplementation for 18 months resulted in significant increases in total body and spinal bone density in 94 adolescent girls (mean age of 11.9 ± 0.5 years), as measured by dual energy X-ray absorptiometry (DXA).

Calcium intake and BMD, measured via DXA, were found to be positively correlated in the L1-L4 vertebrae of 49 girls aged 8-18 years (59), and in the radius, measured via SPA, of 706 girls aged 18-22 years (22). Ruiz et al. (61) found calcium intake to be a significant determinant of the BMD at the lumbar spine (L1-L4), measured via DXA, of 151 children and adolescents, aged 7-15.3 years. A positive correlation was

found between dietary calcium and vertebral density, during the prepubertal period, independently of age, body weight, gender, or sexual maturation, as shown by multivariate analysis (61).

Many other researchers have found calcium intake to have a significant positive influence on BMC and BMD in children and adolescents (1,3,4,62,64). Cross-sectional studies have suggested that intake of milk may be an important determinant of peak bone mass (14,65). Retrospective studies have demonstrated that calcium consumption in childhood and adolescence were positively associated with peak bone mass development (8,58). A comparison of two areas in Yugoslavia found a higher peak bone mass in the community with a high calcium intake as compared to the community with a low calcium intake (65). The high calcium intake was two times greater than that of the low calcium intake (65). Matkovic et al. (65) suggested that the difference in bone mass of the Yugoslavian participants was already present at the age of 30 years, suggesting that the main protective effect of calcium intake was during earlier bone growth. Variations in calcium nutrition early in life may account for as much as a 5-10% difference in peak adult bone mass (49). Such a difference could contribute to more than 50% of the difference in the hip-fracture rate later on in life (49,65).

In contrast, other researchers have found no significant correlations between calcium intakes and bone mass parameters (33,35,67-68,75). This lack of correlation between calcium and bone mineral does not indicate that this factor is irrelevant to bone mass, recognizing that calcium intake is necessary for growth (35,67). Calcium intake had

no significant effects on the mineral densities of the lumbar vertebrae, radius, femur, or total skeletal bone, measured via DXA and DPA, in 45 girls aged 9-21 years (35). All but four of these subjects consumed well below the recommended daily calcium allowance of 1200 mg/day for adolescents (35). Calcium intake was also found to have no significant effects on the BMC nor BMD of the lumbar spine (L2-L4) or femoral neck of 84 Finnish children aged 6-19 years (67) or 74 Canadian children and adolescents aged 9-16 years (99).

Children and adolescents need to be in positive calcium balance to meet the needs of skeletal growth and consolidation (9). Since serum calcium homeostasis takes precedence over skeletal homeostasis, an adequate consumption of calcium is an imperative requirement for optimal skeletal growth (71). The best value for positive calcium balance and optimal peak mass, however, is unknown (9,64,66). Matkovic et al. (1) estimated that most children between infancy and puberty are able to meet the calcium requirements necessary for adequate skeletal calcium retention.

It has been suggested that a higher RDA for calcium for children ages 2-8 years and adolescents ages 10-20 ought to be considered (1,3). Levels of calcium intake in excess of the Recommended Dietary Allowance (RDA) increased rates of bone mineralization by 3-5% at most sites in prepubertal twins (3).

Heaney (100) reported that sufficient exogenous calcium must be present to sustain density during growth and to maintain skeletal mass later in life. Additional calcium, however, above the threshold will not produce more bone than is required either

genetically or by the effects of mechanical loading (100). Matkovic and Heaney (101) analyzed data, consisting of approximately 500 studies, and found clear evidence of a balance threshold at all stages of growth. A threshold intake is defined as the level below which skeletal accumulation is a function of intake, and above which skeletal accumulation is constant, irrespective of further increases in intake (101). The threshold calcium intake is estimated to be approximately 1390 mg/day for individuals 2 to 8 years of age, and 1480 mg/day for individuals 9 to 17 years of age (101). These values are higher than the current RDAs for calcium (800 mg/day for ages 2 to 8, and 800-1200 mg/day for ages 9 to 17). Current recommendations for calcium intake in the United States are based on estimates of obligatory calcium loss, absorption rates, and calcium accretion during growth (102,103).

Fleming and Heimbach (103) recently reported that the RDA for calcium is met only by females who are under one year or 6-11 years of age. In a study of school-age twins, Miller et al. (104) found that the average intake in boys was 991 mg/day, while the average intake was 864 mg/day in girls (66). These average values are in general agreement with those reported by Nelson et al. (105) and the Bogalusa Heart Study (106). After age 11, no age group of females achieved even 75% of the recommended levels (103). Between the ages of 12 and 29, the period peak bone mass development, women consumed <60% of the RDA (103).

Prevention of osteoporosis can be undertaken by maximizing the accumulation of bone tissue during growth (96). Attention to factors that lead to the development of peak

bone mass is warranted. Childhood and adolescence are the most critical periods of skeletal mineralization (107). Approximately 60% of bone deposition is accrued during puberty (108). Therefore, dietary inadequacy may impose far greater constraints on bone formation at this time of life than at other times.

Slemenda et al. (40) found that prepubertal children engaging in weight-bearing physical activity had rates of mineralization four percent to seven percent greater than those not actively participating in exercise. Slemenda et al. (40) suggested that the effects of calcium supplementation combined with the increased mineralization experienced with weight-bearing physical activity might together account for as much as one standard deviation difference (about 10%) in bone mass by age 18 years. An increase of one standard deviation in femoral neck BMD would be expected to result in a 50% or greater reduction of proximal femur fractures in adults (40). As more research is conducted in children to determine the effects of specific parameters on BMD, progress can be made to clarify many of the uncertainties accompanying the disease of osteoporosis.

Dietary Assessment Techniques

Several methods have been proposed to measure dietary intake for accurate estimation of nutrient intake. The methods most commonly used include diet history, diet recall, three- or seven-day food records, and food frequency questionnaires. Accuracy of all of these techniques relies on the memory and ability of the subject to precisely calculate foods consumed (109). The food frequency questionnaire appears to be inaccurate in estimating absolute amounts of nutrients in comparison with other methods (110). Over-

reporting of food intake is common with this method (111). Food frequencies also fail to reflect the variety of foods actually consumed by subjects (112). The 24-hour recall provides an estimate of nutrient intake based on the individual's recollection of food consumed the previous day (111). This method is more successful for group use. The recall method, however, is less valid for individual use because the 24-hour period assessed may not be representative of the usual diet (111).

Three-day to seven-day food records are criticized because they may not accurately reflect actual intake of an individual due to the observer effect (113). The three-day record was found to be a reasonable approach for measuring qualitative nutrient data (112). The seven-day food record provides more accurate information than a three-day record due to the inclusion of both weekend and week-day meal patterns (111).

Even when an appropriate method is utilized, Nordin et al. (114) suggested it is difficult to find relationships between dietary calcium and bone status because calcium intake is only weakly related to calcium balance in careful balance studies (114). Heaney (100) noted that even when one knows to the gram exactly how much of what foods went into an individual's mouth, chemical analysis invariably reveals a somewhat different figure from the entries in food database estimates for those same foods. Interestingly, Heaney (100) also expressed that researchers often pay great attention to the accuracy, sensitivity, and specificity of bone mass measurements, yet there remains low accuracy of the means to quantify what is postulated to be the independent variable (calcium). Although these criticisms have been proposed, no alternate solutions have been suggested.

Another method of analysis is urinary calcium. Measurement of urinary calcium is not always accurate and reliable because during childhood, only a weak relationship between urinary and dietary calcium exists. This is because rapidly growing individuals retain absorbed calcium in the skeleton rather than excreting it in the urine (9).

Body Composition -- Fat Mass

Findings relating peak bone mass to body composition have varied. Several researchers have demonstrated a positive relationship between fat mass (FM) and BMD in postmenopausal women. Reid et al. (41) reported that total body fat is the most significant predictor of BMD throughout the skeleton in normal postmenopausal women, utilizing measurement of BMD of the whole body, lumbar spine, proximal femur, and body composition (total body fat and lean mass) measurements with DXA. Likewise, Kin et al. (45), measuring BMD of the lumbar spine (L2-L4) via DXA, found a positive correlation between obesity and BMD, particularly in this age group. Hassager and Christiansen (69) also demonstrated a significant correlation between FM and total body bone mineral (TBBM)/body height, thus concluding that body fat may have a preservative effect on bone mass in postmenopausal women. Total body bone mineral, lean tissue mass (LTM), and FM were measured by DPA.

Glauber et al. (36) found adiposity, measured by bioelectrical impedance (BIA), to exert more important effects at non-weight-bearing sites, making more substantial independent contributions to BMD at the radial measurement sites in elderly women, as

measured by SPA. Lumbar spine and proximal femur were measured with DXA, and FFM was calculated by mathematical formula.

Riis et al. (115) found that fast bone losers have a lower anthropometrically calculated FM than slow bone losers in the early postmenopausal years. It has been generally observed that fatness is positively associated with skeletal density, both at weight-bearing sites (i.e., femur) and sites where mechanical forces are less likely to be important (i.e., radius) in postmenopausal women (36). Much of the explanation for increased fat tissue being highly correlated with BMD relates to increases in the direct effects of transmitted gravitational forces, and the ability of adipose tissue to convert androgens to estrogens (41,69,70-72).

Explanations for the positive relationship between FM and BMD, however, have varied. Reid et al. (41) suggested that the fat-BMD relationship is not explicable in terms of either estrone production in fat tissue or the dependence of skeletal load-bearing on FM. This relationship was independent of a much weaker correlation between circulating estrone and BMD, indicating that it could not be explained in terms of adipocyte estrogen production (41). Reid et al. (41) also demonstrated that the relationship between weight and BMD was entirely accounted for by a relationship between FM and TBMD. Once FM was taken into account, there was no relationship between LTM and BMD (116). Reid et al. (41) concluded that the weight-BMD relationship could not be explained in terms of skeletal load-bearing. If this were the case, fat and lean masses would both influence BMD (43).

Reid et al. (43) also found that the fat-BMD relationship seen after menopause is also present in younger women, but that it is much less marked in men. Dual energy X-ray absorptiometry was used to measure TBMD, LTM, and FM. This analysis was repeated using BMD/height as an index of "true" density because BMD is an areal density, not a volumetric density (41,43). Lindsay et al. (70) found FM [measured via skinfold, BIA, and body mass index (BMI)] to be well correlated with TBBM (measured via DPA) in both pre- and postmenopausal women, but not more strongly in the latter. In fact, TBBM was more dependent on body fat percentage in pre- versus postmenopausal women. Lindsay et al. (70), like other researchers (36,69), had anticipated that FM may have a greater effect among postmenopausal women because of its responsibility in the conversion of adrenal steroid precursors to estrogens, which are quantitatively more important in postmenopausal women.

Houtkooper et al. (72) also demonstrated that only FM and changes in FM were positively associated with TBMD change in premenopausal women, measuring BMD of total body, spine (L2-L4), and three femur sites, FM, and LTM with DXA . Increases in FM were associated with increases in TBMD (72). Nishizawa et al. (42) demonstrated that BMD values correlated significantly with body weight and percentage of fat, indicating that fat itself was a determinant of BMD, particularly in weight-bearing bones, in subjects aged 14-66 years (mean age = 34.4 years). Regional and TBMD and TBMC were measured using DPA. Lean body mass, FM, and percent fat were measured via DXA.

Other researchers have found differing results. Sowers et al. (44) found that FM was only protective of BMD when associated with substantial muscle. Body composition was measured using four-point BIA, and values for fat and lean compartments were categorized into tertiles. Bone mineral density of the proximal femur, including femoral neck and trochanter, were measured using DPA. In contrast, Lindsay et al. (70) showed a negative relationship between body fat and radial BMC corrected for soft tissue variability, suggesting that low body fat, along with its accompanying variables, is protective of radial mineral content at both the primarily cortical and cancellous sites. Total body bone mineral and bone mass in the lumbar spine and femoral neck were measured by DPA. Fat mass and lean body mass (LBM) were also measured via DPA. Body fat was calculated via skinfold; FM and LBM, via bioelectrical impedance; and body fat, via BMI. Lindsay et al. (70) noted that this regional area responded differently, however, to all other skeletal sites, including TBBM, with regard to this body fat relationship (70).

Again, studies are limited with children. Zamboni et al. (117) demonstrated that an alteration of mineral metabolism occurred in obese children, resulting in a reduced BMC. Thus, whereas obesity may appear to offer a protective effect through increases in BMD in postmenopausal women, the same may not apply to children. McCormick et al. (5) also found a reduced lumbar spine BMD in 23 obese children.

De Schepper et al. (116), however, found normal lumbar spine (L2-L4) values in 59 obese children between the ages of 6 and 15 years, using DPA. These children had a normal BMD for age, but a rather lower BMD than expected for their body weight.

Obesity does not appear to induce abnormally increased bone mineralization during the growth of children, as might have been expected by the positive correlation between BMD and body weight in normal children (116).

Bell et al. (107) demonstrated an alteration of the vitamin D endocrine system in obese subjects which was characterized by secondary hyperparathyroidism. It was suggested that such a negative metabolic force may counterbalance the physical forces in the accretion of mineral content in obese children (116). Increases in fatness showed weak positive correlations with skeletal mineralization in prepubertal children, and negative correlations with mineralization in peripubertal children (40). However, Reid et al. (39) suggested that in adolescence, higher FM is associated with early menarche and increased skeletal maturity, which may have lasting effects on bone mass.

Could body fat distribution affect these values, too? Heiss et al. (118) demonstrated a significant positive correlation between abdominal body fat and BMD in postmenopausal women. Body fat distribution was assessed via waist-to-hip ratio, and abdominal fat and BMD were determined by DXA. No studies were reviewed examining such relationships in children or adolescents.

Body Composition--Lean Tissue Mass

BMD has also been found to be closely related to LTM. Explanations for such relationship vary. Several researchers (12,46,71) have suggested that muscular contractions induce strains in the bones to which muscles are attached, suggesting that perhaps the forces a muscle exerts on a bone can influence bone mass. A reduction or

increase in muscle weight results in corresponding loss or increase of bone (12). Doyle et al. (46) demonstrated a basic quantitative relationship between the weights of bones and their attached muscles. The ash weight of the 3rd lumbar vertebral body and the weight of the left psoas muscle were significantly correlated (46). Slemenda (37) suggested two possible explanations for the positive association between LTM or muscle strength and skeletal mass: first, muscle mass may increase in response to external stimuli and directly load the skeleton, and second, many intrinsic factors may influence the growth of both skeletal and muscle tissues, including growth hormone, insulin, and androgens among others.

Sowers et al. (44) found that BMD of premenopausal women had a stronger relation with their muscle compartment than with their fat compartment. Low lean mass was found to be a risk factor for low BMD (44). Bevier et al. (119) demonstrated that although both FM and LBM were associated with BMD of the lumbar spine, stepwise multiple regression indicated that only the lean mass contributed significantly to the prediction of spinal BMD. Likewise, Aloia et al. (73) found that the major determinant of bone mass in healthy women aged 24-79 years was fat-free mass (FFM). Body composition was measured via a multicomponent approach, including DXA, prompt gamma-neutron-activation analysis, inelastic neutron scattering, titrated water dilution, and whole-body counting. Nichols et al. (74), utilizing DXA to determine BMD and body composition, found regional LTM was a better predictor of BMD than regional FM in college females.

A recent study by Slemenda et al. (40) found that in peripubertal children (and prepubertal children to a lesser degree), after adjusting for growth, there were strong positive correlations between increases in an index of muscle mass and all skeletal sites. In a study conducted by Ogle et al. (120) involving 265 subjects aged 4-26 years, a strong relationship between LTM (measured by DXA) and BMC was found for each sex. In another study conducted on children aged 8-16 years, Faulkner et al. (6) found a significant correlation between bone-free lean tissue (BFLT) and BMD in both girls and boys; weight did not account for any additional variance in predicting TBMD. Both bone mineral and soft tissue values were determined by DXA. Bone free lean tissue and LTM are synonymous, and LTM will be used throughout this paper.

Several items could be responsible for the discrepancies observed in the explanations of the effects of FM versus LTM on BMD (39). First, different methods were used for the assessment of body composition. Second, definition of indexes of bone density, dependent vs. independent on body size, were controversial among researchers (39). Sample sizes, sample selection, and differing sample criteria also could contribute to the differences.

Weight

Weight is strongly associated with BMD, but the mechanism of this effect is not well understood (36). As is evidenced by the two previous sections, both FM and LTM affect the skeleton, and both contribute to weight. Slemenda (37) reported that there is a

well-established relationship between body size and bone density which must be determined at least in part by skeletal responses to mechanical forces.

Rico et al. (38) examined postpubertal women and found that weight, not body fat, was the main determinant of bone mass. Both bone density and body composition values were obtained via DXA. Several other investigators have also reported weight as most important in the determination of bone mass in women (121-122). Similarly, Glauber et al. (36) found weight was a major determinant of BMD at all skeletal sites examined in a study of 6730 postmenopausal women. BMD was measured via SPA and DXA; adiposity was calculated through BIA; and FFM was calculated via a formula. Lindsay et al. (70) found TBBM was significantly related to height and weight in both premenopausal and postmenopausal women. Several researchers found changes in bone mass that paralleled weight gain and weight loss (123-125).

Fehily et al. (34) indicated that body weight was the strongest determinant of female BMD in young adults. Lloyd et al. (12) found the strongest combined predictors of prepubertal TBMD and TBMC were body weight, followed by height and pubertal development. Bone density measures were calculated via DXA, and body fat was measured with calipers. Katzman et al. (35) also showed a strong correlation between body weight and BMD in adolescent girls. Dhuper et al. (33) demonstrated body weight to be highly correlated with BMD of the spine, wrist, and foot in girls aged 13-20 years. DXA and DPA were utilized for bone mineral measures. Bone mineral apparent density (BMAD), which is BMC normalized to a derived bone reference volume, was formulated

for comparisons (33). Bone mineral apparent density was calculated as BMC divided by an estimate of bone volume obtained from densitometry-derived area and other skeletal length measurements (33). Reid et al. (41) concluded that body weight was more closely related to BMD than any of the biochemical or life-style parameters.

In a study of 218 children (134 girls and 84 boys) aged 1-19 years, results of multiple regression analysis showed that Tanner's stage and weight were the best predictive indicators of bone mass and BMD (7). The influences of age, sex, race, physical activity, and diet were not significant when Tanner's stage and weight were controlled (7). Kelly et al. (27) determined the best correlation coefficients with the lowest standard error of the estimate for BMC and BMD were found with weight in twins. BMD of the lumbar spine (L2-L4) and proximal femur were measured with DPA. Similarly, using multiple regression analysis , body weight and age were cited as the best predictors of spinal BMC in children and adolescents, measured by DXA (75,126).

Contrary to these results, however, Heiss et al. (118) found body weight was not a significant predictor of bone density in a regression analysis, suggesting there exists an association between body fat distribution and BMD, with the android distribution having the higher bone mineral densities in postmenopausal women. It therefore becomes important to determine the mechanisms by which the skeleton responds to forces and other potential influences on skeletal density. Abdominal obesity has been found to be

associated with abnormalities including disturbed glucose-insulin homeostasis and lipid abnormalities not only in adolescents and adults (127), but also in prepubertal children (128).

Bone Mineral Density Measures

Several measures for body composition and BMC have been utilized in the past. There are four basic techniques currently used for noninvasive assessment of bone mass or density: SPA, DPA, DXA, and quantitative computed tomography (QCT). The validity of other traditional methods of measuring anthropometric indices, such as skinfold thickness, arm circumference, and height has been questioned (129). These techniques only indirectly evaluate the child's body composition and may be invalid in determining body composition (130). Although total body electrical conductivity and BIA may be used to assess body FM and FFM, their validity requires confirmation when used in a wide range of pediatric subjects (129).

SPA is a method of passing a beam of photon energy through a site on the forearm wherein the mass of the bone mineral present is directly proportional to the amount of photon energy absorbed by the bone (131). The main problem with this method of measurement is that error may occur if significant soft tissue is present at the site of measurement (131). DPA allows for the measurement of the axial skeleton, while correcting for soft tissue mass. DPA is most commonly applied to the spine and upper femur, but it can also be used to measure bone mass of total body (132). Both a low- and a high-energy beam are used in DPA. This allows detection between bone, which yields a

higher contrast at lower energies than higher energies, and soft tissue contribution (132). Conventional DPA has been used widely in adult research and clinical research, but the scanning time of 20-40 minutes and poor resolution are the major factors limiting applicability in children (129,133).

Dual energy X-ray absorptiometry is based on the same type of principle as the DPA, but DXA utilizes X rays instead of isotopes. Because of its accuracy, precision, and low radiation exposure, DXA has become a popular method for estimating soft lean tissue mass (SLTM), LTM, body FM, and BMC in children and young adults (6,7,77,126,129, 134). DXA has several distinct advantages that favor its use in children: Precision of the measurement is from 1-2%; radiation dose is less than 3 mrem; and regional scans can be performed rapidly, some in under one minute (134). DXA was shown to compare well with chemical analysis in body-composition assessment of pigs weighing 35-95 kg (135) and 5-35 kg (126). The latter weight range is representative of children 1-12 years of age. DXA has the ability to measure BMD both regionally and in the total body (135). Computed tomography is a precise and accurate method; however, it exposes the pediatric subject to unnecessary high radiation (129).

Turnover is greater in trabecular bone than in cortical bone and therefore more sensitive to changes in bone metabolism (133). For this reason, BMD determinations preferably are made in areas of high trabecular bone content (133). One major advantage of the DXA method over SPA is the ability to evaluate the metabolically active trabecular bones, as in the lumbar region (129). One difficulty, however, is that the DXA technique

does not correct for the anterio-posterior depth, so the data are not representative of true density and are, for example, influenced by the size of the vertebra (75).

Ellis et al. (126) warned that in cross-sectional studies of body composition in children in which comparisons are made between subjects of the same age without taking into consideration their differences in body weight, caution should be taken. Variations can be due to the instrument's calibration procedure and not necessarily true biological variability (126). DXA size (mass) estimates for the three body compartments, LTM, FM, and bone mineral were found to be dependent on the analysis of the software version being utilized (126).

Houtkooper et al. (72) suggested that the DXA technique, in combination with adequately precise dietary intake assessment, allowed investigation of the relationship between short-term nutrient intake patterns and BMD changes at many stages in the life-cycle. Chan (129) demonstrated the accuracy of the DXA method for small quantities of bone, FM, and LTM. Ilich et al. (136) conducted a precision study which demonstrated that the DXA had better reproducibility than SPA.

The literature reflects controversy concerning precision of measurement and definitive terminology. BMC is a measurement expressed in grams (134), and has been shown to be highly correlated with total-body calcium (137). BMD is obtained by dividing BMC by bone area or width (138), generally providing size normalization (137). BMD is expressed as g/cm^2 (134).

BMD values do not account for changes in bone thickness (35,134). When bone thickness is constant, as in adults, this does not create a problem (134). In children and adolescents, however, the bones increase in longitudinal and transverse dimensions and thickness, and this will create an apparent increase in the computed BMD when the actual bone density is unchanged (75,134). Prentice et al. (138) warned that BMD is not a measure of true density because absorptiometry fails to provide information about the depth of bone, as well as failing to distinguish between osseous and nonosseous areas within the bone envelope.

Prentice et al. (138) demonstrated that the association between calcium intake and areal bone density in young adults disappeared when bone density was corrected for by size. The effect of calcium on peak bone mass might be mediated through effects on bone size versus bone density (139). Thus, a warning is given concerning making associations between BMD and variables which are related to bone size through their dependence on overall body size (i.e., dietary intake) (138).

To avoid the possibility of size-related artifacts in the analysis of bone mineral data, the suggestion was made to use BMC as the dependent variable and to include bone area (bone weight), weight, and height as independent variables in all multiple regression models (138-139), especially when continuous variables have been converted to natural logarithms. This still, however, will not provide true volumetric density.

Reid et al. (41,43) used BMD/height as an index of "true" density. However, Prentice et al. (138) warned that this correction for body size is inappropriate because the

data are forced to fit predetermined relationships, and thus it is not possible to judge whether any residual effects of bone and body size remain once the correction has been applied.

CHAPTER III

METHODS

Approval

Approval for this study was granted by the Human Subjects Review Committee of Texas Woman's University, Denton, Texas (Appendix A).

Data from the dissertation study of Coni Francis, Ph.D., not previously analyzed, was used to estimate the relationships between TBMD and FM, LTM, calcium intake, body weight, and abdominal body fat mass. The following reflect the data as previously collected.

Participants

The participants included 40 normal-weight children between the ages of six and ten years, in Tanner's stages one or two. Tanner's stages one and two are prior to the adolescent growth spurt and the corresponding hormonal changes of puberty that may confound body composition differences between sexes (48). Mothers were provided an education session for instruction on identifying the various Tanner's stages utilizing written descriptions and pictures of each developmental stage. Tanner's stage for children was determined by their mothers after completion of the training session.

Anthropometrics

Height and weight of children were measured. Height was measured to the nearest 0.1 cm. on a stadiometer (Perspective Enterprises), and weight to the nearest 0.1 kg. on a beam balance (Health-O-Meter) scale without shoes, in lightweight clothing. Body Mass Index (BMI) was calculated for the children as weight in kilograms divided by height in meters squared. Normal weight for children was defined as those within the tenth to ninetieth percentile range for weight by gender and age on the National Center for Health Statistics (NCHS) percentile weight curves for assessing growth of children in the United States (140).

Bone Mineral Density and Body Composition

Fat mass, fat-free mass, and total body bone mineral density estimates of the children were measured via one whole body scan (Figure 1) with a model DPX-L dual energy X-ray absorptiometer (DXA) by Lunar Radiation Corporation, Madison, WI, according to the protocol supplied by the manufacturer. Extended research analysis was performed for abdominal body fat analysis. The abdominal region on each whole body scan (Figure 2) was specified as follows: the lower border was established at the first lumbar vertebrae (L-1); the upper horizontal border was established at the twelfth thoracic vertebrae (T-12); and the lateral borders were established just outside the soft tissue. The total body scan exposed participants to a small amount of radiation (<5 mR). This is far less than the 30 mR of a chest X ray, and quite low when compared to normal background

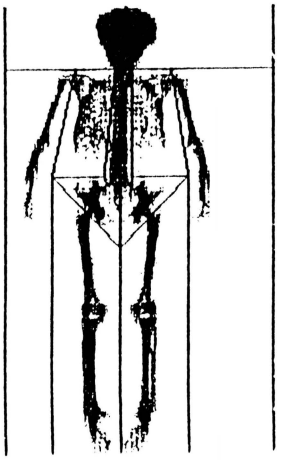

Figure 1. DXA Total Body Scan

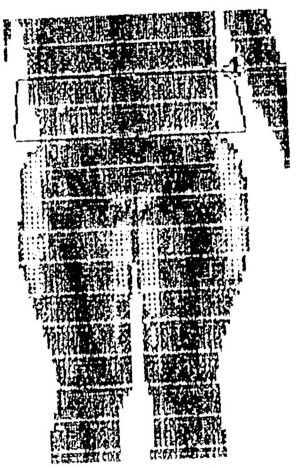

Figure 2. DXA Abdominal Body Scan

radiation. The same technique demonstrated by Nichols et al. (74) examining 58 college females (mean age = 19.9 ± 2.1 years) was utilized to obtain TBMD and abdominal fat measurements .

Food Diaries

A seven-day food diary, compiled by mothers, was collected for the children's food intake. Good agreement between estimates of energy intake, from seven-day food diaries completed by parents, and estimates of total energy expenditure measured by doubly-labeled water have been found in children aged seven to nine years (145). Mothers were provided with an education session for instruction in recording children's daily food and beverage consumption. Food record forms were provided to mothers with instructions on how to record all foods and beverages that children consumed. Food models, measuring cups and measuring spoons, bowls and written materials were used to graphically assist mothers in validating the accuracy of portion sizes. Food diaries were analyzed for nutrient content using the computer program Nutritionist IV, Version 2.0, 1992 (n-Squared Computing, Salem, OR). Seven-day food diaries were averaged. Dietary items analyzed included total calories, protein, carbohydrate, fat, sodium, phosphorus, caffeine, and calcium. Five of the children did not complete their dietary histories and therefore they were excluded from the nutrient analysis.

Calcium intake was the only nutrient utilized to measure calcium correlates with TBMD. Urinary calcium was not used. Although dietary calcium is correlated with urinary calcium excretion in adults, the relationship between dietary calcium and urinary

calcium is not as obvious in rapidly growing children (95). In growing individuals the relationship between dietary calcium and urinary calcium is almost negligible (9,49). One explanation is that a greater fraction of the absorbed calcium in children goes into the skeletal compartment rather than into the urine (95).

Participant Recruitment and Confidentiality

Participants for the study were recruited via physician referral and announcements in newspapers, and on public television and radio. All participants received oral and written information as to the nature of the study, and the risks involved with exposure to radiation. Adequate time for questions and answers was allowed. Written consent was obtained from the parent or legal guardian, and the child. Data were coded for confidentiality, and stored in a locked file in a separate area from the key to the coded information.

Statistical Analysis

Spearman rank order correlation was used to examine the associations between TBMD and estimated FM, estimated LTM, calcium intake, body weight, and abdominal body fat mass.

CHAPTER IV

RESULTS

Demographics and Anthropometric Characteristics

Demographic characteristics of the participants are shown in Table 1. Values reported in tables and text are presented as means ± standard error of the mean (SEM). Participants (n=40) were Caucasian, with the exception of one child who was Hispanic. No history of chronic diseases such as heart disease or diabetes mellitus were reported for the children. The mean ± SEM age of the participants was 8.42 ± 0.2 years, with a range of 6 to 10.5 years. The mean height was 130.65 ± 1.5 cm, with a range of 112.60 to 145.50 cm. The mean weight was 28.26 ± 1.1 kg, with a range of 18.1 to 42.8 kg. The mean BMI was 16.29 ± 0.3, with a range of 13.8 to 20.8.

Dual energy X-ray absorptiometry was utilized to measure TBMD, TBMC, bone calcium, LTM, FM, FFM, percent body fat, percent abdominal fat, abdominal FM, and abdominal LTM. The mean TBMD was 0.85 ± 0.01 g/cm^2, with a range of 0.77 to 0.96 g/cm^2. Mean TBMC was .97 kg ± .03 kg, with a range of .62 to 1.5 kg. Mean bone calcium was 369.35 ± 13.37g, with a range of 234 to 554 g. Mean LTM was 20.67 ± .61 kg, with a range of 13.91 to 28.72 kg. Mean FM was 5.58 ± .53 kg, with a range of 1.79 to 15.61 kg. Mean FFM was 21.65 ± .65 kg, with a range of 14.53 to 30.05 kg. Mean abdominal FM was .469 ± .06 kg, with a range of .12 to 1.9 kg. Mean abdominal LTM

Table 1

Demographic and Anthropometric Characteristics of Prepubescent Children[a]

Characteristic	Value (n=40)	Range
Age (years)	8.4 ± 0.2	6.0 - 10.5
Height (cm)	130.7 ± 1.5	112.6 - 145.5
Weight (kg)	28.3 ± 1.1	18.1 - 42.8
Body Mass Index[b]	16.3 ± 0.3	13.8 - 20.8
Total Body Bone Mineral Density[c]	0.8 ± 0.0	0.8 - 1.0
Fat Mass (kg)	5.6 ± 0.5	1.8 - 15.6
Fat-Free Mass (kg)	21.7 ± 0.7	14.5 - 30.1
Body Fat (%)	19.3 ± 1.1	9.0 - 37.5
Total Body Bone Mineral Content (kg)	1.0 ± 0.0	0.6 - 1.5
Lean Tissue Mass (kg)	20.7 ± 0.6	13.9 - 28.7
Abdominal Fat (%)	15.7 ± 1.3	6.2 - 42.2
Abdominal Fat Mass (kg)	0.5 ± 0.1	0.1 - 1.9
Abdominal Lean Tissue Mass (kg)	2.3 ± 0.1	1.5 - 3.2

[a]Mean ± SEM
[b](kg/m^2)
[c](g/cm^2)

was 2.28 ± .07 kg, with a range of 1.48 to 3.23 kg. Mean percent body fat was 19.32 ± 1.1%, with a range of 9 to 37.5%. Mean percent abdominal body fat was 15.72 ± 1.3%, with a range of 6.2 to 42.2%.

Dietary Intake Analysis

Descriptive statistics for dietary intake can be found in Table 2. Mean kilocalorie intake was 1856.46 ± 73.9, with a range of 1209 to 2770. Mean fat intake was 66.85 ± 3.6 g, with a range of 34.95 to 112.90 g. Mean protein intake was 60.30 ± 2.0g, with a range of 41.3 to 88.8 g. Mean carbohydrate intake was 262.49 ± 11.5 g, with a range of 162.1 to 495.5 g. Mean sodium intake was 2845.03 ± 131.0 mg, with a range of 1590 to 4789 mg. Mean phosphorus intake was 1075.23 ± 41.7 mg, with a range of 698.6 to 1590 mg. Mean caffeine intake was 17.01 ± 3.5 mg, with a range of 0 to 110.7 mg. Mean calcium intake was 862.43 ± 40.22 g, with a range of 418.9 to 1250.0 g.

Relationships Between TBMD and Anthropometric Variables

The correlations between TBMD and anthropometric values can be found in Table 3. TBMD was moderately and positively associated with weight (.75), FM (.54), and abdominal FM (.47) in the children. TBMD was strongly and positively associated with LTM (.81) and FFM (.79).

Relationships Between TBMD and Nutrient Intake

The correlations between TBMD and nutrient intake can be found in Table 4. There were no strong correlations between nutrient intake and TBMD in the children.

Table 2

Dietary Intakes of Prepubescent Children[a]

Nutrient	Participants (n=35)	Range	RDA (102)
Kilocalories	1856 ± 73.9	1209 - 2770	2000
Fat (g)	67 ± 3.6	35 - 113	---
Protein (g)	60 ± 2.0	41 - 89	36
Carbohydrate (g)	262 ± 11.6	162 - 495	---
Sodium (mg)	2845 ± 131.0	1590 - 4789	---
Phosphorus (mg)	1075 ± 41.7	698 - 1590	800
Caffeine (mg)	17 ± 3.5	0 - 110	---
Calcium (mg)	862 ± 40.2	418 - 1250	800

[a] Mean ± SEM

Table 3

Correlation Coefficients[a] Between Bone Mineral Density and Anthropometric Measurements in Prepubescent Children

Anthropometric Measurement	Bone Mineral Density r_s
Weight (kg)	.75
Fat Mass (kg)	.54
Fat-Free Mass (kg)	.79
Lean Tissue mass (kg)	.81
Abdominal Fat Mass (kg)	.47

[a]Spearman rank order

Table 4

Correlation Coefficients[a] Between Bone Mineral Density and Nutrient Intakes in Prepubescent Children

Nutrient	Bone Mineral Density r_s
Kilocalories	.30
Carbohydrate	.31
Protein	.24
Fat	.26
Sodium	.36
Phosphorus	.19
Caffeine	.19
Calcium	.28

[a]Spearman rank order

TBMD was weakly and positively associated with kilocalories (.30), carbohydrate (.31), protein (.24), fat (.26), sodium (.36), phosphorus (.19), caffeine (.19), and calcium (.28).

CHAPTER V

DISCUSSION

Total Body Bone Mineral Density, Lean Tissue Mass, and Fat Tissue Mass

Not much information has been published concerning normative data for BMD and body composition variables in prepubertal children. Three recent studies reported normative data on DXA-derived measurements of body composition in children (6,141, 142). Compared to values found by Zanchetta et al. (142), values of the present study are normal: weight (25.8, this study vs. 28.3 kg, Zanchetta et al.), height (130.7 cm vs. 124.4 cm), BMI (16.3 vs. 16.4), BMD (.8 vs. .8 g/cm^2), LTM (20.67 vs. 20.36 kg), FM (5.58 vs. 6.78 kg), and TBMC (.97 vs. .95 kg) (Table 5). A sample was taken from Zanchetta's data that corresponded to the age group examined in the current study, 6 to 10 year-old children. Faulkner et al. (6) also found similar results: TBMD (.8 g, this study vs. .8 g, Faulkner et al.), LTM (20.67 kg vs. 23.43 kg), FM (5.58 vs. 7.34 kg). Faulkner's study included children and adolescents 8 to 16 years of age. A select sample of Faulkner's data was also used to make comparisons (8 to 10-year old children). The 43 participants studied by Gutin et al. (141) ranged in age from 9 to 11 years; therefore, higher values were found in comparison to the present study, which included children 6 to 10.5 years of age (Table 6).

Table 5

Comparison Between Demographic and Anthropometric Characteristics of Prepubescent Children[a]

Characteristic	Zanchetta et al. (142)	Values
Height (cm)	124.4 ± 5.9	130.7 ± 1.5
Weight (kg)	25.8 ± 4.1	28.3 ± 1.1
Body Mass Index[b]	16.4 ± 1.5	16.3 ± 0.3
Total Body Bone Mineral Density[c]	0.8 ± 0.1	0.8 ± 0.0
Fat Mass (kg)	6.8 ±3.2	5.6 ± 0.5
Total Body Bone Mineral Content (kg)	1.0 ± 0.2	1.0 ± 0.0
Lean Tissue Mass (kg)	20.4 ± 2.3	20.7 ± 0.6

[a]Mean ± SEM
[b](kg/m^2)
[c](g/cm^2)

Table 6
Comparison of Demographic and Anthropometric Characteristics of Prepubescent Children[a]

Characteristic	Gutin et al. (141) (n=43)	Values (n=40)
Age (years)	10.3 ± 0.6	8.4 ± 0.2
Height (cm)	144.4 ± 6.5	130.7 ± 1.5
Weight (kg)	39.6 ± 10.1	28.3 ± 1.1
Body Mass Index[b]	18.8 ± 3.7	16.3 ± 0.3
Fat Mass (kg)	10.0 ± 6.8	5.6 ± 0.5
Fat-Free Mass (kg)	28.6 ± 4.3	21.7 ± 0.7
Body Fat (%)	23.9 ± 9.8	19.3 ± 1.1
Total Body Bone Mineral Content (kg)	1.2 ± 0.3	1.0 ± 0.0
Lean Tissue Mass (kg)	27.4 ± 4.2	20.7 ± 0.6

[a]Mean ± SEM
[b](kg/m^2)
[c](g/cm^2)

Dual energy X-ray absorptiometry was utilized to obtain TBMD, TBMC, LTM, FM, FFM, percent body fat, percent abdominal fat, abdominal FT, and abdominal LTM. The relationship between TBMD and the above parameters was explored. TBMD is thought to be more accurate than other anatomical region measures in estimating general bone mass, and it has a good correlation with total body calcium content (6,143). In comparison to only measuring bone density of the spine, TBMD includes both cortical and trabecular bone. Rico et al. (144) suggested that since the major component of TBBM is cortical bone (80% vs. 20% of trabecular bone), the assessment of TBBM could minimize differences that are only evident when the trabecular component is taken into account.

There currently exists no widely accepted technique or absolute for measurement of body composition in children (141). Gutin et al. (141) recently evaluated DXA in measurements of body composition of 9-11-year-old children. The techniques examined included DXA, BIA, and skinfold thickness measurements. DXA was found to be especially well-suited for repeated measures studies in which small differences need to be detected in children (141). DXA provided percentage of fat values slightly higher than those derived from skinfold thickness measurements and BIA (141). Laskey et al. (145) reported that the model DPX-L DXA by Lunar Radiation Corporation could determine FM and LTM precisely and accurately in whole body scanning. DXA requires little effort of the children, providing objective and reliable data (141).

Tanner's Stages and Body Composition

All but four of the children in this study were in Tanner's stage one. Controlling for Tanner's stages limits the variation in size which can be brought about by differences in age and pubertal development (48). Use of subject groups closely matched by pubertal stage reduces the likelihood that differences in pubertal progression will obscure interpretation of the bone measurements (48). Rico et al. (144) found no differences in TBMD between Tanner's stages one and two in boys or girls. In a study conducted by McCormick et al. (5), sex differences in spinal BMD were found only after the age of ten. Glastre et al. (75) found no marked differences in BMD until the age of 12. Geusens et al. (146) reported no gender differences in 3-9-year-old children in BMD or BMC at the lumbar spine, arms, and legs -- nor in TBMD and TBMC. Deurenberg et al. (147) also found no differences in FFM between children in Tanner's stages one or two utilizing DXA.

Dietary Intake

Livingstone et al. (148) found good agreement between reported seven-day estimates of dietary intake, recorded by parents, in 7-9-year-old children when compared to total energy expenditure measured by doubly-labeling water. In the present study, both children and mothers were trained and provided literature on how to complete daily food diaries. Some of the older children took an active role in completing their food diaries, especially with foods consumed away from the home. Analysis of nutrient intake was done with Nutritionist IV, Version 2.0, 1992 (n-Squared Computing, Salem, OR). In

deciphering menus, any questions concerning reported intake were clarified via phone conversation with parents of the children. Many parents also provided food labels and recipes to assist in the proper identification of foods. This aided in the careful coding of food diaries. Whenever an individual is asked to record intake, there always exists the question of accuracy. Strict weighing of all food items would have provided a more accurate picture as to the child's intake. However, complete precision and accuracy are difficult unless food is distributed, weighed, and measured in a controlled environment. The possibility remains that some of the food diaries provided by the children were not accurate.

The dietary intakes of the children came close to meeting the US Dietary Goals (149). Carbohydrate contributed approximately 56% of kilocalories, protein contributed approximately 12% of kilocalories, and fat contributed approximately 32% of kilocalories. Although protein contributed only approximately 12% of total kilocalories, every child in the study consumed protein in excess of 138% (range 138-370%) of the Recommended Dietary Allowance (RDA) (102) for their age.

The mean calcium intake was 862.4 ± 40.52 mg, which is above the RDA for calcium (800 mg/day) (102) for this age group. Matkovic et al. (1) estimated that most children between infancy and puberty are able to meet their calcium requirements necessary for adequate skeletal calcium retention. Fleming and Heimbach (103) recently reported that the RDA for calcium is met only by females who are under one year or between 6-11 years of age. In a study of school-age twins, Miller et al. (104) found that

the average intake in boys was 991 mg/day, while the average intake was 864 mg/day in girls (66). These average values are in general agreement with those found in the present study (934 mg/day in boys, and 815 mg/day in girls), as well as those reported by Nelson et al. (105) and the Bogalusa Heart Study (106). Dietary surveys in the United States have shown that the median calcium intake for children conforms to the current RDA (49,149).

It has been suggested that a higher RDA for calcium for children ages 2-8 years and adolescents ages 10-20 years should to be considered (1,3,49). National Institutes of Health reported that the optimal calcium intake for children 6-10 years of age is 800 to 1200 mg/day. The threshold intake, a level below which skeletal calcium accumulation is a function of intake, and above which skeletal accumulation is constant, irrespective of further increases in intake, is 1390 mg/day for 2 to 8 year olds, and 1480 mg/day for 9 -17 year olds (101).

Current RDAs for calcium have been established at levels of intake below the calculated thresholds for the vast majority of individuals during growth (49). Andon et al. (150) reported that the latest revision to the RDA occurred before the establishment of the threshold intake of calcium for growth and the advent of controlled trials of calcium intervention and bone mass acquisition during childhood. Andon et al. (150) also suggested that current RDAs are insufficient to support optimal bone mass gain during growth and development, and recommend an RDA of 1250 mg/day during childhood, based on recent intervention trials.

In a three-year clinical trial of monozygotic prepubertal twins, supplementation of calcium citrate malate to approximately 1612 mg/day resulted in an increase in BMD of the radius (5.1%), spine (2.8%), and proximal femur (3.2%) over the values for the control twins who averaged approximately 900 mg/day (3). In a recent analysis of calcium balances, children on calcium intakes of 800 or 1390 mg/d had calcium retentions of 110 and 250 mg/d, respectively (9,101). The fact that children are able to retain more calcium with further increases in intake is important (9,49,101). This could mean higher bone mass and density are possible but not being achieved (3,9). Matkovic and Heaney reported that calcium balance and presumably skeletal calcium retention increase during adolescence with intakes up to approximately 1500 mg/day, suggesting that when confounding variables are controlled, dietary calcium intakes greater than the RDA result in increased peak bone density and mass (101). The mean intake of the children in this study was 862 mg. Only two children reached levels above 1200 mg. The lowest level of calcium consumed was 418 mg. Sixteen of the 35 participants consumed less than 800 mg (range of 418 mg -773 mg) of calcium daily. No child consumed extremely low levels of calcium. At this range, an effect of calcium intake may not be demonstrated.

Relationships Between TBMD and Anthropometric Variables

Weight was moderately and positively associated with TBMD in the children (.75). Some of the literature reflects weight as being strongly and positively associated with BMD. Lloyd et al. (12) found body weight to be highly correlated (r=.90) with

prepubertal TBMD. Katzman et al. (35) also found a strong correlation between TBMC and body weight in adolescent Caucasian girls. Dhuper et al. (33) found weight to be highly correlated with BMD of the spine, wrist, and foot of adolescent girls aged 13-20 years.

Total body bone mineral density was strongly and positively associated with LTM (.81) and FFM (.79). This finding agrees with other research which has found BMD to be closely related to LTM. Slemenda et al. (40) found strong positive correlations between muscle mass and all skeletal sites in prepubertal children. Faulkner et al. (6) also found a significant correlation between LTM and BMD in both girls and boys aged 8-16 years. Another study conducted by Ogle et al. (120) involving 265 participants aged 4-26 years found a strong relationship between LTM and BMC for each sex.

Faulkner et al. (6) suggested that the amount of LTM is more important than overall body mass in predicting TBMD. Bone is a dynamic tissue aligned with the muscular system, and it exhibits changes similar to those observed in muscle (6). Thus, it is likely that muscle tissue mass would be a better predictor of TBMD than simply body weight (6). Other possible explanations for the observed positive association between LTM and skeletal mass include that muscle and skeletal mass may increase in response to the same types of external stimuli, in particular activities which load the skeleton and strain musculature (37). Also, many intrinsic factors may influence the growth of both skeletal and muscle tissue, including growth hormone, insulin, and androgens among others (37). Sowers et al. (44) suggested further study should be done to determine whether the

association of muscle with bone is primarily a function of physical loading or hormonal growth factors.

Fat mass was moderately and positively associated with TBMD (.54). Slemenda et al. (40) found weak positive correlations between fatness and skeletal mineralization in children. Based on the literature, explanations for the positive relationship between body fat and BMD are varied. Many studies have found a strong positive correlation between FM and BMD in postmenopausal women. It has been suggested that increased fat tissue is correlated with BMD due to increases in the direct effects of transmitted gravitational forces, and the ability of adipose tissue to convert androgens to estrogens (41,69,70-72). Geusens et al. (146) found no differences in BMC nor BMD between males and females at any site in prepubertal children, indicating that prepubertal growth factors acting on bone are probably not sex-steroid dependent.

Abdominal FM was moderately and positively associated with TBMD (.47). Percent abdominal fat ranged from 6% to 42%. Abdominal FM ranged from 118 g to 1948 g. Heiss et al. (118) found abdominal fat weight to be a significant predictor in regression models for all BMD parameters in postmenopausal women. Such a relationship was not found in normal-weight, prepubertal children.

Relationships Between TBMD and Nutrient Intake

There were no strong correlations between nutrient intake and TBMD. Kilocalories, sodium, phosphorus, caffeine, and calcium were all weakly and positively correlated with TBMD. Calcium would be expected to have a positive relationship to

BMD, but many researchers have tried to determine to what degree bone mass and loss are related to dietary calcium intake. Several have hypothesized that better calcium nutrition during childhood and adolescence may optimize, within genetic boundaries, peak bone mass (47-49,56,59). Optimization of peak bone mass by life-long adequate calcium intake is recognized as the most effective prophylaxis to reduce the risk of developing osteoporotic fractures later in life (56). One reason for this is because serum calcium homeostasis takes precedence over skeletal homeostasis; therefore, an adequate consumption of calcium is a requirement for optimal skeletal growth and maintenance (71). Sandler (71) suggested that calcium functions in an "enabling" mode in that it permits the skeleton to respond appropriately to genetic, physiologic, and mechanical cues.

Similar to the results of the present study, Glastre et al. (75) found no association between BMD and calcium intake in children aged 1-15 years. Kroger et al. (67) also found no correlation between dietary calcium intake and BMD in healthy children and adolescents aged 6-19 years. The consumption of calcium in these studies, however, was around the RDA value of 800 mg/day. A cross-sectional study is less likely to show the effects of calcium intake on bone mass, particularly if sample size is not adequate, and/or subjects are not exposed to habitually different calcium intakes (151). Results of Johnston et al. (3) suggest that additional calcium can increase the rate of bone gain in skeletal mineral, even in children whose dietary intake is nearly 1000 mg/day.

Results of the effects of caffeine consumption on BMD have varied (152). Because the average caffeine consumption among the participants in this study was relatively low (17 mg), possible negative effects might not have been seen. Bunker (152) reported that a moderate caffeine intake may have a calciuric effect, but this is probably only of importance in the elderly or when associated with a low dietary intake of calcium. Caffeine only appears to exert a significant effect at low intakes of calcium (152).

Phosphorus is important for bone health, but recent concerns of the nutrition community have centered around whether its presence in the diet might be excessive, with consequent harm (100,153). Very high phosphate intakes with concomitant low calcium intakes sometimes cause hyperparathyroidism, which (if lasting long enough) could have a permanent negative effect on bone mass (95). Calvo et al. (154) demonstrated significantly elevated parathyroid hormone concentrations in females on a low-calcium, high-phosphorus diet (1:4). In this study, the average phosphorus intake was 1075.23 mg, while the average calcium intake was 862.4 mg. The mean calcium-phosphorus ratio of .8:1 was weakly and positively correlated with BMD. Calvo et al. (154) suggested that a chronic high-phosphorus intake adversely affects calcium retention through stimulation of resorptive activity by PTH. A longer study period and higher phosphorus intakes with decreased calcium intakes would probably be necessary to show an effect of increased phosphorus intake.

Fat, protein, and carbohydrate intake were also weakly and positively correlated with BMD. Interestingly, each participant in this study had a protein intake >138% of

standard RDA (102). It might be expected that such a high protein intake would result in a negative correlation with BMD. Young individuals, contrary to adults, are in strong positive nitrogen balance, and therefore, for the same protein intake, more amino acids will be utilized for body building (151). It has been established that supplementation of diets with purified proteins increases the urinary calcium loss and increases calcium requirements (152). The problem with this, however, is that the increase intake in dietary protein is typically accompanied by an increase in other nutrients (152). Dietary breakdown of the present study revealed that carbohydrate contributed approximately 56% of kilocalories, fat contributed approximately 32% of kilocalories, while protein contributed approximately 12% of kilocalories.

High protein intake has produced negative calcium balance from increased urinary calcium excretion if phosphate intake is kept low, but if the phosphate intake increases with the protein intake the effect of a high protein intake on calcium metabolism is minimized (100,150). The data regarding the effects of protein and phosphorus on calcium metabolism are contradictory (152).

A study published by Recker et al. (63) showed that calcium intake was positively and protein intake was negatively associated with bone gain in young women. However, when the two intake variables were combined as the calcium to protein ratio, the association with bone gain became highly significant, and turned out to be the most important determinant of rate of bone gain (63). The calcium to protein ratio of 14.38 in

this study resulted in a weak, positive correlation (.14) with BMD. The suggested ratio is at least 16:1 (14,100).

Because this study was limited in size, the finding that a particular variable is unrelated to BMD does not mean its relationship does not exist. Also, it might be that some correlates of BMD may be related to some other unidentified factor which has an impact on the skeleton. Research has shown that there is no simple relationship between dietary constituents and bone health. Thus, it is difficult to draw conclusions based on individual nutrients. For example, although dietary calcium was not strongly correlated to BMD in this study, the positive effects of calcium nutrition may not be evidenced immediately, but may play a significant role in the prevention of fractures later in life.

Lean tissue mass, however, was strongly and positively correlated with BMD. This result is consistent with other research (6,12,37,44,46,71,73,119,120), and thus should be acknowledged in attempts to attain peak BMD. Data suggest that BMD has potential for modification by increased muscle mass. The ability to influence BMD could have important ramifications for minimizing osteoporosis in later years.

Conclusions

In response to the research question asked in chapter I, the following conclusions were reached:

1. There was no strong relationship between BMD and estimated FM in normal-weight prepubertal children.

Additional research to investigate the relationship between FM and BMD in obese children would be warranted.

2. There was a strong relationship between BMD and estimated LTM in normal-weight prepubertal children.

Further study to examine types of physical activity which best influence LTM and BMD accretion are needed.

3. There was no strong relationship between BMD and calcium intake in normal-weight prepubertal children.

More research is needed to determine the relationship between dietary intake and BMD. Studies designed to examine longitudinal effects of calcium supplementation are also necessary. It is important to investigate whether the effects of calcium supplementation persist after intake of calcium is reduced. The possibility of raising the current RDA values for calcium for the periods of childhood and adolescence is another area that warrants additional research.

4. There was no strong relationship between BMD and body weight in normal-weight prepubertal children.

5. There was no strong relationship between BMD and abdominal FM in normal-weight prepubertal children.

Further studies are needed to elucidate the relationship between abdominal fat stores and BMD. Research examining the effects of a slender body habitus on BMD is also necessary. By clarifying such relationships, further insight into the prevention of

osteoporosis will be gained. There appears to be substantial evidence in the literature to suggest that increased muscle mass and increased calcium intake will lead to improved BMD values.

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APPENDICES

APPENDIX A
Glossary of Terminology

GLOSSARY OF TERMINOLOGY

BFLT	Bone-Free Lean Tissue; Lean Tissue Mass; fat-free soft tissue; total non-mineral, non-fat lean mass
BMAD	Bone Mineral Apparent Density; which is BMC normalized to a derived bone reference volume; calculated as BMC divided by an estimate of bone volume obtained from densitometry-derived area and other skeletal length measurements (33).
BMC	Bone mineral content. The absolute amount of hydroxyapatite (calcium phosphate crystal) present in measured bone (108).
BMD	Bone mineral density (BMC/bone area); an area density, expressed in g/cm^2, that is conventionally used to describe the bone mass per unit of projected bone area, or the average mass per pixel (155).
BW	Body Weight; BMC + LTM + FM; FFM + FM
FM	Fat Mass; total body fat
FFM	Fat-Free Mass; BMC + LTM
LTM	Lean Tissue Mass; fat-free soft tissue; total non-mineral, non-fat lean mass. Also, an indication of muscle mass (155).
TBBM	Total Body Bone Mineral. Corresponds to ash weight of total skeleton and correlates closely to total skeletal weight and to total body calcium (155).
TBMC	Total Body Bone Mineral Content. Correlates highly with actual skeletal mass and with total body calcium by neutron-activation analysis in vivo because calcium is a constant fraction (approximately 37%) of the mineral component, or calcium hydroxyapatite (155).
TBMD	Total Body Bone Mineral Density; an area density, expressed in g/cm^2, that is conventionally used to describe the bone mass per unit of projected bone area, or the average mass per pixel of the entire body (155).

APPENDIX B
Human Subjects Approval

TEXAS WOMAN'S
UNIVERSITY
DENTON · DALLAS · HOUSTON

HUMAN SUBJECTS
REVIEW COMMITTEE
P.O. BOX 22930
Denton, TX 76204-0930
Phone: 817 898-3377

December 6, 1995

Jenifer Kruse
C/O Dr. Betty Alford
Nutrition & Food Sciences

Dear Jenifer Kruse: Social Security #:

The HSRC has received the memo dated November 29, 1995, from Dr. Betty Alford informing the committee that you will be analyzing data collected for the study "Serum Lipid and Lipoprotein Concentrations, Fasting Glucose, Body Composition, Resting Energy Expenditures, Activity Levels and Dietary Intake of Normal Weight Children of Obese and Normal Weight Biological Mothers."

Because this study was originally approved by the HSRC on August 7, 1995, and you will be conducting a portion of the study which was originally approved with Dr. Alford as the PI, the HSRC is exempting this study from any further review. According to HHS regulations, another review by the Committee is required if your project changes.

Special provisions pertaining to your study are noted below:

_X_ The filing of signatures of subjects with the Human Subjects Review Committee is not required.

_X_ Your study is exempt from further TWU Human Subjects Review because it is part of a larger study that has previously been approved.

____ No special provisions apply.

Sincerely,

Pam Englehart

Chair
Human Subjects Review Committee

cc: Graduate School
Dr. Betty Alford, Nutrition and Food Sciences

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