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Title: Relationship Between Total, Axial and Peripheral Bone Mineral Density,

Lifetime Milk Consumption and Lifetime Physical Activity in Elderly Mothers

and Their Premenopausal Daughters

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Osteoporosis is a bone disorder affecting millions of elderly people in the United States, particularly women. Since possibilities for treatment are limited, more research is directed towards possible preventive measures.

This study investigated the effect of lifetime and current milk consumption and of lifetime physical activity on total, axial and peripheral bone mineral density in 25 elderly women and their premenopausal daughters. We also investigated the extent to which the bone mass of mothers and their daughters was correlated. Furthermore, we investigated whether health attitudes were related to bone mineral density, current or lifetime milk consumption, or physical activity. Other factors assessed for their effect on bone density were anthropometric variables, hormonal and reproductive variables and behavioral variables. Bone mineral density (BMD) of the total, axial and peripheral skeleton was measured by dual energy X-ray absorptiometry. Lifetime milk consumption and lifetime physical activity were assessed retrospectively by questionnaire and interview. A qualitative distinction was

made between weight-bearing and non-weight-bearing physical activities as well as between exercise-related and non-exercise-related physical activities.

We did not observe any correlation between lifetime or current milk consumption, or lifetime physical activity and total, axial, peripheral or relative BMD in elderly mothers. There was also no combined effect of these two variables on BMD. However, mothers' calcium intake from supplements over age 60 was positively associated with several measurements of BMD. Other factors associated with higher BMD in mothers were body weight and related anthropometric parameters, estrogen replacement therapy and former birth control pill intake. Mothers' BMD decreased with increasing age. Multiple regression models including mothers' body weight, age, estrogen replacement therapy, birth control pill intake and calcium supplementation over age 60 explained between 50% and 90% of the variation in BMD among mothers.

Among daughters, several measures of lifetime physical activity were correlated with BMD. Weight-bearing exercise was more strongly associated with bone parameters than total exercise (weight-bearing and non-weight-bearing exercise). Total weight-bearing activity, which included non-exercise-related physical activity was more strongly correlated with total and relative BMD than weight-bearing exercise. Variables of lifetime physical activity were the factors most strongly correlated with daughters' BMD and were usually the only variable entering the multiple regression analysis.

We found a positive relationship between the peripheral BMD of mother/daughter pairs after adjusting for major factors influencing mothers' and daughters' BMD (mothers: weight, age, estrogen replacement therapy, birth control pill intake; daughters: lifetime total weight-bearing activity). We also observed similarity in lifetime milk consumption between mothers and their

daughters. Current milk consumption was in both groups positively correlated with lifetime milk consumption.

The lack of association between lifetime milk consumption and bone variables among the daughters in this sample can be explained partly by a uniformly very high calcium intake among the daughters. The observed positive correlation between lifetime physical activity and BMD among premenopausal women provides support for recommendations to increase physical activity as a measure for osteoporosis prevention. Exercise itself, as well as its role in increasing lean body mass, appeared to positively affect BMD in premenopausal women. Our results in mothers suggest that anthropometric parameters such as body weight or body mass index, as well as estrogen from exogenous sources, have a predominant influence on bone mass during postmenopausal years.

Relationship Between Total, Axial and Peripheral Bone Mineral Density,
Lifetime Milk Consumption and Lifetime Physical Activity
in Elderly Mothers and Their Premenopausal Daughters

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INTRODUCTION

Osteoporosis is a condition in which bone mass decreases, resulting in an increased bone fracture susceptibility, in the absence of other recognizable causes of bone loss (1). Osteoporosis affects as many as 15 to 20 million people in the United States, and 1.3 million fractures annually in individuals over age 45 and older in the United States can be attributed to osteoporosis (1). After age 50, the lifetime risk of any fracture of the hip, spine or distal forearm equals almost 40% in Caucasian women and 13% in Caucasian men (2). One-third of Caucasian women, and 17% of Caucasian men living to age 90 will suffer a hip fracture. In the United States, the annual cost of osteoporosis has been estimated at 3.8 billion dollars annually, which contributes significantly to increasing health care costs (1). Since the American population of this age group is increasing, osteoporosis poses a growing public health problem (3-6).

Recommendations for treatment, published in the National Institutes of Health conference on osteoporosis statement, include estrogen replacement therapy in postmenopausal women, adequate calcium intake (1,000-1,500 mg per day) and a program of modest weight-bearing exercise (1). Since the possibilities for treatment are limited, prevention of osteoporosis is the primary goal. It is argued that prevention should focus on achievement and maintenance of a maximum bone mineral density throughout life.

Bone mass is determined by a number of "internal factors", or genetic determinants, as well as by a number of "external factors", like nutrition and life-style. Current research suggests that adequate calcium intake (7-39) and physical

activity (9, 10, 13, 15, 17, 19, 40-63) during times of skeletal growth are major factors contributing to a maximum peak bone mass, and may play an important role for maintenance of bone mass throughout life. However, genetic determinants, such as ethnicity and gender (64-71), strongly influence body frame as well as peak bone mass. Other factors believed to affect bone mass are estrogen from endogenous and exogenous sources (27, 57, 72-77), reproductive and lactation history (78-80), anthropometric parameters (8, 55, 73, 81-84), smoking and alcohol intake (85-90), fluoride intake (91-100), vitamin D status (101, 102), eating disorders (103-106), and various drugs and medical conditions (4, 6, 107-109).

It is known that calcium intake, physical activity, and genetic factors each influence bone densities at specific sites in women. However, few studies have assessed total bone mineral density (BMD) and none have evaluated bone density of the axial skeleton as a unit (bones forming the head and trunk) and bone density of the peripheral skeleton as a unit (bones forming the extremities) before. Lifetime calcium intake and lifetime physical activity patterns have been estimated only in a few studies (11, 13, 17, 27, 50), and seem, despite the difficulty of their assessment, more reliable predictors of BMD than data about current behavior. This study was the first to examine lifetime calcium intake and lifetime physical activity among elderly mothers and their premenopausal daughters. The age group chosen (mothers >64 years) is the oldest ever investigated for effects of lifetime milk consumption and lifetime physical activity, and the oldest one ever investigated in intergenerational comparisons of bone densities.

This study was designed to investigate to what extent lifetime milk consumption and level of lifetime physical activity were related to bone density of the total, axial, and peripheral skeleton in elderly women and their premenopausal daughters. We also investigated the extent to which the bone

mass of premenopausal daughters was correlated with that of their elderly mothers. In addition, the relationship between health attitudes, as measured by the Multidimensional Health Locus of Control Scales, and the health behaviors of lifetime milk consumption, current milk consumption and lifetime physical activity was investigated. The influence of a number of possible intervening variables on milk consumption, physical activity and bone density was also examined.

REVIEW OF LITERATURE

MEASUREMENT OF BONE MINERAL DENSITY AND BONE MINERAL CONTENT: COMMON SITES AND TECHNIQUES

Bone mineral content is measured by most techniques in grams of calcium hydroxyapatite. Bone mineral density is defined as the bone mineral content per unit of bone area, measured as grams of hydroxyapatite per square centimeter. The units do not describe a true three-dimensional density.

Four general methods are currently used for noninvasive measurement of bone mineral density and bone mineral content at various sites, both in the axial skeleton, which consists of bones forming the axis of the body, and the peripheral skeleton, which is defined as bones of the extremities (110). These are single-photon absorptiometry (SPA), dual-photon absorptiometry (DPA), dual energy X-ray absorptiometry (DEXA) and quantitative computed tomography (QCT). All these measurement techniques differ in the particular anatomic sites they measure (DPA, DEXA and QCT: all sites; SPA: forearm only), in their precision and accuracy, in duration of measurement and radiation dose.

DEXA is the most precise measurement technique today (precision error <0.5% to 2%) and enhances our ability to monitor bone density changes in individuals over time. DEXA is more accurate than DPA, and measures comparable sites, applies lower radiation (<1 μSv) and requires a shorter scanning time (<10 min lumbar spine, femur, <20 min total body).

Typical measurements performed by DEXA, as well as by DPA are lumbar spine (cortical: trabecular bone=C:T=50:50, axial skeleton), proximal femur (C:T=60:40, peripheral skeleton) and total body (C:T=80:20) bone mineral. Trabecular bone is honeycomb-structured with a high surface-to-volume ratio

and is considered to be more metabolically active than cortical bone (consisting of compact plates around nutrient canals) (111). Both DEXA and DPA allow distinction between specific regions of the femur; femoral neck, Ward's triangle and trochanter. Data for each body skeletal's bone variables are provided as well. In addition, both techniques provide accurate body composition measurements. Additional bone sites measured (mostly by SPA) are distal third of the radius (C:T=95:5), which is frequently referred to as radial bone mineral density, and ultradistal radius (C:T=60:40), both found in the forearm. Os calcis (C;T=5:95), the heel bone, is also used for bone measurements (110).

CHANGES IN LIFETIME BONE MASS

Bone mass is built up during childhood, adolescence and young adulthood until reaching its peak (peak bone mass, PBM). This peak is characterized by the greatest mineral content and density of bone reached in life (112). There is some controversy concerning the age range during which PBM is reached as well as the age of onset and rate of bone loss which characterizes the remainder of life. Both the onset and rate of mineral loss from a skeletal site appear to depend on the ratio of trabecular to cortical bone at this specific site. Age-related architectural changes of bone loss include the complete loss of trabecular ties as well as the thinning of remaining trabeculae, trabeculation of previously cortical bone and the development of intracortical porosities (111, 113). There is unanimous agreement that trabecular bone is lost with age, and that axial density is substantially lower in older persons than in younger ones (53).

Several limitations need to be considered when evaluating studies which investigate age-related changes in bone mass: Most data originate from cross-

sectional studies (66, 73, 81, 83, 84, 114-118), since only a limited number of longitudinal studies have been conducted (79, 116, 119-121). Only a few population-based studies (66, 121) have been conducted. A variety of bone sites have been measured and several measurement techniques have been used in assessing bone mineral density (84, 114-116, 118). Various risk factors have been assessed and sometimes were screened for (84, 114, 115, 120). Cross-sectional data are available from a number of researchers (66, 73, 81, 83, 84, 114-118)) involving sample sizes from 48 to 7,659 subjects among both sexes and among all age groups. Longitudinal studies (79, 116, 119-121) have been conducted in premenopausal women (79, 116), women close to menopause (119) and in postmenopausal women (76, 116, 120, 121).

Two cross-sectional studies suggest that peak bone mass of the lumbar spine is reached at the beginning of the thirties (114, 115). However, other researchers' cross-sectional data suggest that peak bone mass of the lumbar spine is reached before age 25 (122). Aloia et al (116) in a longitudinal study observed a significant decrease in lumbar spine during premenopausal years (age range 20-45 years). Other cross-sectional studies, however, did not show a decline in lumbar spine BMD during premenopausal years (81, 83). In a 2 yr longitudinal study among 75 women close to menopause no significant changes could be observed over time at that site (119).

Sowers et al (79) conducted a 5 yr longitudinal study on radial bone mineral density involving 181 pre- and postmenopausal women. They found a significant decrease in radial bone mineral density among women who were premenopausal or on postmenopausal estrogen replacement therapy, and a much larger decrease among postmenopausal women who were not taking estrogen. In this study, bone loss at the mainly cortical radius was observed even

in the youngest group of study subjects (25-29 years old), suggesting that PBM at this site is achieved before age 25.

Cross-sectional data suggest that bone mass of the femoral neck and Ward's triangle is lost to a small extent throughout the premenopausal years (83, 114). Cross-sectional data from two studies involving 1,154 and 7,659 postmenopausal women (66, 118) indicate that there is a significant linear decrease in bone mass at the proximal femur and proximal radius (both composed mostly of cortical bone) (66) as well as at the distal radius, proximal radius and os calcis and separate femoral regions during postmenopausal years (118). In these studies, bone seemed to be lost to a lesser degree from the spine than from the radius and os calcis (118). No significant decrease could be observed at the ultradistal radius (66). Data from these two large studies indicate that bone loss occurred from the mid sixties into the late eighties among their women. Women undergoing estrogen replacement therapy were not excluded from these two studies. Estimates for yearly loss of bone mass were highest in Ward's triangle (-1.3%). The Framingham osteoporosis study (66) also included male study subjects and found a decrease at the femoral regions similar to the one observed in females.

A significant age-related decline in total body bone mineral density measured by DEXA was observed in 140 postmenopausal women who participated in a cross-sectional study (73).

A cross-sectional study by Nordin et al (117) among 485 postmenopausal women, out of which 87 pairs were matched with respect to years since menopause, investigated the comparative influence of age and years since menopause on radial bone mineral density. Their results indicate that the menopausal component results in an exponential loss of bone mass, whereas the influence of age was best described by a linear model. These authors suggest

that after age 70 the age-related function is dominant, resulting in continuous linear loss of bone mass.

Longitudinal studies conducted by Sowers et al (79, 120) report substantial decreases in radial bone mineral density among postmenopausal women: More than 65% of women lost radial bone mineral density in excess of 1% per year and 30% of women lost at least 2% per year (follow up period: 5 yrs) (120). A longitudinal study conducted in Denmark reported vast differences in rate of radial bone loss among early postmenopausal women (Fast losers: 26.6% in 12 yrs, slow losers: 16.6% in 12 yrs) (121). In none of the above studies was itreported whether the women who participated were undergoing estrogen replacement therapy.

RELATIONSHIP BETWEEN CALCIUM INTAKE AND BONE MASS

The skeleton is the repository for 99% of total body calcium and can support plasma calcium levels at times of need. Adequate calcium intake is of major importance for bone development and maintenance especially in times of growth. A low calcium intake in childhood, adolescence and possibly early adulthood may be a major limiting factor in achieving maximal genetically determined adult bone mass (7-13).

Positive correlations between current calcium intake, bone mineral content and bone mineral density in young women, (8, 9, 10, 14, 15), adolescents and children (7, 16) have been reported. Calcium intake has usually been assessed by dietary records kept for at least two days (7, 9, 14-16) or by quantitative food frequency questionnaires (8, 10). Most studies have investigated radial bone density as the dependent variable using SPA. Chan (16) investigated 98 boys and 76 girls between 2 and 16 years old and found that the children with a calcium

intake larger than 1000 mg per day had higher radial bone mineral content than those with lower calcium intakes. A study conducted in children and adolescents found a positive correlation between current calcium intake and vertebral bone density (7), and Lutz and Tesar (14) observed correlations between current calcium intake and bone density of the L2 vertebrae, femoral neck and trochanter in their subsample of young women. Kanders et al (9) assessed bone mineral density of the radius and of lumbar spine in relation to current calcium intake. Radial bone mineral density was significantly related to calcium intake while the correlation between the vertebral site and calcium intake became significant only after adjustment for physical activity. Kanders' results of vertebral bone mineral density also suggested the existence of a threshold effect for calcium intakes above 800 to 1000 mg/day. Up to this amount a strong positive correlation was observed between calcium intake and vertebral bone mineral density; with calcium intakes higher than 800-1000mg per day no significant increase in vertebral bone mineral density could be observed. A logarithmic model for calcium intake better described vertebral bone mineral density.

Some studies failed to detect a correlation between current calcium intake and bone variables in young women (12, 17, 18). Matkovic et al (12) conducted a 2 yr calcium supplementation intervention study among adolescent females and attributed their lack of any significant associations between radial or vertebral bone mineral density and current calcium intake to their small sample size (n=31).

For adults over age 30, findings on the influence of current calcium intake, from food or supplements, on bone densities are not consistent. The effects of calcium on bone density after age 30 might be site specific (19) and may be dependent on estrogen status. Two studies failed to find a significant influence

of current calcium intake on bone mineral density among premenopausal women (20) and a group of pre- and postmenopausal women (14). Baran et al (21) conducted a 3 yr intervention study among 20 premenopausal women, who were age- and weight-matched with a control group. The intervention group, taking calcium supplements of 610 mg per day, did not show a significant decrease in lumbar spine bone density, whereas women in the control group lost in average 2.9% of vertebral bone mineral density. Similar results obtained from a 4 yr intervention study (22) investigating the effects of calcium supplementation of 1500mg/day on bone loss in 35 premenopausal women over age 35 found significantly reduced rates of bone loss in the left humerus (upper arm).

Several studies found no correlations between dietary calcium intake and rates of bone loss in perimenopausal and early postmenopausal women (23-25). Dawson-Hughes et al (26) did not find an effect of 500 mg of calcium supplementation among postmenopausal women, who were five years or less postmenopausal. These results suggest that another factor, possibly estrogen deficiency, is a more important determinant of bone loss during the earliest stages of menopause than calcium intake (27).

A number of cross-sectional studies among postmenopausal women show a positive influence of calcium intake on bone mineral density (28-31). Buchanan et al (28) reported that higher calcium intake was associated with lower prevalence of vertebral crush and wedge fractures and tended to be correlated with higher vertebral bone mineral density (p=.08) in a study among 63 postmenopausal women. A significant correlation between vertebral bone density and calcium intake was observed by Andon et al (29) among 131 postmenopausal women. Sowers et al (31) found in a sample of 324 postmenopausal women, that bone mineral density was greater in women meeting both the RDA for calcium (800 mg/day) and the RDA for vitamin D

(400 I.U./day, assessed as a combination between dietary and supplemental vitamin D intake as well as sunlight exposure) than in other women. A longitudinal study among 76 postmenopausal women (32) showed that women with a daily calcium intake lower than 405 mg lost bone mineral density at the spine at a higher rate than women with more than 777 mg calcium per day during a seven month period. Intervention studies among postmenopausal women showed a positive impact of calcium intake on bone mineral density at several sites (19, 22, 26, 33). Dawson-Hughes' group (26) investigated the effect of 500 mg supplemental calcium on bone mineral density among 301 postmenopausal women with low dietary calcium intake during a 2 yr trial. Among women who ingested less than 400 mg calcium daily from dietary sources, supplementation substantially decreased bone loss from the spine, femoral neck and radius compared to women in the placebo group. A 4 yr intervention study (22) among 82 postmenopausal women showed a decrease of bone loss from peripheral sites (radius, ulna, humerus) among women taking 1500 mg supplementary calcium per day compared to their controls taking none. Supplementation of 831 mg calcium per day during a 1 yr intervention study was found to increase femoral neck bone mineral density in a sample of 36 postmenopausal women (19). Calcium supplementation (1200 mg/day) used as therapy for osteoporosis in a study of 39 women (mean age 69 years) with severe osteoporosis improved bone mineralization (33). Calcium supplementation in combination with exercise and/or estrogen replacement therapy was shown to be effective in reducing bone loss among 120 postmenopausal women (34).

From a meta-analysis of 37 papers investigating the relationship between calcium intake and bone mineral density, Cumming (35) concluded that calcium supplementation had a consistent preventive effect on the rate of bone loss in postmenopausal women, especially when their baseline calcium intake was low.

This supports the hypothesis of a threshold for calcium intake beyond which its effect on bone density is reduced.

Since peak bone mass appears to be a major determinant of bone mass in senescence and since calcium intake at early ages may be a determining factor of peak bone mass, lifetime calcium intake is more likely than current calcium intake to be reflected in bone mineral density at older ages. All studies investigating the influence of historical or lifetime calcium intake (11, 13, 27) except for one (17) found a positive association between calcium consumption in earlier life stages, esp. childhood and adolescence, and current bone mineral density. Cauley et al (27) used milk consumption history for estimating lifetime calcium intake among 174 postmenopausal women who were an average of 57 years old and found positive correlations with radial bone mineral density, measured by computed tomography. The same methodology was used by Sandler et al (11) who observed a positive effect of lifetime calcium intake on radial bone mineral density among 255 postmenopausal women between 49 and 66 years old. Halioua and Anderson (13) estimated lifetime calcium intake by a quantitative food frequency questionnaire and observed associations with distal and midshaft radial bone mineral density and bone mineral content among premenopausal women who were between 20 and 50 years old.

Whenever discussing calcium intake or supplementation, one must take into consideration the degree of calcium absorption which exerts a major influence on the proportion of dietary calcium actually available to the body. Bioavailability of calcium is low and absorption fractions may be less than 20% (123). Calcium is continuously excreted in urine, feces, and sweat as an obligatory loss (124). Several studies conducted by Heaney and his colleagues indicate that calcium absorption is highly dependent on daily intake levels (123) and calcium load per meal (125). Absorption also decreases with age and with

lower estrogen status (123). Heaney's group also investigated a woman's absorptive consistency across differing calcium loads, differing intervals, and substances of differing intrinsic absorptability (126). They found that women's calcium absorption rates in response to specific test situations were consistent over several years. This suggests the existence of an individual absorptive setpoint. Since the rate of calcium absorption decreases with age and is associated with estrogen status as well as with a variety of dietary factors, medical conditions and drug use, studies evaluating the efficacy of different types of calcium supplements on bone mineral density (26, 127) are especially important to provide knowledge about possible intervention methods.

REVIEW OF METHODOLOGY LITERATURE FOR LIFETIME CALCIUM INTAKE

The assessment of past lifetime dietary patterns is difficult, because of limited recall ability of individuals. Halioua and Anderson (13) used a quantitative food frequency questionnaire and found that milk was the primary calcium source among premenopausal Caucasian women. Sandler et al (11) and Cauley et al (27) estimated calcium intake in childhood, adolescence and adulthood, retrospectively, from responses concerning frequency of milk consumption at various stages of life. Since the primary source of dietary calcium is milk, particularly in children (128) and in young women (129), as well as in 19-50 year old women (106), the retrospective estimation of calcium intake measured as frequency of milk consumption seems to be an appropriate method.

RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND BONE MASS

Evidence that physical activity positively influences bone variables at different bone sites is available from many studies (9, 19, 40-46). Excessive levels

of exercise, however, which induce amenorrhea, have a detrimental effect on bone mass which outweighs the positive influence of physical activity (43, 47, 48). A histomorphometric study in pigs suggests, that the primary effect of increased exercise appears to be osteoblast activation (49). During peak bone mass development physical activity is important in achieving maximal bone mineral density (17, 40). Later in life, physical activity is essential to maintain bone mass (13, 50-53).

A study conducted by Slemenda et al (40) investigated physical activity levels of 118 children, 5 to 14 years old. They used a questionnaire addressed to the children and their mothers which asked how many hours per week children spent on specific physical activities. There were positive correlations between total hours of weight-bearing activity per week and bone density of the radius, femoral neck, Ward's triangle and trochanter. No significant association was found between weight-bearing activity and bone density at the lumbar spine. Several authors have reported positive associations between physical activity levels at younger ages and current bone parameters in pre- or postmenopausal women (10, 13, 15, 46, 50). McCulloch et al (17) observed that premenopausal women, who reported being "very active" during their childhood, had significantly higher bone densities than those who had been "less active" children. Halioua and Anderson (13) found a significant positive association between high lifetime physical activity (at least 45 minutes of moderate to strenuous exercise, 4 times/week) and distal and midshaft radius parameters (bone mineral density, bone mineral content and bone width). Kriska et al (50) observed a significant correlation between lifetime physical activity after age 14 (leisure time physical activity during four time periods) and bone area in the radius.

Several questionnaire-based cross-sectional studies among premenopausal women reported positive correlations between levels of physical activity and radial bone mineral content (15, 54) and vertebral bone mineral density (9). In studies assessing current physical activity levels by biomechanical means (accelerometer, pedometer, sensors), one group (42) reported a positive impact of higher physical activity on total body calcium and bone mineral density in the lumbar spine, whereas another group (55) involving larger sample size (n>200) did not observe such correlations.

A number of intervention studies have been undertaken in order to investigate the effectiveness of different exercise regimens on bone mineral content and bone mineral density at various sites. Back strengthening exercises performed on the floor with specific weights did not increase spinal bone density in postmenopausal women (56); however, intervention (57, 41) as well as cross-sectional data (44, 58) showed higher lumbar spine bone mineral density among subjects participating in primarily non-weight-bearing muscle building activities on machines. Intervention studies which used weight-bearing exercise have found that this exercise had a significant positive influence on lumbar spine bone density in pre- (41) and postmenopausal (19) women and on radial bone mineral density (45, 58) in postmenopausal women. Studies observing the influence of weight-bearing activity on lumbar spine bone mineral density failed to detect an effect on femoral bone mineral density or total body calcium among postmenopausal participants in a 1 yr walking program (19) or on femoral bone mineral density between young women involved in regular jogging or weightlifting and control subjects (41). Since the duration of these intervention studies was a maximum of 12 months, it is not surprising that changes in bone mass were first observed in sites composed mostly of trabecular bone (e.g. spine), where bone remodeling occurs at the highest rate. It is also possible, that

habitual loading of the femur during daily activities like standing and walking is so great that additional loading exercises contribute only a small proportion to the overall daily loading effect on the femur.

Results from prospective cohort studies involving 1415 and 3595 study subjects (59, 60) and from case control studies (61, 62) suggest that a higher level of physical activity is accompanied by a lower risk of sustaining a hip fracture.

REVIEW OF METHODOLOGY LITERATURE FOR LIFETIME PHYSICAL ACTIVITY

Physical activity is difficult to assess retrospectively, because of the limited recall ability of individuals (130). Several methods of assessing physical activity retrospectively over long time periods have been tried. Zhang et al (46) categorized subjects according to their participation in school sports teams; McCulloch et al (17) used a scale with four points to evaluate self-reported levels of childhood physical activity ("sometimes active" to "very active"). For investigating current physical activity and physical activity during youth in postmenopausal Japanese women, Lacey et al (131) included participation in sports, household activities and job activity level (high job activity: nurse, waitress; very high: farmer) in their estimate of physical activity. Hirota et al (15) asked whether their young female study subjects currently liked sports, and also assessed the duration of exercise during high school (years regularly active in sports three or more times per week). Halioua and Anderson (13) used extensive information on physical activity habits during different life periods and categorized their subjects from sedentary (<2 h/wk moderate intensity activity) to active (≥ 45 min at least 4x per week moderate to strenuous activity). Kriska et al (50) used a survey instrument dividing the life span into four periods (ages 14-

21, 22-34, 35-50, 50+) and asking about participation in leisure time physical activity for each time period. Subjects were asked whether they participated in a range of sport activities and, if so, the number of months per year and the duration of each sport were recorded. Each activity was converted to kilocalories of energy expenditure depending upon the estimated intensity level. A modified version of this questionnaire was used by Fehily and colleagues (10).

All these approaches have limitations: Closed-ended questions exclude additional information which is not asked for specifically. Open-ended questions decrease reproducibility, are more difficult to categorize and can be more time-consuming. Recall ability of subjects is enhanced by providing reference points, which can be either specific physical activities or specific time frames (50). In any case, a questionnaire tailored for the specific activity patterns of a study population increases its usefulness (10, 50). Among middle-aged or elderly women, the assessment of non-exercise physical activity as part of the overall activity pattern might be important (131).

COMBINED EFFECTS OF PHYSICAL ACTIVITY AND CALCIUM INTAKE ON BONE MASS

Several researchers have investigated the combined effect of physical activity and calcium intake on bone mineral density, with varying results (9, 10, 13, 15, 17, 19, 33, 40, 131). The findings of a 1 yr walking program in combination with calcium supplementation in postmenopausal women suggest that moderate exercise and dietary calcium have different effects at various skeletal sites (19): exercisers versus non-exercisers showed an increase in spinal bone density, whereas high dietary calcium increased femoral neck bone density. No combined synergistic effect of calcium and physical activity on any of the

investigated skeletal sites was observed. Similar site specific effects in response to calcium intake and physical activity were observed by Kanders et al (9) among sixty premenopausal women. Vertebral bone mineral density was significantly positively related to activity pattern, and, after adjusting for this factor, was positively related to calcium intake. The results of these two studies (9, 19) suggest that calcium intake predominantly affects bone density at the peripheral sites, whereas physical activity positively influences bone density at axial sites. Radial bone mineral content was significantly affected by calcium intake only.

Fehily et al (10) found that sports activity during adolescence was a stronger positive determinant of radial bone mineral density in young women than was current calcium intake. Both contributed significantly to higher bone density at the radius. The results of a study among Asian young women indicate an additive effect of current calcium intake and physical activity during adolescence (measured as years of regular activity in sports three or more times a week) (15). Halioua and Anderson (13) observed a synergistic effect of lifetime calcium intake and lifetime physical activity on radial bone mineral density. When both values were at least intermediate (calcium intake 500-800 mg/day, physical activity at least 2 hours moderate intensity physical activity per week) maximal bone mineral density and bone mineral content at radial sites were achieved. An intervention study among 120 postmenopausal women revealed that a combined program of exercise and calcium supplementation resulted in a decreased rate of bone loss compared to a group of exercising women who did not take calcium supplements (33). Other authors either failed to observe a correlation between calcium intake and bone variables alone (17, 55) or between physical activity and bone variables alone (55, 131) and did not observe a combined effect of these variables.

HEALTH ATTITUDES

The Multidimensional Health Locus of Control questionnaire was developed by Wallston et al in 1978 (132) and measures the degree to which individuals believe that their health is a result of their own behavior (internal control), is a matter of chance, or is under the control of powerful others. High scores on the internal control scale is believed to be associated with positive health status.

GENETIC INFLUENCES ON BONE MASS

Gender (64-66), ethnicity (65-71) and other genetic influences are major determinants of peak bone mass as well as rate of bone loss. The two types of osteoporosis, postmenopausal and senile osteoporosis, affect predominantly women (90). Women frequently are affected by postmenopausal osteoporosis, which is characterized mainly by trabecular bone loss and vertebral crush fractures. The underlying causes for this type of osteoporosis are related to declining serum estrogen levels with menopause (90). For senile osteoporosis, which occurs at later ages and is characterized by trabecular and cortical loss resulting in vertebral wedge and hip fractures, the female:male ratio equals 2:1. Causes are factors related to aging, which are uniform among both genders (90).

Recent studies among dizygotic twins (4) and children and adolescents (65) suggest that before menopause females have a bone density higher than expected on the basis of muscle strength or body size and weight, which is sometimes higher than in males. Results from the Framingham Osteoporosis Study indicate that there is also no significant difference in rate of bone loss at the femur or radius between elderly women and elderly men (both groups' participants older than 68 yrs) (66). Significant changes in bone mass might

occur during early postmenopausal years in women. Nordin et al (117) observed exponential bone loss after menopause and a linear decrease in bone mass at higher ages. These results suggest that women's higher risk of sustaining osteoporotic fractures is highly associated with factors related to menopause.

Early studies investigating patterns of osteoporosis worldwide (68) and comparing a Puerto Rican with a North American population (69) indicated that ethnicity influences bone variables significantly. Bone mass is greater in black than in white (65, 70) and Hispanic children and these differences in bone mass continue throughout life (71).

Familial resemblance in bone mass has been investigated by several studies involving twins, siblings and parent or mother/daughter pairs (8, 12, 14, 133-138). The results of a twin study (133) indicate a high degree of heritability for bone mass, which is not reduced by adjustments for height, age and environmental factors. A second twin study (137) reported significant genetic determination of circulating osteocalcin levels, an indicator of bone formation. Matkovic et al (12) observed high correlations between bone mass/bone density of both parents and their adolescent daughters. Mother/daughter studies conducted by Lutz and others (14, 134), Tylavsky et al (8) and Hansen et al (135) found significant resemblance between the lumbar, femoral and radial bone mineral densities of pre- and postmenopausal mothers and their premenopausal daughters. In these studies, postmenopausal women were screened for estrogen replacement therapy. One study involving mother/daughter pairs as well as sibling sets failed to detect any familial similarity in radial bone mineral density (138). Biochemical markers of bone turnover were found to be correlated between siblings (135) but not between mothers and daughters. The correlations between bone variables of premenopausal daughters and postmenopausal mothers decreased with the mothers' increasing postmenopausal age (135). This

decline in familial resemblance could be explained by increasing heterogeneity of factors affecting bone loss with increasing postmenopausal age (139).

The same researchers did not observe lower bone densities in the daughters of women with a moderate state of osteoporotic fractures (135). Seeman et al, however, found lower bone mineral density at the lumbar spine and almost significantly lower bone mineral density at femoral regions among premenopausal daughters of women with osteoporotic fractures (140).

ESTROGEN LEVELS AND ESTROGEN REPLACEMENT THERAPY

Low endogenous estrogen levels are related to low bone density (27, 73, 74) and amenorrheic premenopausal women have significantly lower bone mineral density than control subjects (141). Estrogen replacement therapy is currently considered the most promising approach for the prevention and treatment of postmenopausal osteoporosis during later ages (142). The protective effects of estrogen replacement therapy, which can be observed at all skeletal sites, continue for as long as treatment is administered (75, 142). The mode of action of estrogen treatment is not clear: The findings of Hurley et al (143) contradict the theory of increased plasma-calcitonin levels in treated women. Estrogen-binding and estrogenic responses were observed in human osteoblast-like cells, which suggests that the skeleton itself is a target organ for estrogen (144).

Estrogen replacement therapy has been shown to successfully prevent bone loss in postmenopausal women (76), and patients participating in regular exercise programs in addition to estrogen replacement therapy had increasing bone mass at different sites (57, 72). New approaches to treatment include transdermal estrogen therapy in combination with adequate calcium intake (145).

Studies investigating the effect of oral contraceptive use on bone mineral density have not had consistent results. Neither Lloyd et al (146), Tylavsky et al (8), or Mazess and Barden (55) observed a significant effect of long-term premenopausal oral contraceptive use on trabecular bone density, measured at the spine with computed tomography. However, Stevenson and colleagues (82) reported a positive correlation between former oral contraceptive use and higher bone mineral density at the lumbar spine and femoral regions among 172 postmenopausal women.

Early menopausal age (73, 83, 147) and late menarche (81) have been associated with decreased bone mass, possibly because of relatively lower estrogen levels during certain ages.

OTHER FACTORS AFFECTING BONE MASS

Other factors influencing bone variables are reproductive history, possibly lactation history, anthropometric measurements, smoking and excessive alcohol intake, fluoride intake, endogenous and exogenous vitamin D, possibly caffeine intake and dietary fiber intake, a history of eating disorders and various drugs and medical conditions.

Nulliparity seems to be associated with lower bone density (73, 79) and long-term lactation may negatively affect bone mineral density (80), especially when associated with low calcium intake, but this effect might be outweighed by increased intestinal calcium absorption during pregnancy and lactation, resulting in no change in bone density (78).

Bone mineral density has been found to be highly positively correlated with body weight, height, or body mass index in a number of studies (8, 55, 73, 81-84). Total body fat mass and total body lean mass have also been observed to

influence bone mineral density (73, 83). All variables describing body frame are associated with weight-bearing effects on the skeleton. Body composition might exert additional influence due to possible correlations between fat mass and endogenous estrogen levels, and between lean body mass and physical activity.

Smoking has been shown to exert a negative influence on bone mineral density (86-88) and smoking has been associated with younger age at menopause (89). Excessive alcohol intake reduces bone mass; however, it is not clear whether a linear relationship exists between the amount of alcohol consumed and a decrease in bone density, or whether there is a threshold effect of alcohol consumption (6, 85, 90).

The effect of fluoride intake is controversial, and seems to depend on the actual dose of fluoride. Cross-sectional data suggest that bone mineral density of individuals exposed to high fluoride levels in drinking water may be lower than bone mineral density among residents living in a community with optimal fluoride levels in water supply (91, 98). Overall fracture risk of women living in a high-fluoride community was significantly higher than the risk of those living with a water supply with optimal fluoride levels (91). Recent results from a community-based cohort study indicated a higher risk of hip fractures among residents exposed to artificial fluoridation of 1 ppm than to residents of communities with less than 0.3 ppm fluoride in their water supply (100). Cross-sectional data from Finland contradict these findings and found lower fracture incidence among residents of a community with 1ppm water fluoridation (99). Unfortunately, no longitudinal data are available for optimal fluoride levels among premenopausal women. Fluoride has been shown to significantly increase bone mineral density in osteoporotic women, particularly when administered in combination with calcium and other minerals (92-94). However, an increase in hip fracture risk was observed in osteoporotic patients receiving

treatment with fluoride supplements (96). These controversial results may be due to the different architecture and "quality" of bone incorporating high levels of fluoride. Whereas absolute bone mineral density increases, fracture risk may increase as well. One of the principal skeletal effects of fluoride is the stimulation of osteoblast activity, mostly by inducing an increase in osteoblast numbers (97).

Dietary factors influencing bone mass are vitamin D status (102), which contributes to increased intestinal calcium absorption and might also have direct effects on bone remodeling; fiber content of the diet, which may diminish calcium absorption or influence sex hormone levels (101); and possibly excessive caffeine intake, resulting in decreased calcium absorption (148). Moderate caffeine intake did not change fractional calcium absorption, endogenous fecal calcium, or urine calcium in a randomized trial (149); however, there was evidence of altered bone remodeling, including slight decreases in bone accretion, bone resorption and calcium pool turnover. Tesar et al (150) did not observe any difference between postmenopausal lacto-ovo-vegetarian women and omnivorous women. Marsh et al (30), however, found higher bone density among postmenopausal lacto-ovo-vegetarian women, who consumed a diet with a higher calcium:phosphorus ratio and whose diet had a higher pH compared with omnivorous women.

Eating disorders have a negative influence on bone mass, because of inadequate dietary intake, as well as a high prevalence of amenorrhea among patients (103-106, 151). Long-term treatment with glucocorticoids can induce osteoporosis (109) and results in decreased bone mineral density at various sites (107). A number of other drugs and medical conditions can influence bone densities, frequently mediated by changes in calcium or estrogen metabolism (4, 6, 108, 152, 153).

METHODS

RESEARCH DESIGN

This cross-sectional study evaluated the relationship between the independent variables: lifetime milk consumption, current milk consumption, lifetime level of physical activity and health attitudes; and the dependent variables: total, axial, and peripheral , as well as relative, bone mineral densities in 25 mother/daughter pairs. The study also compared total, axial, and peripheral bone mineral density between mothers and their biological daughters. The effects of three kinds of possible intervening variables were assessed and when possible accounted for. They were:

anthropometric:

body weight, height, body mass index, percentage body fat, total body fat mass and total body lean mass;

hormonal/reproductive:

estrogen replacement therapy, birth control pill intake, age and years since menopause, number of children, breast feeding;

behavioral:

smoking, alcohol consumption, calcium supplement intake, frequency of dieting.

SAMPLE

Twenty-five mothers, some of whom were participants in an earlier study ("Women's Diet and Activity study"), and their biological daughters were recruited for this study by phone. Volunteer mother and daughter pairs were screened via telephone interview and were selected based on five criteria: Mothers had to be at least 64 years old. Daughters had to be premenopausal with no possibility of pregnancy. Both mothers and daughters could not smoke more than 1/2 package cigarettes per day, and not have taken asthma or thyroid medication within the last year. Both had to be able to come to Oregon State University for one three-hour session including measurements and a review of their questionnaire, and daughters had to be willing to keep a 3-day food record.

One mother had had a right side hip replacement. Her axial bone mineral density, therefore, could not be computed, and only n=24 mothers were available for analyses involving axial BMD, axial BMC and total BMC. Upon advice from the Department of Exercise and Sport Science, her peripheral bone mineral density was computed by substituting data from the right leg with data from the left leg. Total bone mineral density was also adjusted for this factor (right leg instead of left leg). Her total BMC could not be adjusted. One mother had a breast implant on the right side. For computing her axial and total bone mineral density, data from the right ribs were substituted with data from the left ribs.

DATA COLLECTION AND PROCEDURES

Data collection took place between July and October of 1992. All study participants were sent a questionnaire to fill out regarding their current health, lifetime milk consumption, and lifetime physical activity. They had a scheduled appointment at the Bone Research Laboratory in the College of Health and Human Performance at Oregon State University to which they brought their questionnaires. During approximately three hours, measurements were made of waist and hip circumference, weight, height and bone mineral density. Their questionnaire was reviewed in order to clarify any questions and more specific information regarding lifetime physical activity was obtained during an interview. A nutritionist instructed the daughters in keeping 3-day dietary records. These records were kept by the daughters on three days (including one weekend day) closely following their visit and were returned to Oregon State University by mail. All procedures had been approved by the Human Subjects Committee at Oregon State University.

MEASUREMENTS

BONE DENSITY MEASUREMENTS

The measurement of total, axial, and peripheral bone mineral density was conducted at the Bone Research Laboratory in the College of Health and Human Performance using dual energy X-ray absorptiometry (DEXA) (Hologic QDR 1000/W, Waltham MA). The Hologic QDR 1000/W X-ray bone densitometer is a state-of-the-art scanner (FDA approved) and is currently housed in over 600 universities (160 in the US). The device uses quantitative

digital radiography to measure rapidly and accurately the bone mineral content (BMC) in grams hydroxyapatite and bone mineral density (BMD) in grams/cm^2 (as g hydroxyapatite/cm^2 bone). Precision error for whole body BMD measurement is 0.50% C.V. at 1.0g/cm^2. Measurement accuracy of an anthropomorphic spine phantom of known mineral content is 0.5% (154).

Axial and peripheral BMD were computed from the measured bone sites. Axial bone mineral area was defined as the sum of the following bone areas: right ribs, left ribs, lumbar spine, thoracic spine and pelvis. To obtain axial BMD, axial bone mineral area was divided by axial bone mineral content in grams (consisting of the sum of the bone contents at the axial sites).

Axial BMD = Axial BMA/Axial BMC

$$\text{Axial bone mineral density} = \frac{\text{Axial bone mineral area}}{\text{Axial bone mineral content}}$$

$$\frac{(\text{BMA right ribs + BMA left ribs + BMA thoracic spine + BMA lumbar spine + BMA pelvis})^*}{(\text{BMC right ribs + BMC left ribs + BMC thoracic spine + BMC lumbar spine + BMC pelvis})}$$

* BMA= Bone mineral area in cm^2 ; BMC= Bone mineral content in grams

Peripheral bone mineral density was computed accordingly from bone mineral areas and bone mineral contents of the peripheral skeleton (extremities).

Peripheral BMD = Peripheral BMA/Peripheral BMC

$$\text{Peripheral bone mineral density} = \frac{\text{Peripheral bone mineral area}}{\text{Peripheral bone mineral content}}$$

$$\frac{(\text{BMA right arm + BMA left arm + BMA right leg + BMA left leg})}{(\text{BMC right arm + BMC left arm + BMC right leg + BMC left leg})} \quad *$$

* BMA= Bone mineral area in cm^2 ; BMC= Bone mineral content in grams

The axial skeleton consists mainly of trabecular bone, whereas the peripheral skeleton includes bone sites formed predominantly from cortical bone.

Relative bone mineral density was calculated by comparison of each subject's total bone mineral density with the average total bone mineral density of women her age previously measured with the Hologic QDR (155). Relative bone density is expressed as a percentage of the mean total bone mineral density of women the same age, and is therefore equivalent to the z-score.

DETERMINATION OF LEVEL OF LIFETIME MILK CONSUMPTION

Milk consumption during six life stages ranging from childhood to late adulthood was assessed retrospectively in mothers and daughters from responses to questions on a written questionnaire concerning frequency of milk consumption during specific age ranges:

childhood	through age 12	(13 years)
teens	13-19	(7 years)
earlier adulthood	20-39	(20 years)
mid adulthood	40-59	(20 years)
later adulthood	over 60	(dep. on age)
past two years specifically	past 2 years	(2 years)

For each of these age periods, subjects indicated how often they drank at least one glass of milk or ate at least one cup of yogurt:

with most meals	(at least 3 times/day)	value=4
frequently	(at least once/daily)	value=3
sometimes	(a few times a week)	value=2
rarely or never	(less than once/week)	value=1

The variable "lifetime milk consumption" was calculated by averaging milk consumption over life stages for each subject. The age periods "childhood" and "teens" were averaged to make them comparable to the other periods.

Lifetime milk consumption= $\dfrac{\text{Sum of milk cons. values for each period}}{\text{Number of periods}}$

= (1/2 x milk consumption during childhood + 1/2 x milk consumption during teens +

milk consumption during early adulthood + milk consumption during mid adulthood +

milk consumption during late adulthood)/ number of periods applicable for specific subject.

In addition, a variable "youth milk consumption" was computed by adding the values for childhood and teens:

Youth milk consumption=

milk consumption during childhood + milk consumption during teens/2.

CALCULATION OF A NEW VARIABLE

A variable "lifetime calcium intake" combining lifetime milk consumption and lifetime calcium supplementation was created: For each age period the four categories of milk consumption were collapsed into two categories, distinguishing between subjects consuming "at least one glass of milk per day" (=2) and subjects consuming "less than one glass of milk per day" (=1). The average amount of calcium supplementation intake during that age period was also categorized as "at least 250 mg Ca/day" (=2) or "less than 250 mg /Ca per day" (=1). The two variables were multiplied by each other to obtain an interactive value. This process resulted in a significant loss of information from both data sources.

Example: A person who drinks less then one glass of milk per day (1), but takes more than 250 mg Ca/day (2) in form of supplementation has a combined value of 1 x 2=2.

CURRENT CALCIUM INTAKE

Current calcium intake was assessed using two different methods. The first was a beverage frequency questionnaire which indicates with what meals and snacks milk and other beverages are consumed daily. The second method was a food frequency questionnaire of calcium-rich foods.

Validity of the beverage frequency and food frequency questionnaire was estimated in the daughter sample by comparison with 3-day food records. Current calcium intake, estimated in daughters by 3-day dietary records, was positively correlated with current milk consumption (estimated by beverage frequency, $r=.42$ $p<.05$) and milk consumption during past two years ($r=.57$ $p<.01$). There was no correlation between current calcium intake from daughters' dietary records and their scores on the calcium food frequency questionnaire.

Current milk consumption estimated by beverage frequency and current calcium intake estimated by the calcium food frequency questionnaire were previously validated for elderly women using nine days of diet records (156).

COMPARISON OF MEASURES OF CURRENT CALCIUM INTAKE

In both groups, mothers and daughters, current milk consumption assessed by the beverage frequency questionnaire as number of glasses of milk during a typical day was highly correlated with milk consumption during the past two years, assessed by the milk history questionnaire (M: $r=.79$, $p<.0001$; D: $r=.67$, $p<.0005$). In mothers, current calcium consumption estimated by scores on the food frequency questionnaire was positively correlated with current milk consumption, assessed by the beverage frequency ($r=.56$ $p<.005$). There was no such correlation for daughters.

DETERMINATION OF LEVEL OF LIFETIME PHYSICAL ACTIVITY

In a manner similar to the estimation of lifetime calcium intake, lifetime physical activity was assessed retrospectively from responses to questions concerning regular exercise during various life stages:

childhood	through age 12	(13 years)
teens	13-19	(7 years)
earlier adulthood	20-39	(20 years)
mid adulthood	40-59	(20 years)
later adulthood	over 60	(dep. on age)
past two years specifically	past 2 years	(2 years)

For each age period subjects were asked how often and for how long each time they engaged in regular exercise. They were asked, in an open ended question, to describe what kinds of physical activities they performed (Appendix D). Later an interviewer probed for the number of years, number of months per year and number of hours per week a subject performed a

certain activity. Although questions were open-ended, specific initiating questions were asked of each subject (physical education classes at school, walking to school or school bus, having a dog, having a garden). An average of 30 minutes were spent for review and discussion of historical physical activity with each subject.

From the responses an average for the hours per week spent on a specific activity throughout the whole age period was computed:

Hours/week for whole age period=

hours activity/week x adjusting factor for # months/year x adj. factor for # years/period =

$$\text{hrs of activity/week} \quad \times \quad \frac{\text{\# months}}{12} \quad \times \quad \frac{\text{\# years}}{\text{\# years in this period}}$$

From the number of hours obtained from this computation, subjects' physical activity was classified as

* high	> 4/hrs per week	value=3
* middle	2-3 hrs per week	value=2
* low	< 1 hr per week	value=1

People who fell between categories (e.g. 3.5 hrs per week, which is between the categories "middle" and "high") were categorized considering the main types of activities performed: If low-intensity physical activities (e.g. walking) accounted for most of the total activity hours, the lower of the two categories was chosen, if high-intensity physical activities (e.g. basketball) dominated, the higher one of the two categories was chosen.

QUALITATIVE DISTINCTION BETWEEN PHYSICAL ACTIVITY MEASUREMENTS

Weight-bearing and non-weight-bearing activities were distinguished between. The sum of hours/week of weight-bearing and non-weight-bearing activities was calculated from the hours/week for the whole age period in a specific activity.

Examples of weight-bearing activities	Examples of non-weight-bearing activities
jogging, ball-games, walking, tennis, etc.	bicycling, horseback riding, swimming, rowing etc.

Professions involving physical activity (e.g. nurse, farming), household activities much higher than the average, and the number of children raised were also taken into consideration in calculating weight-bearing activity values. These were evaluated by the interviewer with advice from the Department of Exercise and Sport Science and labeled "non-exercise physical activity". They were based on statements of the study subjects like "I am on my feet all day" as well as on hours per week reported working in a physically active occupation.

To evaluate the influence of different types of physical activity (weight-bearing, non-weight-bearing; exercise vs. non-exercise) on BMD various physical activity variables were created:

Weight-bearing exercise: All exercise performed on your feet.

Total exercise: All exercise including weight-bearing and non-weight-bearing physical activities.

Total weight-bearing   All weight-bearing physical activity,

activity: including exercise and non-exercise (e.g.

                                        profession, children).

In addition, subjects were asked by an interviewer, how they themselves perceived their level of physical activity for each age period. The interviewer recalled in terms of key words the activities they had reported for a certain age period. Then each subject was asked to rate her level of physical activity on a scale from 1 to 5:

                                   1=very lightly active

                                   2=lightly active

                                   3=moderately active
                                       (average person)

                                   4=active

                                   5=very active

The obtained variable was labeled **perceived physical activity.**

These computations resulted in categorical variables (high, middle, low) for each specific age period, each qualitatively distinguished by the type of activity. Lifetime variables were continuous, but based on the original categorical distinction.

Categories	Age periods	Qualitative distinctions
high	childhood	weight-bearing exercise
medium	youth	total exercise
low	earlier adulthood	total weight-bearing activity
	mid adulthood	perceived physical activity
	later adulthood	
	past two years	

HEALTH ATTITUDES

Health attitudes were assessed using the Multidimensional Health Locus of Control scales (MHLC) (125). The "internal" score in the MHLC expresses to what degree subjects thought that they had "internal" control over their health, i.e. control through their own behavior.

ADDITIONAL VARIABLES

Anthropometric measurements:

Weight:	measured on a balance scale to the nearest 100g (in indoor clothing)
Height:	measured against a tape on the wall to the nearest centimeter
Body Mass Index:	computed as BMI=weight (kg)/height (m)^2
Total fat mass:	body fat mass in grams, measured by the Hologic QDR 1000/W
Total lean mass:	body lean mass in grams, not including bone mass, measured by the Hologic QDR 1000/W
Percentage body fat:	measured by the Hologic QDR 1000/W

Hormonal/reproductive:

Estrogen replacement therapy: (postmenopausal women only)	yes = currently, for one year or longer, at what ages taken, for how many years
Birth control pills:	at what ages taken, for how many years
Age at menopause:	to the nearest whole year
Years since menopause:	to the nearest whole year
Number of children:	as number of births
Breast feeding:	ever, for one month or longer

<u>Behavioral</u>:

Smoking: ages, number of years, cigarettes per day

Alcohol consumption: currently regular alcohol consumption, number of drinks per week

Calcium supplements: mg/day, at what ages

Frequency of dieting: computed by averaging the sum (1=Yes) of all decades for which a subject indicated weight reduction dieting

Beard and colleagues (157) assessed the accuracy of self-reported risk factors relevant for bone-related studies by comparing interview data with medical records of postmenopausal women. They found that there was substantial agreement for self-reported and medical record reports of peptic ulcer disease, estrogen replacement therapy, oral contraceptive use, and cigarette and alcohol exposure. Moderate agreement was seen for hysterectomy and thyroid medication. Poor agreement was observed with respect to corticosteroid use.

DATA ANALYSIS

All of the statistical analyses were performed using the software Statistical Package for the Social Sciences (SPSS^X, 158). Initial Student's t-tests (for investigating yes/no variables) and Pearson Correlation Coefficients evaluated associations between any of the possible intervening variables: body mass index, birth control pill intake, estrogen replacement therapy, age at menopause, years since menopause, smoking, alcohol consumption, number of children, frequency of dieting, calcium supplementation, and a number of medications and medical conditions and BMD, independent of milk consumption or physical activity. The significance level chosen throughout all correlation analyses was $p \leq .05$ for a two-tailed test of significance.

Relationships between each of the independent continuous variables: lifetime milk consumption, lifetime physical activity and "internal" score in the MHLC and the dependent continuous variables: total, axial, and peripheral BMD were investigated using correlation and linear regression analysis. In addition, we investigated the relationship between lifetime milk consumption and bone variables with Student's t-test for comparisons between the means of subjects with lowest lifetime milk consumption and the means of subjects with highest lifetime milk consumption (25th and 75th percentile, 33rd and 67th percentile).

The data for mothers and daughters were investigated separately. One of the statistical assumptions made in correlation analysis is independence of the subjects. Since the mothers and their biological daughters can not be considered to represent independent populations, it was not appropriate to combine the groups in order to obtain a larger sample for investigating the

effects of milk consumption on bone density. Mother and daughter groups differed significantly from each other with respect to bone characteristics as well as confounding factors, and therefore pooling seemed not to be appropriate.

Single and multiple regression analysis and ANOVA were used to investigate the combined effect of lifetime milk consumption and lifetime physical activity on total, axial and peripheral BMD.

Lifetime milk consumption and current milk consumption in mothers and daughters were compared by correlation analysis. The relationship between the independent variable "internal" score in the MHLC and the dependent variables (health behaviors): current milk consumption (in daughters only), lifetime milk consumption and lifetime physical activity (in mothers and daughters) was investigated by correlation analysis.

For all comparisons between mothers and daughters, paired t-tests were performed initially to determine differences between means. The relationship between mothers' and daughters' total, relative, axial and peripheral BMD was investigated by correlation analysis and single and multiple regression analysis. Partial correlation coefficients were used to investigate the relationship between mothers' and daughters' bone variables after adjusting for major confounding factors. The significance level chosen for partial correlation coefficients was .05 for a one-tailed test of significance. It was appropriate to chose a one-tailed test, since the direction of the expected correlation was given. The relationship between mothers' and daughters' lifetime milk consumption, lifetime physical activity and score in the Multidimensional Health Locus of Control was investigated by correlation analysis.

RESULTS

BONE MINERAL PARAMETERS

Both bone mineral content and bone mineral density were measured, although the scope of this thesis included only analyses of bone mineral density data. Bone mineral content data, however, are included in the tables for readers who are interested in this information. Information about correlations between bone mineral content and bone mineral density in total, axial and peripheral bone are in the appendix (Appendix A).

STUDY SAMPLE

Our study sample consisted of 25 postmenopausal women and their biological, premenopausal daughters. Their characteristics are described in Tables 1 and 2.

Mothers:

The mean age of the mothers was 72 years and their mean height was 158 cm. Mothers weighed an average of 63.3 kg, their average body mass index was 25.5 kg/m^2 and percentage body fat average was 30.0%. The mean age at menopause for mothers was 52 years, and they were on average 20 years postmenopausal. Mothers were well educated. Seventy-two percent enjoyed at least some college education and 46% held a college degree. Except for one Native American mother, all women were Caucasian. Forty percent reported being on estrogen replacement therapy currently and for at least the past year and 12% had taken birth control pills during their later adulthood. Mothers' mean relative BMD was higher than 100% of the mean among women their age (155).

Table 1 Description of study sample: age, anthropometric parameters, menopause history, health attitudes and bone variables

	Mothers mean +/- s.d.(range) (n=25)	Daughters mean +/- s.d.(range) (n=25)
Age (years)	72 +/- 5 (64-82)	41 +/- 5 (28-50)
Height (cm)	158 +/- 11 (133-175)	167 +/- 8 (156-183)
Weight (kg)	63.6 +/-.9.0 (42.9-82.2)	64.2 +/- 8.8 (46.5-83.0)
Body Mass Index (kg/m^2)	25.5 +/- 4.0 (19.3-35.8)	23.1 +/- 2.6 (18.8-28.0)
Percentage Body fat (%)	30.0 +/- 6.1(23.8-46.9)	26.2 +/- 4.3 (18.0-34.2)
Age at Menopause	52 +/- 4 (40-56)	-
Years since Menopause	20 +/- 6 (9-30)	-
MHLC, Internal Score	27 +/- 5 (6-34)	28 +/-4 (16-35)
Bone variables:		
Total BMD (g/cm^2)	1.016 +/- .117 (.800-1.256)	1.112 +/- .081 (.954-1.241)
Axial BMD (g/cm^2)[1]	.785 +/- .104 (.597-1.020)	.867 +/- .072 (.742-1.000)
Peripheral BMD (g/cm^2)	.922 +/- .123 (.674-1.155)	1.034 +/- .073 (.915-1.156)
Relative BMD (% of mean)	107 +/- 11 (87-131)	105 +/- 8 (89-116)
Total BMC (g)[1]	1618 +/- 395 (868-2326)	1969 +/- 274 (1459-2446)
Axial BMC (g)[1]	456 +/- 138 (234-699)	621 +/- 102 (398-866)
Peripheral BMC (g)	1039 +/- 255 (501-1449)	1231 +/- 184 (886-1630)

[1] n=24

Table 2   Description of study sample: education, ethnicity and lifestyle variables

	Mothers n (%) (n=25)	Daughters n (%) (n=25)
Education		
high school or less	5 (20)	2 (8)
some college	7 (28)	5 (20)
college degree	11 (46)	18 (72)
no information	2 (8)	0
Ethnicity:		
Caucasian	24 (96)	25 (100)
Native American	1 (4)	0
History of smoking	9 (36)	9 (36)
Ever taken birth control pills	3 (12)	20 (80)
Estrogen replacement therapy (ERT), currently	10 (40)	-
years on ERT	11.9 +/- 6.9 (10-21)*	-
History of dieting	15 (60)	18 (72)
Ever taken Ca-supplements	21 (84)	8 (32)
Amount of Ca-supplementation among takers (mg)		
during early adulthood (age 20-39)	-	108 +/-110 (13-265)[1] n=4
during mid adulthood (age 40-59)	207 +/- 176 (30-420)[1] n=6	300 +/- 132 (200-450)[1] n=3
during late adulthood (over age 60)	388 +/- 354 (13-1200)[1] n=19	-
past two years	640 +/- 446 (100-1500)[1] n=19	214 +/- 248 (38-750)[1] n=7
Regular alcohol consumption	7 (28)	9 (36)
Symptoms of lactose intolerance	6(24)	1(4)

[1] mean +/- s.d. (range)

Daughters:

Daughters' mean age was 41 years and their mean height was 167 cm. They weighed an average of 64.2 kg and had an average body mass index of 23.1 kg/m^2 and 26.2% body fat. Ninety-two percent had attended college and 72% had obtained a degree. Thirty-six percent were former smokers, but only one daughter smoked currently, at the rate of four cigarettes per day. Eighty percent of the daughters had taken birth control pills and two of them took them currently. Eight of the daughters (32%) had taken supplements containing calcium; however, only three of them took a significant amount (>100 mg) for several years. Nineteen daughters had between one and five children, and six were nulliparous. Daughters' mean relative BMD was also higher than 100% of the mean among women their age (155).

MILK CONSUMPTION AND BONE MINERAL DENSITY

Mothers:

Data regarding lifetime milk consumption, current milk consumption and current calcium intake among mothers are in Table 3. In addition to lifetime milk consumption, several age periods were investigated separately (Table 3). In mothers, no correlations between any of the measures of milk consumption and total, axial or peripheral BMD were observed. We found, however, that the amount of calcium supplementation (mg) after age 60 was positively related to total, peripheral and relative BMD (Table 4) although calcium supplementation at age 40-59 was not correlated with BMD. The amount of calcium taken in supplements from 40-59 years was negatively correlated with lifetime milk consumption (Table 5) and the negative relationship between calcium taken in

Table 3   Lifetime and current milk consumption in mothers and daughters

	Mothers mean +/- s.d. (range) (n=25)	Daughters mean +/- s.d.(range) (n=25)
Milk consumption[1] during		
childhood (through age 12)	3.0 +/- 1.2 (1-4)	4.0 +/- .2 (3-4)
teens (13-19)	2.9 +/- 1.2 (1-4)	3.7 +/- .5 (3-4)
earlier adulthood (20-39)	2.8 +/- 1.0 (1-4)	3.1 +/- .8 (2-4)
mid adulthood (40-59)	2.7 +/- 1.0 (1-4)	2.8 +/- .7 (1-4)[2]
later adulthood (over age 60)	2.7 +/- 1.0 (1-4)	-
past two years specifically	2.8 +/- .9 (1-4)	3.0 +/- .8 (1-4)
Lifetime milk consumption[1]	2.8 +/- .8 (1.0-4.0)	3.4 +/- .5 (2.0-4.0)
Current milk consumption (Glasses of milk/day)	1.4 +/-1.1 (0-3)	1.5 +/- 1.0 (0-3)
Calcium intake (mg) 3-day dietary record[3]	-	1032 +/- 340 (332-1656)

[1] on a scale from 1 to 4 (4=with most meals 3=frequently 2=sometimes 1=rarely or never)

[2] n=12

[3] not including calcium intake from supplements

Table 4 **Correlation between calcium supplementation and bone variables in mothers (n=25)**

Pearson Correlation Coefficients for calcium supplementation:

	Calcium Supplementation age 40-59 (mg)	Calcium Supplementation over age 60 (mg)
Total BMDensity (mg/cm^2)	.20	**.44***
Axial BMDensity[1](mg/cm^2)	.10	.32
Peripheral BMDensity (mg/cm^2)	.24	**.45***
Relative BMDensity (% of mean)	.20	**.43***
Total BMContent[1] (g)	.18	.27
Axial BMContent[1] (g)	.18	.21
Peripheral BMContent (g)	.19	.35

* p ≤.05
[1] n=24

Table 5 **Correlation between calcium supplementation and lifetime milk consumption in mothers (n=25)**

Pearson Correlation Coefficients for calcium supplementation:

	Calcium Supplementation age 40-59 (mg)	Calcium Supplementation over age 60 (mg)
Lifetime milk consumption	**-.41***	-.38

* p ≤.05

supplements over age 60 and lifetime milk consumption was close to significance (p=.06). Non-dietary calcium sources (supplements) contributed significantly to mothers' daily calcium intake during older ages.

As a result of this finding, a new variable "lifetime calcium consumption" was compiled including both lifetime milk consumption and lifetime calcium supplementation data. This new variable was not correlated with any measurement of BMD among mothers.

Daughters:

Data regarding lifetime milk consumption, current milk consumption and current calcium intake among daughters are in Table 3. In addition to lifetime milk consumption, several age periods were investigated separately. Among daughters, there also was no correlation between any milk consumption variable, including daughters' current calcium intake estimated from 3-day food records, and total, axial or peripheral BMD. Only a small proportion took calcium-containing supplements and these were not related to bone variables.

Adjusting for factors which were found to be associated with BMD among our study group (mothers: body weight, age, estrogen replacement therapy, birth control pill intake (Tables 9, 10, 11,12, 13); daughters: physical activity (Table 7) did not reveal any correlation between current or lifetime milk consumption and bone variables.

PHYSICAL ACTIVITY AND BONE MINERAL DENSITY

Descriptive data for lifetime physical activity of mothers and daughters are listed in Table 6.

Mothers:

Among mothers there was no significant correlation between any lifetime physical activity variable and BMD. In order to evaluate whether a relationship existed between level of physical activity at specific ages and elderly women's BMD, each age period was then investigated separately. For their teen years, mothers fell naturally into two categories: high (>4 hours of total exercise per week, weight-bearing + non-weight-bearing) and moderate (2-3 hours of total exercise per week, weight-bearing + non-weight-bearing). Mothers with a high level of total exercise during their teens had significantly higher total and peripheral BMD than those with a moderate level of total exercise. After adjusting for age (which was negatively correlated with mothers' BMD and which was negatively associated with estrogen use), this relationship disappeared. Women who reported high levels of exercise during their teen years were significantly younger than those who reported moderate exercise during their teens (mean ages=69.6 yrs and 75.1 yrs, p=.00).

Daughters:

Among daughters a variety of lifetime physical activity variables were positively correlated with specific bone variables (Table 7). Weight-bearing exercise was positively correlated with total and peripheral BMD, as well as with relative BMD. Total exercise (weight-bearing + non-weight-bearing) was positively correlated with relative BMD only. Total weight-bearing activity

Table 6   Lifetime physical activity among mothers and daughters (n=25)

	Mothers mean +/- s.d. (range) (n=25)	Daughters mean +/- s.d.(range) (n=25)
Lifetime weight-bearing exercise[1]	2.0 +/- .3 (1.4-2.8)	2.0 +/- .5 (1.0-3.0)
Lifetime total exercise[1]	2.2 +/- .4 (1.8-3.0)	2.2 +/- .6 (1.0-3.0)
Lifetime total weight-bearing activity[1]	2.4 +/- .3 (1.6-3.0)	2.2 +/- .5 (1.3-3.0)
Lifetime perceived activity [2]	3.6 +/- .6 (2.6-5.0)	3.4 +/- .5 (2.5-4.8)

[1] on a scale from 1 to 3 (3=high 2=middle 1=low)
[2] on a scale from 1 to 5 (5=very active 4=active 3=moderately active 2=lightly active 1=very light)

Table 7   Correlation between different measures of lifetime physical activity and bone variables in daughters (n=25)

Pearson Correlation Coefficients for each measure of lifetime physical acitivity

	Lifetime weight-bearing exercise	Lifetime total exercise	Lifetime total weight-bearing activity	Lifetime perceived physical activity
Total BMDensity (g/cm^2)	.43*	.39	.48*	.33
Axial BMDensity (g/cm^2)	.30	.27	.39	.35
Peripheral BMDensity (g/cm^2)	.43*	.37	.38	.31
Relative BMDensity (% of mean)	.47*	.42*	.50*	.32
Total BMContent (g)	.49*	.57*	.58**	.38
Axial BMContent (g)	.42*	.46*	.49*	.37
Peripheral BMContent (g)	.44*	.55**	.48*	.37

* p≤.05   ** p ≤.01
[1] on a scale from 1 to 3 (3=high 2=middle 1=low)
[2] on a scale from 1 to 5 (5=very active 4=active 3=moderately active 2=lightly active 1=very light)

(weight-bearing exercise + non-exercise activity) was significantly correlated with total and relative BMD, with slightly higher correlation coefficients than those found for weight-bearing exercise. The relationships between total weight-bearing activity and axial and peripheral BMD were close to significance (p=.06 and p=.06, respectively). Figure 1 and Figure 2 show the regression of total BMD on lifetime weight-bearing exercise and lifetime total weight-bearing activity, respectively. Total, axial, peripheral and relative BMD tended to increase with increasing perceived level of physical activity, however none of these relationships was statistically significant (p=.10, p=.08, p=.13, p=.11, respectively). The daughters' lifetime perceived physical activity was positively correlated with their lifetime total exercise indicating that their self-perceptions were valid (Table 8).

COMBINED EFFECT OF MILK CONSUMPTION AND PHYSICAL ACTIVITY ON BONE MINERAL DENSITY

The combined effect of milk consumption and physical activity on bone variables was examined with two methods. The first one was the creation of a number of new variables, combining lifetime milk consumption and the different measurements of lifetime physical activity. None of these combined variables was correlated with BMD in mothers or in daughters. The second method was Analysis of Variance (ANOVA), which allowed the measurement of both the separate and interactive effects of the two variables, lifetime milk consumption and lifetime physical activity on BMD. Each measure of physical activity was examined separately. No combined effect was observed between our two independent variables with this method.
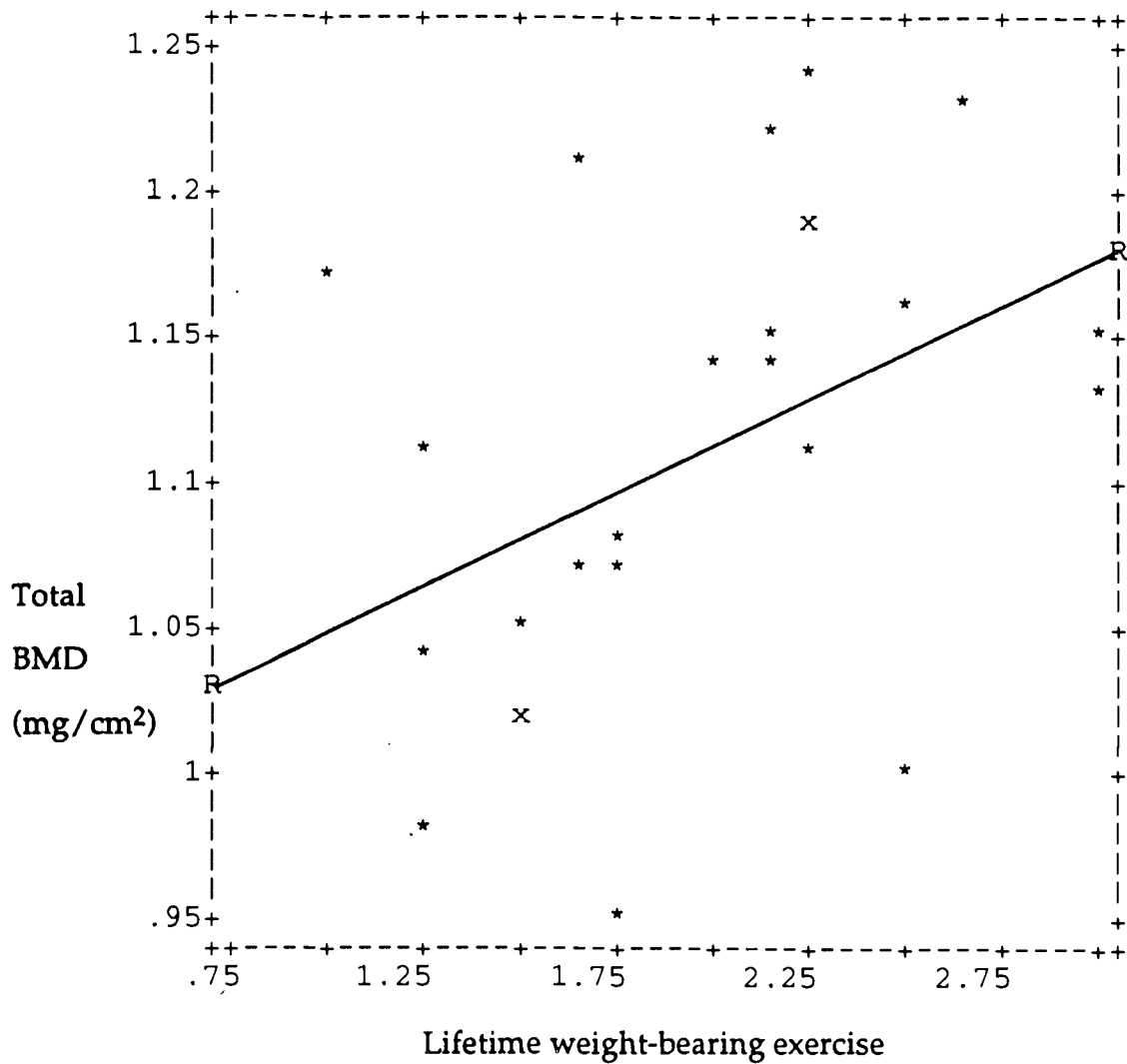
$y=.98488 + .06508x$

Correlation Coefficient r=.43   p=.0300

x = double occurrence

Figure 1   **Linear regression of total BMD on lifetime weight-bearing exercise in daughters (n=25)**
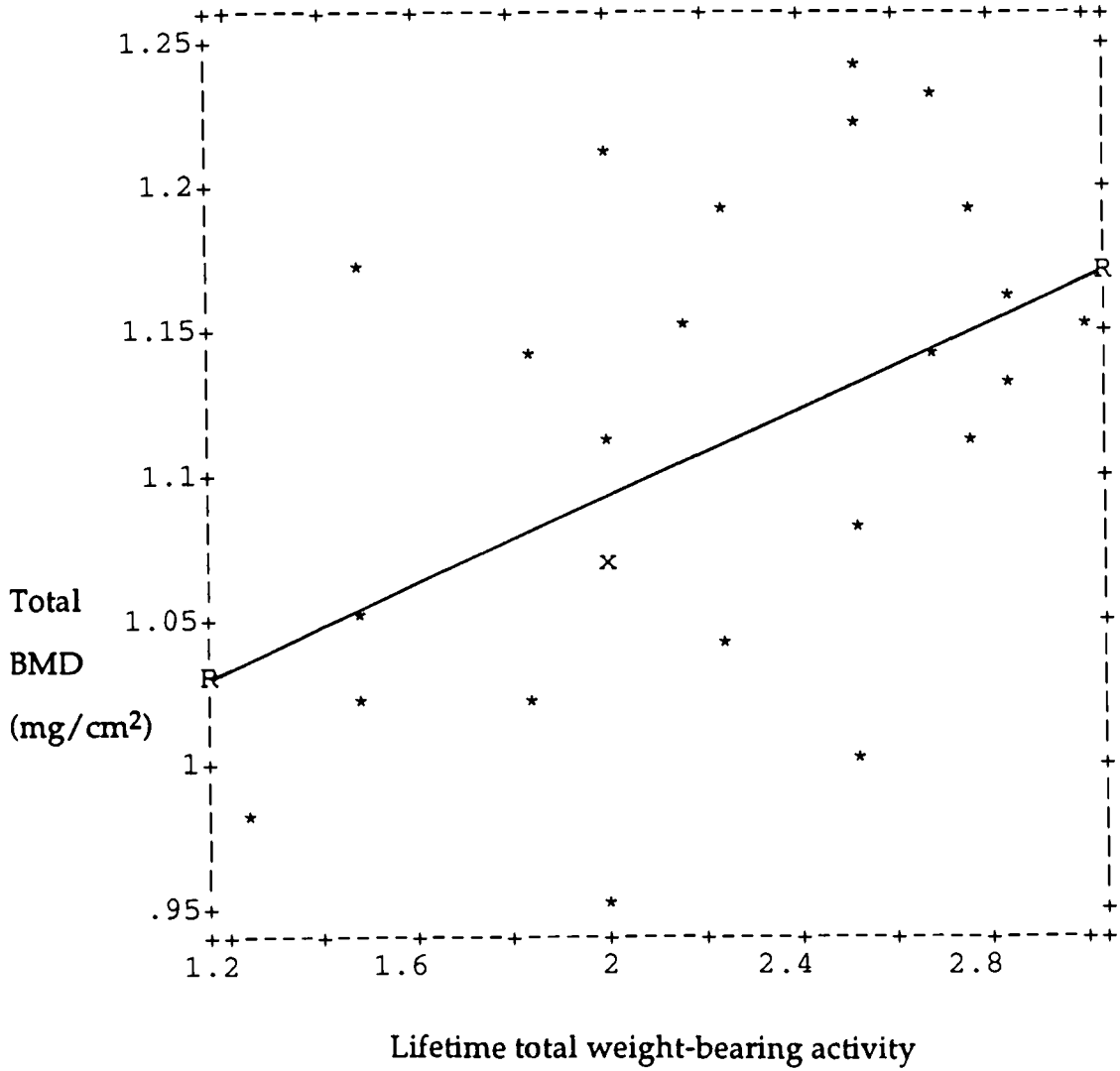
y=.93412 + .08020x

Correlation Coefficients r=.48   p=.0154

x = double occurrence

Figure 2   Linear regression of total BMD on lifetime total weight-bearing
              activity in daughters (n=25)

Table 8   Correlation between lifetime perceived physical activity and other physical activity measurements among daughters (n=25)

Pearson Correlation Coefficients for physical activity measurements:

Lifetime	Weight-bearing exercise[1]	Total exercise[1]	Total weight-bearing activity[1]
Perceived physical activity[2]	.34	.52**	.36

**p$\leq$.01

[1] on a scale from 1 to 3 (3=high 2=middle 1=low)

[2] on a scale from 1 to 5 (5=very active 4=active 3=moderately active 2=lightly active 1=very light)

Relationship between Health Attitudes and Bone Variables, Milk Consumption, and Physical Activity

The internal score on the Multidimensional Health Locus of Control Scale was used to evaluate to what degree subjects thought that they were able to influence their health through their own behavior. In neither group was this score correlated with BMD, or with lifetime and current milk consumption or lifetime physical activity.

Other Factors Correlated with Bone Mineral Density

Mothers

Age and anthropometric parameters were significantly related to mothers' BMD (Table 9). Total and peripheral BMD decreased significantly with increasing age. The factor most strongly associated with BMD in mothers was body weight (Table 9 and Figure 3), which was positively correlated with all bone variables. Body mass index was also positively correlated with each bone variable, but not as strongly as was body weight. Percentage of body fat and total fat mass were positively related to total, peripheral and relative BMD. Total lean mass was positively correlated with axial BMD. After adjusting for weight, the other anthropometric measurements lost their significance (Table 10). This was due to the strong positive correlations between weight and height ($r=.45$, $p \leq .05$); weight and body mass index ($r=.53$, $p \leq .01$); weight and percentage of body fat ($r=.54$, $p \leq .01$); weight and total fat mass ($r=.79$, $p \leq .01$); and weight and total lean mass ($r=.71$, $p \leq .01$). After adjusting for body weight, age lost its significance for total BMD, but the negative correlation with peripheral BMD was still evident. Therefore, body weight was adjusted for first, when investigating relationships between non-anthropometric variables and bone variables.

Table 9   Correlations between age / anthropometric measurements and bone variables in mothers (n=25)

Pearson Correlation Coefficients for age and anthropometric measurements:

	Age (yrs)	Weight (kg)	Height (cm)	Body mass index (kg/m^2)	Percentage Body fat (%)	Total fat mass (g)	Total lean mass (g)
Total BMDensity (g/cm^2)	-.49*	.64**	.06	.58**	.50*	.61**	.31
Axial BMDensity (g/cm^2)[1]	-.34	.63**	.17	.44**	.22	.40	.55**
Peripheral BMDensity (g/cm^2)	-.58**	.64**	.06	.59**	.52**	.62**	.30
Relative BMDensity (% of mean)	-.34	.62**	.03	.59**	.50*	.60**	.28
Total BMContent (g)[1]	-.47*	.83**	.37	.46*	.47*	.73**	.47*
Axial BMContent (g)[1]	-.37	.79**	.38	.41*	.57**	.72**	.43*
Peripheral BMContent (g)	-.62**	.78**	.33	.47*	.52**	.68**	.45*

* p≤.05   ** p ≤.01
[1] n=24
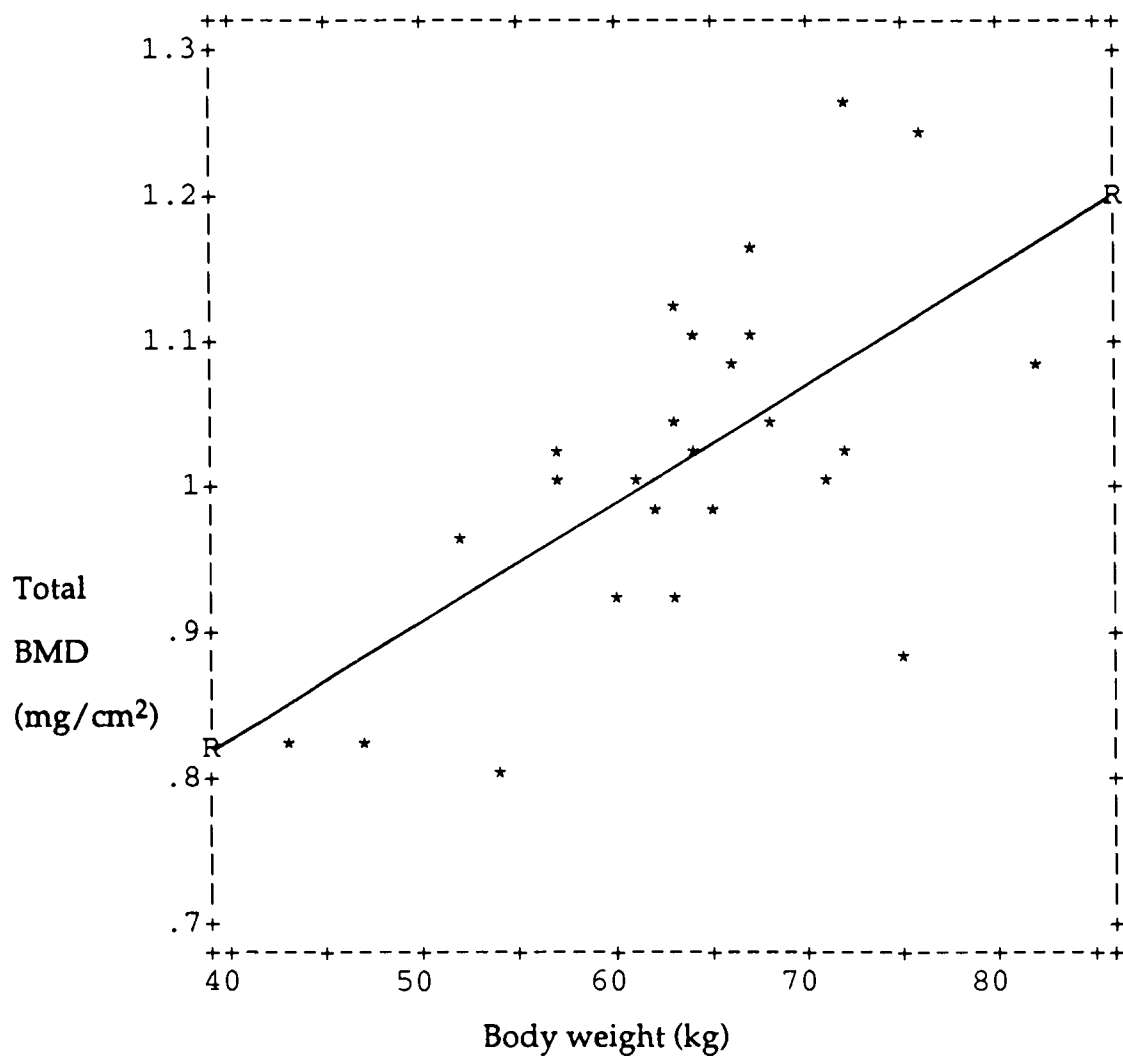
$y = .48868 + .00828x$

Correlation Coefficients r=.64   p=.0006

Figure 3   Linear regression of total BMD on body weight in mothers (n=25)

Table 10 Correlations between age / anthropometric measurements and bone variables <u>adjusted for body</u> <u>weight</u> in mothers

(n=25)

Partial Correlation Coefficients for age and anthropometric measurements:

	Age (yrs)	Height (cm)	Body mass index (kg/m^2)	Percentage Body fat (%)	Total fat mass (g)	Total lean mass (g)
Total BMDensity (g/cm^2)	-.29	-.25	.30	.14	.10	-.12
Axial BMDensity (g/cm^2)[1]	-.11	-.12	.13	-.22	-.23	.23
Peripheral BMDensity (g/cm^2)	**-.43***	-.26	.31	.15	.10	-.13
Relative BMDensity (% of mean)	-.04	-.29	.33	.15	.13	-.15
Total BMContent (g)[1]	-.24	.08	-.02	.21	.20	-.22
Axial BMContent (g)[1]	-.07	.11	-.07	.22	.23	-.23
Peripheral BMContent (g)	**-.52****	.10	-.03	.06	.02	-.06

* p≤.05   ** p ≤.01
[1] n=24)

Other positive influences on mothers' BMD were past birth control pills use and current estrogen replacement therapy. Estrogen replacement therapy was associated with higher total, peripheral and relative BMD (axial BMD close to significance) (Table 11). Estrogen replacement therapy was more common among younger mothers (Table 11).

It was not possible to separate the effects of estrogen replacement therapy from those of age on mothers' bone variables. Age could explain 24% of the variation in total BMD and 33% of the variation in peripheral BMD (Table 12). Estrogen replacement therapy could explain 21% of the variation in total BMD (R^2) and 20% of the variation in peripheral BMD. Combining the two variables increased the amount of variation explained to 30% in total BMD and 37% in peripheral BMD. We observed that estrogen replacement therapy interrupted the expected strong negative correlation between age and BMD in mothers (Figures 4, 5 and 6). Correlations between age and total BMD were:

in all mothers:	r=-.49, p=.01
non-estrogen using mothers:	r=-.71, p=.00
estrogen using mothers:	r= .26, p=.47

Duration of estrogen replacement therapy (in years) among treated women did not have significant influence on BMD.

Past birth control pill intake was significantly associated with higher total and peripheral BMD, as well as higher relative BMD in mothers (Table 13) The difference in axial BMD was close to significance (p=.06). Mothers having ever taken birth control pills did not differ from other mothers with respect to age, height, weight, body mass index, total fat mass or percentage body fat.

Table 11 Bone mineral density and content in mothers undergoing estrogen replacement therapy and those not undergoing estrogen replacement therapy

	Estrogen Replacement Therapy	
	yes (n=10) mean +/- s.d.	no (n=15) mean +/- s.d.
Age (years)	69 +/- 4 *	74 +/- 4
Total BMD (g/cm^2)	1.082 +/- .106 *	.971 +/-.104
Axial BMD (g/cm^2)[1]	.829 +/-.083	.753 +/-.108
Peripheral BMD (g/cm^2)	.991 +/-.085 *	.876 +/-.124
Relative BMD (% of mean)	112 +/- 12*	103 +/-10
Total BMC (g)	1829 +/- 336*	1467 +/- 373
Axial BMC (g)[1]	530 +/- 124 *	403 +/- 125
Peripheral BMC (g)	1186 +/- 174 *	941 +/- 257

* p<.05   **p<.01
[1] n=24

Table 12   Variability in bone mineral variables of mothers which is explained by age and estrogen replacement therapy (ERT) separately and combined (n=25)

	Age (separate R^2)	ERT (separate R^2)	Age, ERT (combined R^2)
Total BMD (mg/cm^2)	.24*	.21*	.30*
Axial BMD (mg/cm^2)[1]	.12	.14	.17
Peripheral BMD (mg/cm^2)	.33**	.20*	.37**
Relative BMD (% of mean)	.11	.16	.19
Total BMC (g)	.22*	.21*	.30*
Axial BMC (g)[1]	.14	.22*	.25
Peripheral BMC (g)	.37**	.22*	.42**

* p<.05   **p<.01
[1] n=24
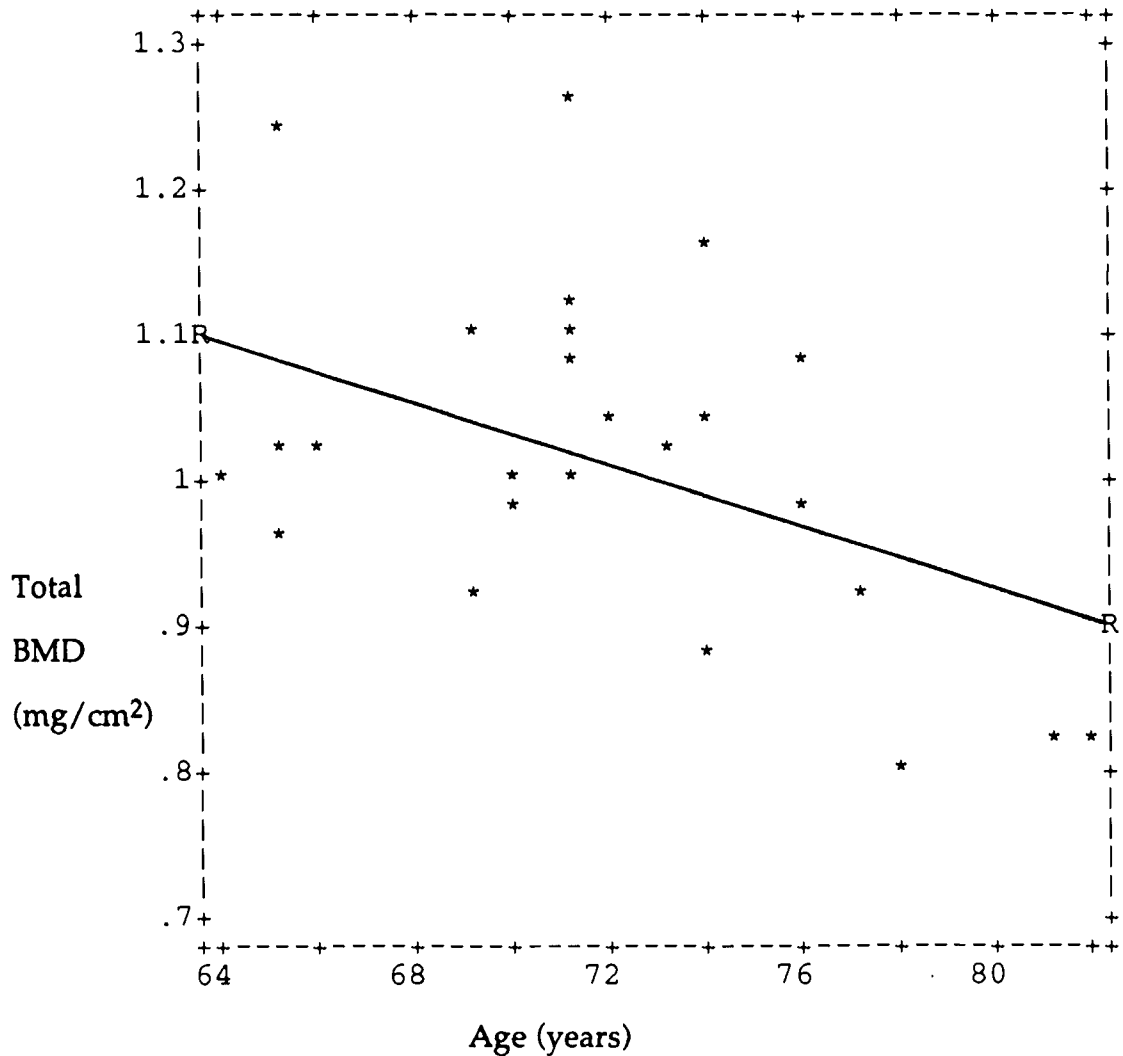
$$y=1.86981 - .01189x$$

Correlation Coefficients r=-.49   p=.0120

Figure 4   Linear regression of total BMD on age among mothers (n=25)
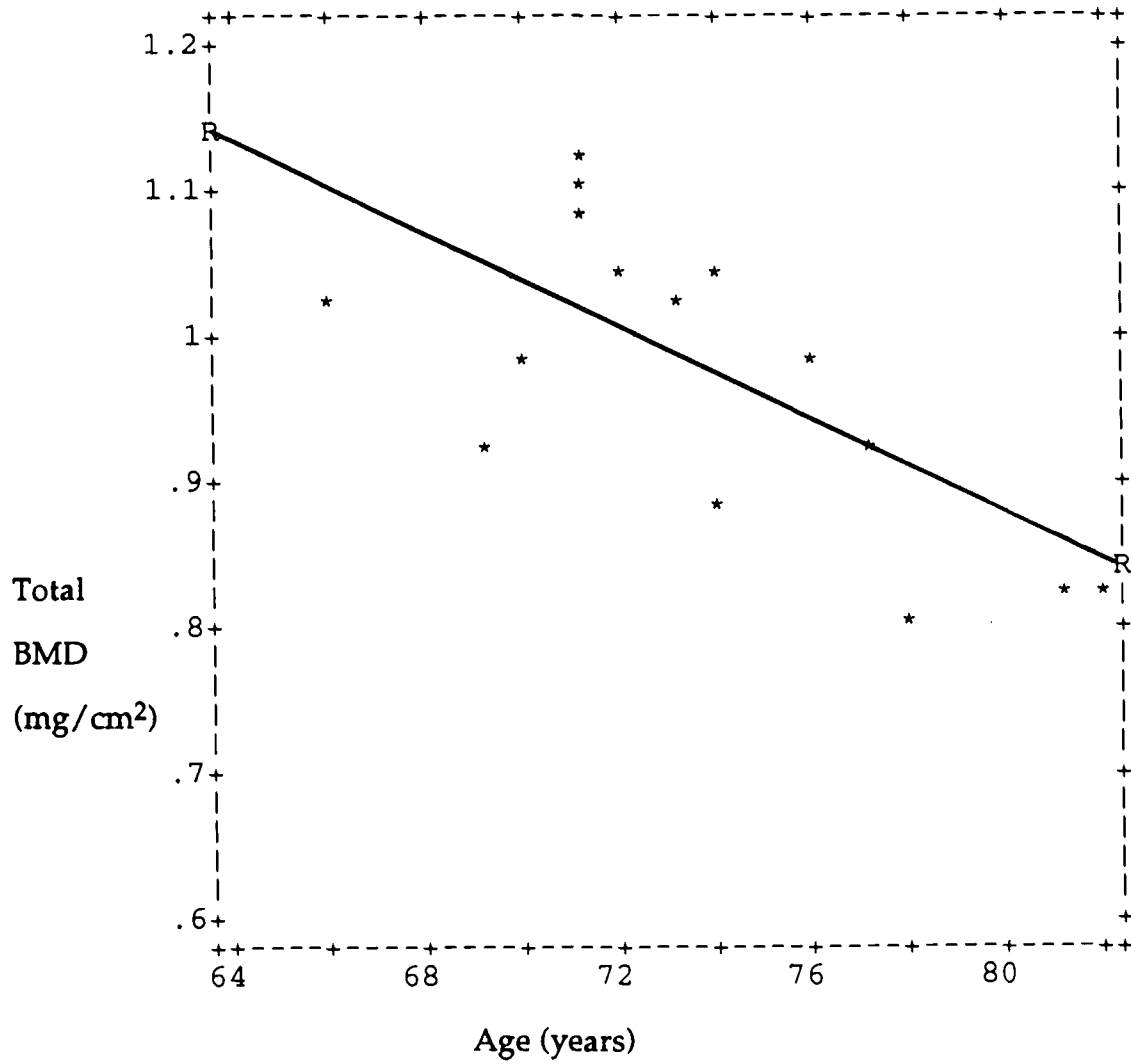
$y=2.19682 - .01664x$

Correlation Coefficients r=-.71   p=.0030

Figure 5   Linear regression of total BMD on age among mothers not
             undergoing estrogen replacement therapy (n=15)
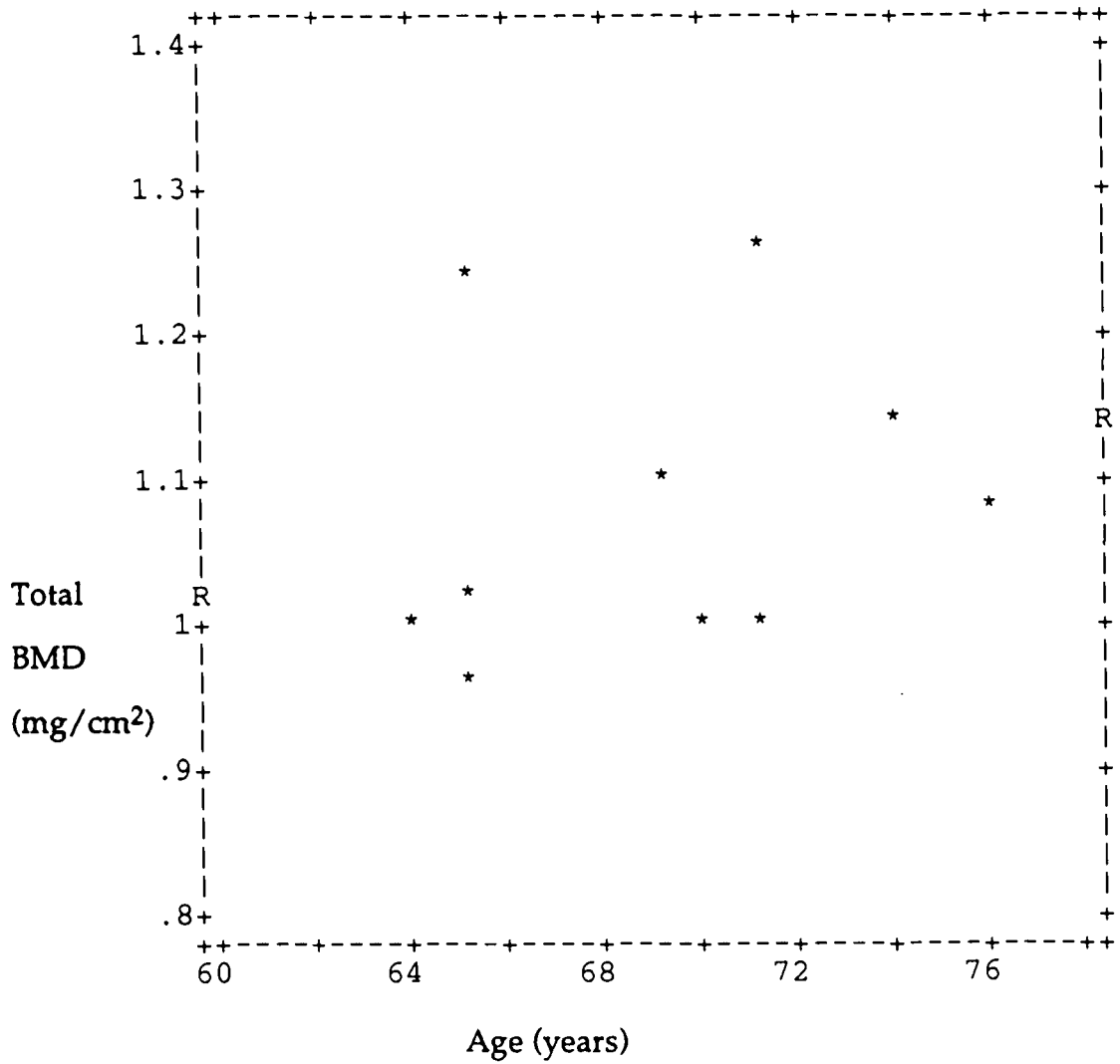
Figure 6   Linear regression of total BMD on age among mothers
            undergoing estrogen replacement therapy (n=10)

Table 13   Bone mineral density and content in mothers having formerly taken and those not having taken birth control pills

	Birth Control pills	
	yes (n=3) mean +/- s.d.	no (n=22) mean +/- s.d.
Total BMD (g/cm^2)	1.172 +/-.076 *	.994 +/-.105
Axial BMD (g/cm^2)[1]	.903 +/-.029	.768 +/-.100
Peripheral BMD (g/cm^2)	1.047 +/-.094 **	.905 +/-.118
Relative BMD (% of mean)	123 +/- 8	104 +/- 10
Total BMC (g)	2107 +/- 210 *	1548 +/- 367
Axial BMC (g)[1]	530 +/- 124	430 +/- 126
Peripheral BMC (g)	1186 +/- 174 **	1002 +/- 247

* p<.05   **p<.01
[1] n=24

Daughters:

Among daughters, total and peripheral, as well as relative BMD, increased with age (Table 14). This was probably an artifact of the almost significant positive correlation between daughters' age and their lifetime physical activity variables (weight-bearing exercise: $r=.39$, $p=.05$, total weight-bearing activity: $r=.36$, $p=.07$).

Fewer correlations were observed between anthropometric parameters and BMD among daughters than among mothers. Daughters' BMD was not significantly correlated with weight, height, body mass index, percentage body fat or total fat mass (Table 14), although axial BMD was positively correlated with total lean mass. Total lean mass was positively correlated with three measures of lifetime physical activity (total weight-bearing activity: $r=.47$, $p<.05$; total exercise: $r=.53$, $p<.01$; perceived level of activity: $r=.42$, $p<.05$). Current or former birth control pill intake was not correlated with bone variables in daughters.

Neither medications and medical conditions nor menstrual cycle irregularities were found to have confounding influence on bone variables in mothers or in daughters.

MULTIPLE REGRESSION MODELS FOR FACTORS INFLUENCING BMD OF MOTHERS AND DAUGHTERS

Since there were many variables associated with BMD and it was difficult to see their relative importance when looking at them separately, we constructed a series of models to investigate several factors at the same time. Multiple regression analysis was used in an attempt to describe total, axial, peripheral and

Table 14   Correlations between age / anthropometric measurements and bone variables in daughters (n=25)

Pearson Correlation Coefficients for age and anthropometric measurements:

	Age (yrs)	Weight (kg)	Height (cm)	Body mass index (kg/m^2)	Percentage Body fat (%)	Total fat mass (g)	Total lean mass (g)
Total BMDensity (g/cm^2)	**.48***	.28	.03	.28	-.21	.03	.39
Axial BMDensity (g/cm^2)	.32	.29	.08	.28	-.25	.00	**.44***
Peripheral BMDensity (g/cm^2)	**.45***	.27	.11	.22	-.14	.05	.36
Relative BMDensity (% of mean)	**.59****	.28	.02	.30	-.15	.06	.36
Total BMContent (g)	.27	**.79****	**.50***	**.43***	-.02	.37	**.79****
Axial BMContent (g)	.16	**.51****	.39	.32	-.17	.16	**.65****
Peripheral BMContent (g)	.26	**.81****	**.60****	**.47***	-.18	**.54****	**.83****

* p≤.05   ** p ≤.01

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relative BMD in terms of models for mothers and for daughters. Variables were entered using the "forward stepwise" method, in which the only variables entering the equation were those with p-values below .05. With every step, the variable most strongly correlated with the dependent variable entered the equation. All other variables and significance levels were adjusted for the effect of this variable on the dependent variable. When no additional variables met the entrance criteria, the model was considered complete.

Mothers:

The variable list tested for multiple regression models of mothers' BMD included all anthropometric variables, lifetime and current milk consumption variables, lifetime physical activity variables, lifestyle variables (smoking, alcohol, ERT, birth control pills, calcium supplementation), age, reproductive variables, and certain medications. Multiple regression analyses for mothers are described in Tables 15-18. The most dominant variable in the models was body weight; other variables regularly entering the equation were ERT (estrogen replacement therapy) and BCP (former birth control pill intake). Since ERT and age are correlated with each other, age usually did not enter the equation. Body mass index was significant for the total and relative BMD models, even after adjustment for body weight. Calcium supplementation over age 60 contributed four to seven percent in explaining the variation between mothers in total and peripheral, as well as relative BMD. The variation in mothers' BMD explained by these models is remarkably high, many times over 80%.

Daughters:

For daughters, the variable list tested for multiple regression models included all anthropometric variables, lifetime and current milk consumption,

Table 15   Multiple regression model for mothers:
              Total bone mineral density (g/cm^2)
              (n=25)

Variables	Coefficient	T	P-value	R^2
Intercept	.387656			
Weight (kg)	.005652	4.605	.0002	.56
BCP, yes/no	.116235	4.407	.0003	.13
ERT, yes/no	.076309	4.320	.0004	.09
Body mass index (kg/m^2)	.008127	3.052	.0069	.08
Calcium suppl, mg (> age 60)	.000073	2.851	.0106	.04
				R^2=.90

Table 16   Multiple regression model for mothers:
              Axial bone mineral density (g/cm^2)
              (n=24)

Variables	Coefficient	T	P-value	R^2
Intercept	.352877			
Weight (kg)	.006642	3.573	.0018	.39
BCP, yes/no	.102183	2.105	.0475	.11
				R^2=.50

Table 17   Multiple regression model for mothers:
            Peripheral bone mineral density (g/cm^2)
            (n=25)

Variables	Coefficient	T	P-value	R^2
Intercept	.282683			
Weight (kg)	.009263	6.553	.0000	.61
ERT, yes/no	.080627	3.318	.0034	.12
Calcium suppl. (over age 60)	.000091	2.595	.0173	.07
				R^2=.80

Table 18   Multiple regression model for mothers:
            Relative bone mineral density (% of mean)
            (n=25)

Variables	Coefficient	T	P-value	R^2
Intercept	47.696613			
Weight (kg)	.486713	3.843	.0012	.52
BCP, yes/no	13.533682	4.973	.0001	.18
Body mass index (kg/m^2)	.876220	3.189	.0051	.07
ERT, yes/no	6.012065	3.298	.0040	.08
Calcium suppl. (over age 60)	.006443	2.450	.0247	.04
				R^2=.89

current calcium intake, lifetime physical activity variables, lifestyle variables, reproductive variables, age, and mothers' bone variables. Multiple regression analyses for daughters are shown in Table 19 through Table 22. Usually only one variable entered the regression equation for describing the variation in daughters' BMD. Lifetime physical activity variables such as total weight-bearing activity and total weight-bearing exercise and total lean body mass were most important in the models. Since lifetime physical activity variables and lean body mass are correlated with each other, they usually did not enter the same equation. Between 19 and 45% of the variability in daughters' BMD could be explained by the models.

RELATIONSHIP BETWEEN CURRENT AND LIFETIME MILK CONSUMPTION

Among both mothers and daughters, current milk consumption, assessed by the beverage frequency questionnaire as number of glasses of milk per day, was highly positively correlated with lifetime milk consumption (mothers: $r=.59$, $p=.00$; daughters: $r=.69$, $p=.00$).

RELATIONSHIPS BETWEEN MOTHER/DAUGHTER PAIRS

Bone variables

The daughters' BMD in the total, axial and peripheral skeleton was significantly higher than that of their mothers (Table 23). Mothers and daughters had similar BMD relative to their age peers (relative BMD), both were higher than 100%. The variability of bone densities was somewhat larger among mothers than among daughters.

Table 19   Multiple regression model for daughters:
            Total bone mineral density (g/cm^2)
            (n=25)

Variables	Coefficient	T	P-value	R^2
Intercept	.952951			
Lifetime total weight-bearing activity	.074392	2.595	.0165	.23
				R^2=.23

Table 20   Multiple regression model for daughters:
            Axial bone mineral density (g/cm^2)
            (n=25)

Variables	Coefficient	T	P-value	R^2
Intercept	.619800			
Total lean mass (g)	.005626	2.354	.0279	.45
				R^2=.45

Table 21　　Multiple regression model for daughters:
　　　　　　　　Peripheral bone mineral density (g/cm²)
　　　　　　　　(n=25)

Variables	Coefficient	T	P-value	R^2
Intercept	.929792			
Lifetime weight-bearing exercise	.055184	2.251	.0347	.19
				R^2=.19

Table 22　　Multiple regression model for daughters:
　　　　　　　　Relative bone mineral density (% of mean)
　　　　　　　　(n=25)

Variables	Coefficient	T	P-value	R^2
Intercept	87.619734			
Lifetime total weight-bearing activity	7.989917	2.739	.0120	.25
				R^2=.25

Table 23   Comparison between mothers' and daughters' bone variables

	Mothers mean +/- s.d.(range) (n=25)	Daughters mean +/- s.d.(range) (n=25)
Total BMD (g/cm^2)	1.016 +/- .117 (.800-1.256)**	1.112 +/- .081 (.954-1.241)
Axial BMD (g/cm^2)[1]	.785 +/- .104 (.597-1.020)**	.867 +/- 072 (.742-1.000)
Peripheral BMD (g/cm^2)	.922 +/- .123 (.674-1.155)**	1.034 +/- .073 (.915-1.156)
Relative BMD (% of mean)	107 +/- 11 (87-131)	105 +/- 8 (89-116)
Total BMC (g)[1]	1618 +/- 395 (868-2326)**	1969 +/- 274 (1459-2446)
Axial BMC (g)[1]	456 +/- 138 (234-699)**	621 +/- 102 (398-866)
Peripheral BMC (g)	1039 +/- 255 (501-1449)**	1231 +/- 184 (886-1630)

Paired t-test: *p ≤ .05   **p ≤. 01
[1] n=24

Using the non-adjusted data, no significant correlation was detected between paired mothers' and daughters' BMD (Table 24). There was also no significant correlation between paired mothers' and daughters' BMD when investigated separately for mothers undergoing estrogen replacement therapy and those not undergoing estrogen replacement therapy. Partial correlation coefficients were used in order to adjust for four confounding factors affecting the mothers' bone variables (weight, age, estrogen replacement therapy, past birth control pill intake) and one confounding factor affecting the daughters' bone variables (lifetime total weight-bearing activity) (Table 25). A partial correlation coefficient measures the relationship between two variables while controlling for possible effects of other variables. Since the populations were generally related, it was reasonable to expect positive relationships. Therefore, a one-tailed test of significance was chosen. Table 25 shows partial correlations observed between mothers' and daughters' bone after controlling for these major intervening factors. A positive correlation was observed between mothers' and daughters' peripheral BMD.

Milk consumption, physical activity, health attitudes

Lifetime milk consumption among daughters was higher than among mothers (Table 26), and younger life stages in which high milk consumption is common (childhood, teens) contributed more strongly to the daughters' lifetime average than to the mothers' lifetime average. Daughters had a higher average milk consumption during childhood and teens than mothers .

Lifetime milk consumption of mothers and their daughters were positively correlated (r=.43, p<.05). The score on the calcium food frequency questionnaire was also significantly correlated between mothers and their

Table 24   Relationship between mothers' and daughters' (paired) bone
variables (n=25 pairs)

Mother/Daughter bone variable		Partial Correlation Coefficients	
		r	P-value[1]
Total BMD (g/cm^2)	Mother/Daughter	.19	.181
Axial BMD (g/cm^2)	Mother/Daughter [2]	.26	.107
Peripheral BMD (g/cm^2)	Mother/Daughter	.12	.285
Relative BMD (% of mean)	Mother/Daughter	.15	.227
Total BMC (g)	Mother/Daugther [2]	.28	.094
Axial BMC (g)	Mother/Daughter [2]	.36	.042*
Peripheral BMC (g)	Mother/Daughter	.14	.253

[1] one-tailed
[2] n=24 pairs

Table 25   Relationship between mothers' and daughters' (paired) bone
variables, controlling for mothers' weight, mothers' age, mothers'
estrogen replacement therapy, mothers' past birth control pill
intake and daughters' lifetime total weight-bearing activity
(n=25 pairs)

Mother/Daughter bone variable		Partial Correlation Coefficients	
		r	P-value[1]
Total BMD (g/cm^2)	Mother/Daughter	.29	.104
Axial BMD (g/cm^2)	Mother/Daughter [2]	.19	.218
Peripheral BMD (g/cm^2)	Mother/Daughter	.43	.028*
Relative BMD (% of mean)	Mother/Daughter	.26	.135
Total BMC (g)	Mother/Daugther [2]	.49	.016*
Axial BMC (g)	Mother/Daughter [2]	.61	.003*
Peripheral BMC (g)	Mother/Daughter	.36	.059

[1] one-tailed
[2] n=24

Table 26   **Lifetime and current milk consumption in mothers and daughters**

	Mothers mean +/- s.d. (range) (n=25)	**Daughters** mean +/- s.d.(range) (n=25)
Milk consumption[1] during		
childhood (through age 12)	3.0 +/- 1.2 (1-4)** [a]	4.0 +/- .2 (3-4)
teens (13-19)	2.9 +/- 1.2 (1-4)**	3.7 +/- .5 (3-4)
earlier adulthood (20-39)	2.8 +/- 1.0 (1-4)	3.1 +/- .8 (2-4)
mid adulthood (40-59)	2.7 +/- 1.0 (1-4)	2.8 +/- .7 (1-4)[2]
later adulthood (over age 60)	2.7 +/- 1.0 (1-4)	-
past two years specifically	2.8 +/- .9 (1-4)	3.0 +/- .8 (1-4)
Lifetime milk consumption[1]	2.8 +/- .8 (1.0-4.0)**	3.4 +/- .5 (2.0-4.0)
Current milk consumption (Glasses of milk/day)	1.4 +/-1.1 (0-3)	1.5 +/- 1.0 (0-3)

** p ≤. 01

[a] paired t-test

[1] on a scale from 1 to 4 (4=with most meals 3=frequently 2=sometimes 1=rarely or never)

daughters (r=.57, p<.01). Current milk consumption, lifetime physical activity and the "internal" score in the Multidimensional Health Locus of Control Scales were not correlated between mothers and their daughters.

DISCUSSION

MILK CONSUMPTION AND ITS RELATION TO BONE MINERAL DENSITY

Mothers:

We did not observe any correlations between lifetime milk consumption, milk consumption at specific life stages, current milk consumption (estimated by beverage frequency), or score on the calcium food frequency questionnaire with total, axial, peripheral or relative BMD in mothers and therefore could not confirm our hypothesis. Sandler et al (11) and Cauley et al (27) reported finding a relationship between radial (peripheral skeleton) postmenopausal BMD and milk consumption in childhood and adolescence. These researchers excluded women undergoing estrogen replacement therapy from their study. Our study included mothers who were or were not undergoing estrogen replacement therapy and our results might have been confounded by this factor. Our study did not assess BMD at any specific site, but focused, rather, on total body, axial and peripheral skeleton. We also measured BMD with a dual energy X-ray bone densitometer, which is a more precise instrument than quantitative computed tomography used by Sandler (11) and Cauley (27).

Calcium supplementation over age 60 (in mg), however, was positively related to total, peripheral and relative BMD among our postmenopausal mothers. Eighty-four percent of the mothers reported having taken calcium containing supplements during their life. Nineteen mothers (76%) took in average 388 mg calcium intake per day when they were over age 60. This result suggests that calcium did have a measurable influence on BMD, possibly through decreased bone loss during the mothers' postmenopausal years. Intervention

studies have shown similar relationships between postmenopausal calcium supplementation and BMD (19, 22, 26).

Calcium supplementation after menopause has been shown to be especially effective in populations with previously low calcium intake (26, 35). In this study, mothers taking higher calcium supplements also tended to have lower lifetime milk consumption. This implies that calcium supplementation later in life may play an important role in bone density, independent of lifetime calcium intake. Since complete dietary records were not kept by the mothers, their current reported milk consumption could not be validated. More detailed information about their current calcium intake might have been helpful in accounting for calcium supplementation, and evaluating its relative importance.

Sandler et al (11) and Cauley et al (27), who reported a positive correlation between milk consumption in childhood and adolescence and radial BMD in postmenopausal women, investigated samples of significantly younger women than ours (mean age=57 yrs and 58 yrs, respectively). The mothers in our study were at least 9 years postmenopausal, and an average of 72 years old. Heterogeneity of the sample increases with increasing age, due to an ever increasing number of lifetime influences on BMD. This was evidenced by our observation of greater variability in bone variables among the elderly mothers than among their daughters. Furthermore, we observed a strong impact of other factors on mothers' bone densities (weight, age, estrogen replacement therapy, former birth control pill use), which might have overshadowed effects of lifetime milk consumption.

Daughters:

We did not observe any correlations between lifetime milk consumption, milk consumption at specific life stages, current milk consumption (estimated by

beverage frequency), score on the calcium food frequency questionnaire, or current calcium intake (estimated by dietary records), or intake of calcium supplements with BMD in daughters. These results were unexpected and are not consistent with those of Halioua and Anderson (13) who found correlations between lifetime calcium intake, estimated with questions based on a quantitative food frequency questionnaire, and radial BMD among 181 premenopausal women. Again, our study did not focus on one specific site, but investigated the total, axial and peripheral skeleton. Findings of studies which measured BMD only at specific sites are therefore not necessarily parallel to ours. A limitation of our study was also the relatively small sample size. Our findings regarding current calcium intake are consistent with those of other authors who failed to detect any correlation between current calcium intake and BMD (14, 20).

A possible explanation for the lack of any association between milk consumption/calcium intake and bone variables among daughters is a lack of variation in calcium intake among the daughters. We observed little variation in levels of milk consumption during childhood and adolescence among daughters. All except one daughter reported having at least three glasses of milk daily during her childhood, and 18 of 25 continued this behavior throughout their teen years. None of the daughters reported milk consumption of less than one glass per day during childhood and adolescence. For ages 20-39, only six daughters indicated having drunk milk less frequently than once per day, and none reported having "rarely or never" drunk milk. Eighty percent of the daughters reported milk consumption of at least one glass per day during the past two years. This reported high level of milk consumption was also reflected in the daughters' three-day food records. The mean intake of 1032 mg calcium per day (range 332 to 1656 mg/day) is substantially higher than the average intake of calcium in this age group in the United States (women age 19-34: 661 mg Ca/day;

age 35-50: 579 mg Ca/day) (159). It is also substantially higher than the 1989 RDA for calcium (800 mg for women over age 25). Higher daily calcium intakes have been reported for women living in the Western part of the United States (women age 19-50: 676 mg Ca/day) than for women living in other parts. Seventy-six percent of the daughters met the RDA for calcium for their age, and only two daughters had calcium intakes of less than 75% than the RDA. It is possible that the consistently high calcium intakes among daughters did not provide a basis on which to discriminate between their bone densities, which at their ages probably represent peak bone mass. Kanders et al (9) suggest that in young women a threshold effect for calcium intake exists. In their study they observed no significant increase in vertebral BMD with calcium intakes higher than 800-1000 mg per day. Our daughters' mean total BMD was more than 100% of that of similar aged women (variable 'relative BMD'). This indicates that their peak bone mass may reflect uniformly adequate calcium intake.

PHYSICAL ACTIVITY AND ITS RELATION TO BONE MINERAL DENSITY

Mothers:

Among mothers, only one physical activity variable was associated with bone parameters. Those reporting higher levels of total exercise (weight-bearing + non-weight-bearing) during their teens had higher total and peripheral BMD than those reporting less exercise at this time of life. This relationship, however, disappeared after adjusting for age. Older mothers had lower BMD and also reported less exercise during their teens than younger mothers. This might be explained by organized school-sports being less popular and common during youth of the older mothers. A possible explanation for the lack of association between measures of lifetime physical activity, or physical activity during other

specific time periods with BMD in mothers, could be that recalled information provided by the elderly women was inaccurate. Furthermore, we observed a strong impact from other factors on mothers' bone densities (weight, estrogen replacement therapy, former birth control pill use), which might have overshadowed effects of physical activity.

Daughters:

Among daughters we observed several correlations between measurements of lifetime physical activity and BMD which confirmed our hypothesis. Weight-bearing exercise and total weight-bearing activity were the physical activity variables most correlated with total, axial and peripheral, as well as relative BMD. For two of the three BMD measurements associated with lifetime physical activity (total BMD and relative BMD), total weight-bearing activity was a stronger predictor than weight-bearing exercise. These results indicate that a measure of physical activity which includes non-exercise physical activity may be a more precise approach to assessing physical activity which affects BMD among premenopausal women than the assessment of exercise-related activity only. This more comprehensive way of describing lifetime physical activity retrospectively among middle-aged women is especially important for recalling the child-bearing and child-raising years, when exercise-related activities often contribute only a small proportion to the overall level of physical activity in some individuals.

Correlations between lifetime total exercise, which includes weight-bearing and non-weight-bearing activities, and the bone variables of total, axial and peripheral BMD, were consistently lower than those observed between weight-bearing exercise or total weight-bearing activity and BMD. Only one significant correlation, a positive one between total exercise and relative BMD

was observed. These findings are consistent with those of Sinaki et al (56), and suggest that weight-bearing activity is the major component of physical activity determining bone parameters. Other studies have shown that non-weight-bearing muscle building activity, mostly on exercise machines, increased lumbar spine BMD (19, 41). It is possible, that some non-weight-bearing activities reported by our sample (e.g. biking, horseback riding, in which a sitting position is held) increased lumbar spine BMD (part of the axial skeleton) in these subjects at a certain point in time; however, that due to the high rate of bone remodeling typical for trabecular bone, these effects might have been discounted over time. Other bone areas contributing to axial BMD, like the rib cage or pelvis, experience different mechanical stress from physical activity than the spine. The combination of these different sites into one variable might have weakened a relationship between axial BMD and non-weight-bearing activity.

Non-weight-bearing muscle-building activity on machines has also been associated with greater radial BMD (151), which suggests that mechanical stress on the forearm results in increased BMD at this specific site. In our study, peripheral BMD was defined as BMD of the upper and lower extremities. Legs' BMD was proportionally stronger reflected in peripheral BMD than BMD of the upper extremities. Therefore, the effects of non-weight-bearing activity on peripheral bone of the upper body are less likely to be detected with our measure of peripheral BMD than weight-bearing activities (like walking), which affect BMD of the lower body.

Correlation coefficients between perceived lifetime level of activity and bone parameters, although not significant, were almost as high as those between weight-bearing exercise and bone parameters among daughters. This suggests that this method of asking a simple question might be a worthy method for large studies. The relatively "objective" method of estimating lifetime levels of

physical activity by evaluating hours per week spent on each activity was a more valuable approach for explaining differences in BMD than the subjective method of asking subjects how they themselves perceived their level of physical activity during each age period. A useful approach for assessing historical physical activity in studies involving large samples might be to include a qualitative question about the types of activities performed and a question about perceived level of physical activity.

Overall, our results for daughters suggest that lifetime physical activity is an important factor influencing bone mineral density in premenopausal women. The relatively lower impact on axial BMD, where no significant correlations were observed with any variable of lifetime physical activity, indicates that lifetime total weight-bearing activity is more important in exerting "stress" on the peripheral skeleton, predominantly on the large cortical bone areas of the legs (e.g. femur), than non-weight-bearing activity. Slemenda et al (40) investigated physical activity of children between 5 and 14 years old and found that hours of weight-bearing physical activity per week were correlated with BMD at a peripheral site (radius) but not with BMD of the lumbar spine, which is part of the axial skeleton.

COMBINED EFFECT OF LIFETIME MILK CONSUMPTION AND PHYSICAL ACTIVITY ON BONE DENSITY

This study was the first to examine lifetime milk consumption and lifetime physical activity among elderly mothers and their premenopausal daughters. The lack of associations between lifetime milk consumption and lifetime physical activity among mothers may be partly explained by the high age of the study group (mean age 72 years), resulting in bone artifacts like scoliosis especially in

the axial skeleton; by limitations inherent in data on lifetime milk consumption; and by difficulty recalling lifetime physical activity for mothers. Since neither lifetime milk consumption nor physical activity alone were correlated with bone parameters in mothers, it is not surprising that no combined effect of these factors on BMD could be observed.

We did not observe a combined effect of lifetime milk consumption and lifetime physical activity on BMD of premenopausal daughters. These results are in contrast with the findings of Halioua and Anderson (13) who reported a combined effect of lifetime milk consumption and lifetime physical activity on radial BMD in premenopausal women. Our lack of association among the daughters may have been due in part to bias in our sample with respect to milk consumption.

RELATIONSHIP BETWEEN HEALTH ATTITUDES AND BONE VARIABLES, MILK CONSUMPTION, AND PHYSICAL ACTIVITY

The Multidimensional Health Locus of Control Scales (MHLC) was used as a tool to evaluate to what degree subjects thought that they had "internal" control over their health, i.e. control through their own behavior. There was no correlation between the "internal" score in the MHLC and total, axial, peripheral or relative BMD among mothers or daughters. Furthermore, we did not observe any relationship between the internal score in the MHLC and the health behaviors lifetime milk consumption, current milk consumption, or lifetime physical activity. A possible explanation for the rejection of our hypothesis is that our study group was quite homogeneous with respect to their Health Locus of control results. They were uniformly "Internal" in outlook and almost all reported relatively high internal scores (mean in mothers: 27+/- 5; mean in

daughters: 28+/-4). It is also possible that level of milk consumption and physical activity are health behaviors which are not greatly influenced by health attitudes as measured by this scale.

OTHER FACTORS CORRELATED WITH BMD IN MOTHERS AND DAUGHTERS AND MULTIPLE REGRESSION MODELS FOR BMD

Using stepwise multiple regression analysis allowed us to create separate models for the determinants of total, axial and peripheral, as well as relative BMD in mothers and in daughters. A large number of variables were tested in the analysis, but a much smaller number consistently contributed to the significant model.

Mothers:

Among mothers, variables entering the multiple regression equation explained a surprisingly high proportion of the variability in BMD. Factors significant in almost all models describing mothers' BMD were their body weight, estrogen replacement therapy and former birth control pill intake. Age was correlated with estrogen replacement therapy and therefore did not enter the equations separately. For total and peripheral, as well as relative BMD, calcium supplementation over age 60 also had a small but significant role in the model.

The predominant role of body weight in describing postmenopausal bone parameters has been observed by other authors as well (82-84). The relative importance of total body fat mass which we observed among our mothers is consistent with the findings of Reid et al (73). This might be due to the weight-bearing effect of fat mass on the skeleton as well as estrogen production in adipocytes. Higher serum estrogen levels are associated with higher BMD (27,

74). However, Reid et al (73), who investigated also serum estrogen levels and total body lean mass, question the above explanations for the strong influence of fat mass on BMD.

The positive effect of estrogen replacement therapy on BMD of postmenopausal women has been shown by many authors. In our sample, estrogen replacement therapy interrupted the usual strong negative correlation between age and bone mass. Since age and estrogen replacement therapy were correlated in our mothers, their effects could not be separated. Our finding regarding former birth control pill intake was interesting. Its impact was highly significant, even though only three mothers were former birth control pill users and this small sample size should be considered when interpreting these data. It is likely that the dosage of oral contraceptives was much higher during the periods when our postmenopausal mothers took them, and that therefore our results might not apply to current use of oral contraceptives during late adulthood years. Similar observations regarding the influence of former birth control pill intake on postmenopausal BMD were made by Stevenson et al (82).

In some equations of mothers' BMD, body mass index was a significant contributor to bone parameters, even when body weight had already entered the model. This stresses the major importance of "frame size" in describing BMD in elderly women. Models for axial BMD should be interpreted with caution, because of the alterations of these variables due to artifacts like scoliosis, calcification, and fractures in the lumbar spine among some mothers.

Daughters:

Among daughters, many fewer variables, frequently only one, entered the model for BMD. The strongest determinant of total, peripheral and relative BMD in daughters was lifetime physical activity, which explained up to 25% of the

variability. For axial BMD, total lean mass was a better predictor of bone mass than lifetime physical activity measurements. Since total lean mass (in grams) was significantly correlated with levels of lifetime physical activity, this value represented both body weight and level of physical activity. This explains why measures of lifetime physical activity were usually not related to BMD after adjusting for total lean mass.

MILK CONSUMPTION OVER THE LIFETIME

Lifetime milk consumption among both mothers and daughters was highly correlated with their current milk consumption, as estimated by beverage frequency. Sandler et al (11) reported similar correlations between lifetime milk consumption and current calcium intake (estimated by three day food records). This observation indicates the importance of early milk drinking patterns for establishing a lifelong pattern. Women who drank less milk during childhood and adolescence and who may not have had an adequate calcium intake during their youth were less likely to have adequate dietary calcium intake later in life, and might therefore be at increased risk for osteoporosis. Preventive measures could aim at a high level of milk consumption during childhood in order to create a lifetime habit.

RELATIONSHIPS BETWEEN MOTHER/DAUGHTER PAIRS

After controlling for major factors influencing mothers' bone parameters (weight, age, estrogen replacement therapy, former birth control pill intake) and factors having impact on daughters' bone parameters (lifetime physical activity), we observed significant correlations between mothers' and daughters' bone density for peripheral bone. Peripheral bone, which consists of primarily cortical

bone, has a significantly lower rate of bone metabolism than does axial bone. Axial bone is prone to short-term adaptations in bone mineral. Therefore, we would expect that genetic similarities are maintained throughout life to a higher degree in peripheral bone than in axial bone. Gillis et al (160) observed similarities in the mother-daughter pairs of our study with respect to femoral BMD (cortical:trabecular bone = 60:40) but not in lumbar spine BMD (axial).

Among a proportion of mothers in our study, spinal artifacts (axial skeleton), whose prevalence usually increases with age, were observed. Artifacts like scoliosis, osteoarthritis, lumbar fusions and spinal crush or wedge fractures alter the results of BMD and BMC measurements (118). Calcification of tissue might occur as well as difficulties in separating lumbar vertebrae. These artifacts might also have decreased similarities between axial BMD of mothers and their daughters.

Levels of lifetime milk consumption and scores on the food frequency questionnaire were positively correlated for mother/daughter pairs. These results suggest that a significant carryover effect exists between mothers and their daughters regarding milk consumption. Other health behaviors, like current or lifetime physical activity, were not correlated among the pairs. These variables may be determined to a greater degree by the respective life style and life situation of each person.

METHODOLOGICAL OBSERVATIONS

All physical activity data were retrospective so there was no means of evaluating their validity through an alternative form of measurement. Nevertheless, our approach of assessing, by personal interview, the duration, the frequency and ages during which a specific activity was performed, seemed to

result in more complete information than is usually obtained by a questionnaire. An average of 30 minutes were spent for review and discussion of lifetime physical activity with each subject. This procedure allowed detailed questioning and some resolution of reporting inconsistencies. The intense interaction between interviewer and study subject also resulted in additional information being remembered during the course of the interview. Although questions were open-ended, the same initiating questions were asked each subject (physical education classes at school, walking to school or school bus, having a dog, having a garden).

A questionnaire designed for the assessment of historical physical activity by Kriska et al (50) is not open-ended, but lists certain activities, typical for the investigated population, and asks for hours spent in a certain type of physical activity during a specific age period. This approach has the advantages of higher reproducibility and recall of all activities listed. One of its limitations is that activities performed other than those listed are less likely to be recalled. Another disadvantage might be that a specific format can result in over-simplification of exercise description. In our study, very individual and complex patterns of lifetime physical activity, which might not have fit into a close-ended questionnaire, were recorded for study subjects. Subjects frequently reported performing certain activities for a number of years, then interrupting them because of childbirth, and resuming the activity again later, with a different intensity. These subtle changes are likely to be lost by using a closed-ended questionnaire, resulting in loss of information. It is also likely that a long questionnaire, which this kind tends to be, leads to inaccuracies because of attention loss. The same could apply to a long interview session; however, in this study, almost all participating women were very interested and cooperative.

There was a qualitative distinction made between measures of physical activity for several reasons: Weight-bearing and non-weight-bearing physical activity have been shown to affect different skeletal sites differently. No one has undertaken the approach of investigating the sum of axial and peripheral bone sites, so it was not known what effects of physical activity on BMD could be detected by this method. It was therefore decided that weight-bearing and non-weight-bearing exercise should be assessed separately. Further distinction was made between information from exercise-related physical activity, which could be quantitatively assessed, and non-exercise physical activity, which was based on impressions of household, job and other physical activity.

The fourth variable of physical activity was the self-perceived level of physical activity during a certain age period. This approach was chosen for two reasons: The first one was that the combination of quantitative assessment of hours of exercise per week and additional assessment of job- and household related physical activity may not sufficiently describe an individual's pattern of physical activity. Some individuals are more vigorous than others, and the same hours of activities like school sports might need to be evaluated differently for different individuals. The subject herself may have a relevant subjective perception of her activity level during different age periods, different from the assessment as exercise hours and job- and household-related physical activity.

The second reason is that in epidemiological studies involving large numbers of study subjects, the approach of personal interviewing might be too time-consuming or not feasible. Therefore, we wanted to investigate the degree to which there was agreement between the subjectively perceived level of physical activity and the more objective estimate by hours/week physical activity. The lifetime variable of perceived level of physical activity was strongly correlated with levels of lifetime total exercise, and tended to be correlated with

lifetime weight-bearing exercise and lifetime total weight-bearing activity. A limitation of our subjective approach was that the study subjects answered the question about their perceived level of physical activity after a recall of the types of physical activity they had reported for a certain age period. Our results suggest that the perceived level of physical activity was a less valuable predictor of BMD in premenopausal women than the more objective method of collecting specific information. However, the correlation coefficients observed between perceived lifetime level of physical activity and levels of lifetime total exercise and the relatively high correlation coefficients between perceived lifetime level of physical activity and BMD suggest that this measure might be a valuable tool in studies involving large samples. If similar correlation coefficients (range between .31 and .35) between perceived lifetime level of physical activity and BMD were observed in a large sample, these results would likely reach statistical significance.

GENERALIZABILITY OF RESULTS TO TOTAL POPULATION

Since this study was not population based, generalizability of the results is limited. Our study sample of 25 women in each of two groups is too small and specialized to generalize to the general population. The procedure of screening for thyroid and asthma medication, smoking and pregnancy, additionally reduced variability among subjects. Only women who were mobile and able to come to the university for the measurements participated. This excluded all elderly women who were too ill or too frail to come to the university. Finally, our study sample consisted of volunteers, which inevitably introduces selection bias. Women participating in this study were very well educated (46% of the mothers and 72% of the daughters held a college degree) and interested in the

topic of osteoporosis. Therefore, it is likely that this study attracted women who were more health conscious and concerned about bone density, and who possibly had a higher level of physical activity and milk consumption than the average population.

PROSPECTIVE FUTURE RESEARCH

Future research should be directed towards the development of a valid and accurate tool for assessment of lifetime physical activity, especially among elderly individuals. Accordingly, a valid tool for the assessment of lifetime calcium intake is needed. It might also be productive to investigate the effect of oral contraceptive use during late premenopausal years on bone mineral density. More research needs to be conducted in order to establish the influence of lifetime calcium intake on bone mineral density in premenopausal and postmenopausal years and to identify the level of dietary calcium intake, which is essential for achieving and maintaining optimal bone mass.

SUMMARY AND CONCLUSIONS

This study investigated the effect of lifetime and current milk consumption and of lifetime physical activity on total, axial and peripheral bone mineral density in 25 elderly women and their premenopausal daughters. We also investigated the extent to which the bone mass of mothers and their daughters was correlated. Furthermore, we investigated whether health attitudes were related to bone mineral density, current or lifetime milk consumption, or physical activity. Other factors assessed for their effect on bone density were anthropometric variables, hormonal and reproductive variables and behavioral variables. Bone mineral density (BMD) of the total, axial and peripheral skeleton was measured by dual energy X-ray absorptiometry. Lifetime milk consumption and lifetime physical activity were assessed retrospectively by questionnaire and interview.

Few studies to date have assessed total BMD and none have evaluated BMD of the axial skeleton as a unit and BMD of the peripheral skeleton as a unit. Lifetime calcium intake and lifetime physical activity patterns have been estimated only in a few studies. Our study was the first to examine lifetime calcium intake and lifetime physical activity among elderly mothers and their premenopausal daughters. The age group chosen (mothers >64 years) is the oldest ever investigated for effects of lifetime milk consumption and lifetime physical activity, and the oldest one investigated in intergenerational comparisons of bone densities.

Our study results provide important information about the influence of lifetime milk consumption and lifetime physical activity on bone mineral density. A state-of-the-art measuring technique allowed the accurate assessment of total, axial and peripheral skeleton, and an intensive,

interactive interview, provided detailed information about lifetime physical activity.

For mothers, the lack of association between lifetime milk consumption or lifetime physical activity and bone variables can be attributed partly to the strong impact of other factors, like body weight and estrogen replacement therapy, on mothers' bone mass; partly to limited recall ability due to the age of our sample; and partly to the heterogeneity of any population in this age range. We did detect, however, a positive relationship between bone density and calcium supplementation over age 60, which suggests that calcium intake plays a role in maintaining or increasing bone mass in postmenopausal years. Multiple regression models including anthropometric variables, lifetime and current milk consumption, age, reproductive variables and certain medication, often explained more than 80% of the variation in mothers' BMD. We suggest that anthropometric parameters like body weight or body mass index, as well as estrogen from exogenous sources, have a predominant influence on bone mass during postmenopausal years.

For the premenopausal daughters in this study, levels of lifetime total weight-bearing activity or weight-bearing exercise were strongly associated with BMD of the total skeleton, and especially the peripheral skeleton. Up to 25% of the variation in BMD in our premenopausal women could be explained by levels of lifetime physical activity. This emphasizes the importance of an overall high activity level throughout all life stages for bone mineral density in premenopausal women. For the daughters in this study weight-bearing activity was more strongly associated with bone mineral density than non-weight-bearing activity. These results contribute valuable information about the kind of physical activity which is likely to positively affect BMD of the total body or peripheral skeleton.

In addition, we showed that non-exercise related physical activities, which are not necessarily of high intensity, were positively related to bone mineral density in the premenopausal years. These results suggest that bone mineral density can be positively influenced by an overall high level of physical activity which does not necessarily include a large proportion of high-intensity exercise. Specifically, among women of childbearing and childrearing age, non-exercise physical activities contributed a significant proportion to total physical activity. This finding should be taken into consideration in future studies assessing physical activity and bone densities among women. Our study provides support for recommendations to increase physical activity as a measure for osteoporosis prevention. Exercise itself, as well as its role in increasing lean body mass, appeared to positively affect BMD in premenopausal women.

The lack of association between lifetime milk consumption and bone variables among the daughters in this sample can be explained partly by a uniformly very high calcium intake among the daughters.

Familial resemblance between mothers' and daughters' BMD was only observed after adjusting for several factors influencing mothers' and daughters' bone mineral density. This again emphasizes the importance of external factors for describing contributors to BMD among pre- and postmenopausal women.

We showed, that high current milk consumption was associated with a lifelong dietary pattern of high milk consumption in mothers as well as in daughters. Milk is the predominant source of calcium among women, and in order to ensure adequate intake of this mineral throughout life, more stress should be laid on childhood and adolescent milk consumption in order to form an early dietary habit. Our finding of high similarity between mothers'

and daughters' lifetime milk consumption shows the importance of maternal influence on the diet of adult women.

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APPENDICES

APPENDIX A

Correlations between bone variables
in mothers and daughters

Tables 27-30

TABLE 27. Correlations between bone variables in mothers (all mothers)
(n=25)

Pearson Correlation Coefficients for bone variables:

	Total BMDensity (g/cm^2)	Axial BMDensity[1] (g/cm^2)	Peripheral BMDensity (g/cm^2)	Relative BMDensity (%of mean)	Total BMContent[1] (g)	Axial BMContent[1] (g)
Total Bone Mineral Density (g/cm^2)	1.00	** all correlations p≤.01				
Axial Bone Mineral Density (g/cm^2)[1]	.85	1.00				
Peripheral Bone Mineral Density (g/cm^2)	.96	.82	1.00			
Relative Bone Mineral Density (% of mean)	.99	.86	.92	1.00		
Total Bone Mineral Content (g)[1]	.91	.72	.88	.89	1.00	
Axial Bone Mineral Content (g)[1]	.86	.72	.82	.86	.98	1.00
Peripheral Bone Mineral Content (g)	.91	.76	.93	.87	.96	.91

[1] n=24

TABLE 28. Correlations between bone variables in mothers (non-estrogen users, mean age=69+/-4 yrs) (n=15)

Pearson Correlation Coefficients for bone variables:

	Total BMDensity (g/cm^2)	Axial BMDensity (g/cm^2)	Peripheral BMDensity (g/cm^2)	Relative BMDensity (% of mean)	Total BMContent[2] (g)	Axial BMContent[2] (g)
Total Bone Mineral Density (g/cm^2)	1.00	** all correlations p≤.01, except [1]				
Axial Bone Mineral Density (g/cm^2)[2]	.80	1.00				
Peripheral Bone Mineral Density (g/cm^2)	.97	.82	1.00			
Relative Bone Mineral Density (% of mean)	.99	.82	.96	1.00		
Total Bone Mineral Content (g)[2]	.87	.60[1]	.83	88	1.00	
Axial Bone Mineral Content (g)[2]	.81	.60[1]	.77	.84	98	1.00
Peripheral Bone Mineral Content (g)	.93	.72	.92	.92	.96	.90

[1] p≤.05
[2] n=14

TABLE 29. Correlations between bone variables in mothers (current estrogen users, mean age =74 +/-4 yrs) (n=10)

Pearson Correlation Coefficients for bone variables:

	Total BMDensity (g/cm^2)	Axial BMDensity (g/cm^2)	Peripheral BMDensity (g/cm^2)	Relative BMDensity (% of mean)	Total BMContent (g)	Axial BMContent (g)
Total Bone Mineral Density (g/cm^2)	1.00	** all correlations p≤.01, except [1]				
Axial Bone Mineral Density (g/cm^2)	.89	1.00				
Peripheral Bone Mineral Density (g/cm^2)	.94	.70[1]	1.00			
Relative Bone Mineral Density (% of mean)	.99	.89	.91	1.00		
Total Bone Mineral Content (g)	.91	.81	.89	.87	1.00	
Axial Bone Mineral Content (g)	.86	.81	.83	.83	.98	1.00
Peripheral Bone Mineral Content (g)	.83	.69[1]	.86	.77	.96	.89

[1] p≤.05

TABLE 30. Correlations between bone variables in daughters (n=25)

Pearson Correlation Coefficients for bone variables:

	Total BMDensity (g/cm^2)	Axial BMDensity (g/cm^2)	Peripheral BMDensity (g/cm^2)	Relative BMDensity (% of mean)	Total BMContent (g)	Axial BMContent (g)
Total Bone Mineral Density (g/cm^2)	1.00	** all correlations are significant at the .01 level				
Axial Bone Mineral Density (g/cm^2)	.91	1.00				
Peripheral Bone Mineral Density (g/cm^2)	.93	.84	1.00			
Relative Bone Mineral Density (% of mean)	.99	.89	.92	1.00		
Total Bone Mineral Content (g)	.79	.78	.74	.77	1.00	
Axial Bone Mineral Content (g)	.72	.82	.71	.69	.90	1.00
Peripheral Bone Mineral Content (g)	.66	.63	.70	.65	.92	.75

APPENDIX B

Application for Approval of the
Human Subjects Board

Application for Approval of the Human Subjects Board

Principal investigator: Constance Georgiou, Assistant Professor

Department: Nutrition and Food Management   Phone: 737-0965

Joint investigator: Christine Snow-Harter, Assistant Professor

Department: Exercise and Sport Science   Phone: 737-6788

Present or Proposed Source of Funding:
Dr. Georgiou: OSU Research Council:
"The exercise, diet and fitness connection; does it apply for elderly women?"
Dr. Snow-Harter: Medical Research Foundation

Project title: Comparison of Bone Mineral Densities, Muscle Strength,
        Lifetime Calcium Intake and Physical Activity in
        Mother/Daughter Pairs.

This project is a joint project between the Dept. of Nutrition and Food
Management and the Dept. of Exercise and Sport Science.
**The bone mineral density, muscle strength, and nutrition aspects of the study
have already been approved separately by the Human Subjects Committee as:
1) Bone mineral density in apparently healthy men and women (12/6/91)
2) The relationship between bone density and muscle strength (4/19/91)
3) The exercise, diet, and fitness connection: Does it apply for elderly women?
(5/2/91).**
Attached you will find a copy of the application forms for the studies regarding
muscle strength and bone densities, including the informed consent documents,
which will also be used in the current study.

Significance of this project:

Osteoporosis is a bone disease characterized by fractures of the vertebrae, wrist
and proximal femur (hip). The disease affects men and women, but is more
prevalent in women, afflicting one in four women over the age of 60 in the
United States. Osteoporosis is caused by a number of factors which include
genetics, reproductive hormone levels, calcium intake and physical activity.
        This research will contribute toward quantifying to what extent lifetime
calcium intake, physical activity, muscle strength and health beliefs influence
bone mineral densities in premenopausal and postmenopausal women. The
comparison between mother/daughter pairs will provide data about the genetic
influence on bone densities. An additional aspect of this study will be evaluation
of the extent to which mothers influence their daughters in terms of health and
diet habits. The study will provide information about some of the physical and
behavioral risk factors for developing osteoporosis.

<u>Methods and procedures</u>:

Assessment of current diet adequacy will be estimated from subjects' self-recorded, 3-day food intake records. Health beliefs will be measured by the Multidimensional Health Locus of Control Scale (attached). Data about lifetime calcium intake, lifetime physical activity, medication and other nutrition- and bone health-related data will be obtained from a 9-page questionnaire (attached). The daughters' questionnaire includes all items which are in the mothers' questionnaire plus some additional ones. Weight, height, hip and waist circumference will be used as indirect estimates of body composition. Body composition and bone mineral densities will be measured by dual-energy x-ray absorptiometry (Hologic QDR 1000, Waltham, MA). The two methods will be compared.

Detailed descriptions for the bone density and muscle strength measurements are listed in the approved Human Subject Committee applications of both studies (attached).

<u>Risks, Benefits</u>:

The risks of bone density and muscle strength measurements are listed in detail in the approved applications of both studies (attached).
There are no known risks involved in the nutritional part of the study or in the measurement of weight, height, hip and waist circumference.

The benefits to subjects will be finding out the nutrient content of their usual diets, their body composition, their bone mineral density and muscle strength compared to others their age. The average cost of bone density assessment is $250-300 and a body composition is $20.

<u>Description of the study population</u>:

The study population will consist of 25 mother/daughter pairs, who volunteer to participate in the study. Mothers will be a minimum of 65 years old, so all daughters will be adults. Only women will be studied since costs will limit the sample size, and since bone health is a topic of major concern for females. Potential subjects will be screened by telephone. Those who currently take thyroid or asthma medication as well as current smokers will be excluded from the study because of the influence of these factors on bone densities. An appointment will be scheduled in a time period during which potential pregnancy of the subject can be excluded. Pregnancy is the only contraindication against bone density measurement.

For further details regarding the bone density and muscle strength measurements see attached copies of the approved applications of both studies.

<u>Anonymity of Subjects:</u>
Screening forms will have the person's name only on a front tear-off sheet. This sheet will be discarded as soon as a number is assigned to the subject. All records of subjects during the study will be connected to their identification numbers and associated with their names only in one secured place for verification purposes.

APPENDIX C

Informed Consent Documents

(Mothers/Daughters)

OREGON STATE UNIVERSITY
DEPT. OF NUTRITION AND FOOD MANAGEMENT

Informed Consent for: body composition measurements
and health and nutrition questionnaire

Thank you for volunteering to participate in a nutrition and bone health research study at Oregon State University. The purpose of this study is to investigate relationships between nutrition, physical activity and bone health. Please read the following agreement carefully and sign it indicating your informed consent to participate in the study.

AGREEMENT:

I agree to complete a confidential questionnaire regarding my diet, activity and health which will be sent to me by mail. To review and clarify my questionnaire I will meet with a nutritionist at OSU.
The meeting will take place in conjunction with measurements of my weight, height, waist and hip circumference, bone mineral density and muscle strength. Therefore, I will be required to come to OSU one time only for approximately 3 hours.

There are separate consent forms for bone mineral density and muscle strength measurements, which I will receive at the lab. I agree to come, by appointment, to the OSU Dept. of Exercise and Sports Science, Women's Building. During approximately 3 hours I will participate in the above mentioned measurements. The appointment will be scheduled during a time period in which pregnancy, the only contraindication against bone density measurements, can be excluded. If my medical records contraindicate muscle strength measurements, this measurement will not be taken.

All of the personal, diet, activity and health information I provide, and all data from the physical measurements taken will be held in strictest confidence. I understand that I will be treated with respect for my privacy during the study. I agree to answer the questions about my age and personal health requested on my Confidential Information Form and I am aware that some people find this stressful.

At the end of the study I will receive the results of my body composition, and my muscle strength. Any questions regarding my bone mineral density report should be directed to my physician.

If I have any questions regarding any part of the study I am welcome to ask Dr. Georgiou (Tel 737-0965) or Dr. Snow-Harter (Tel: 737-6788).
I understand that I can withdraw from the study at any time, but my signature indicates my intention, at this time, to complete all parts of the study.

_____   _____

Participant's signature   Date

_____   _____

Project Leader's signature   Date

OREGON STATE UNIVERSITY
DEPT. OF NUTRITION AND FOOD MANAGEMENT

Informed Consent for: dietary records, body composition measurements and health and nutrition questionnaire

Thank you for volunteering to participate in the Mother/Daughter bone density, diet, and activity study at Oregon State University. The purpose of this study is to investigate relationships between nutrition, physical activity and bone health. Please read the following agreement carefully and sign it indicating your informed consent to participate in the study.

AGREEMENT:

I agree to complete a confidential questionnaire regarding my diet, activity, and health which will be sent to me by mail. To review and clarify my questionnaire I will meet with a nutritionist at OSU. The meeting with the nutritionist will take place in conjunction with measurements of my weight, height, waist and hip circumference, bone mineral density and muscle strength. Therefore, I will be required to come to OSU one time only for approximately 3 hours.

There are separate consent forms for bone mineral density and muscle strength measurements, which I will receive at the lab. I agree to come, by appointment, to the OSU Dept. of Exercise and Sports Science, Women's Building. During approximately 3 hours I will participate in the above mentioned measurements. The appointment will be scheduled during a time period in which pregnancy, the only contraindication against bone density measurements, can be excluded. If my medical records contraindicate muscle strength measurements, this measurement will not be taken.

The nutritionist will also instruct me in how to keep 3-day food records. I will keep a record of everything I eat and drink during the assigned days and will continue to follow my usual eating habits during these 3 days. I will return the records by mail in the envelope provided as soon as they are completed. The nutritionist will telephone me for any clarification needed.

All of the personal, diet, activity and health information I provide, and all data from the physical measurements taken will be held in strictest confidence. I understand that I will be treated with respect for my privacy during the study. I agree to answer the questions about my age and personal health requested on my Confidential Information Form and I am aware that some people find this stressful.

At the end of the study I will receive a summary and explanation of the nutrient content of my usual diet, my body composition, and my muscle strength. Any questions regarding my bone mineral density report should be directed to my physician.

If I have any questions regarding any part of the study I am welcome to ask Dr. Georgiou (Tel 737-0965) or Dr. Snow-Harter (Tel: 737-6788).
I understand that I can withdraw from the study at any time, but my signature indicates my intention, at this time, to complete all parts of the study.

Participant's signature   Date

Project Leader's signature   Date

APPENDIX D

Questionnaire Mothers

TEAR SHEET

This cover sheet is the only reference to your name and will be discarded upon receipt. Your participant identification number only will be used on all research materials.

_____
Number of subject

_____
Last name   First name   Middle

_____
Address. street

_____
City, State

Phone: _____   _____
          work   home

MOTHERS - 2

<div style="text-align:right">

_____

Number of subject
</div>

NUTRITION AND HEALTH QUESTIONNAIRE

Basic questions

1. Date of birth   ____ / ____ / ____
                            month day year

2. Your physician's name: _____
                            name   city

Alcohol consumption

3. Do you drink alcohol regularly ?   1 ___ No
                                       2 ___ Yes

3a. If yes, how many drinks/week?   ____

Smoking

4. Do you or did you ever smoke cigarettes?   1 ___ No
                                               2 ___ Yes

4a. If yes, at which ages?   from age_____ to age_____ how many cig./day_____

Nutritional supplements

5. Do you currently take any calcium. bone meal. VitD or other nutritional supplements
    to influence bone health or have you ever taken any on a regular basis?   1 ___ No
                                                                              2 ___ Yes

5a. If yes, please specify the name of the product and product description as stated on the
    label, how often you took or take each one, and at what ages:

    Brand & specific name (and amount)   number of pills/day   from age   to age
    on the product label

    _____

    _____

    _____

    _____

    _____

Birth control pills

6.   Do you currently or have you ever taken birth control pills ?   1____ No
                                                                          2____ Yes

6a. If yes, please specify at which ages: from age _____ to age ____
                                          and   from age _____ to age ____

6b. Brand name:_____

Estrogen

7.   Do you currently or have you ever taken estrogens for hormone replacement therapy ?
     1____ No
     2____ Yes

7a. If yes, please specify at which ages: from age _____ to age ____
                                          and   from age _____ to age ____

7b. Please specify name and dosage:_____

Medication

8.   Do you currently take any prescription medications on a regular basis?   1____ No
                                                                                2____ Yes

8a. If yes, please specify: medication (name & dosage)   how often taken?

     _____
     _____
     _____
     _____

Diet

9.   Do you follow any kind of special diet (e.g. vegetarian, low sodium, diabetic etc)?
     1____ No
     2____ Yes(please specify)_____

10. In general. would you say the healthfulness of your diet is:
     1____ Excellent   2____ Good   3____ Fair   4____ Poor

11. At the present time do you consider yourself to be:
     1____ Very underweight   4____ Somewhat overweight
     2____ Somewnat underweight   5____ Very overweight
     3____ About the right weight

12. Have you ever been on a diet to reduce your body weight?   1___ No   2___ Yes

12a. If yes, please indicate during which periods of your life you have dieted to lose weight:

1___ 10-20 years old   4___ 40-50 years old

2___ 20-30 years old   5___ 50-60 years old

3___ 30-40 years old   6___ 60+ years old

Health and physical activity

13. At the present time, how would you describe your health?

1___ Excellent   2___ Good   3___ Fair   4___ Poor

14. How would you describe your usual activity pattern **now**? (check only one)

1___ I am sedentary almost all of the time.

2___ I am quite sedentary on most days, but occasionally I exercise vigorously.

3___ I am sedentary during the day, except for my regularly scheduled vigorous exercise.

4___ I am energetic in my usual chores, hobbies and leisure time activities, although I do not participate in any regularly scheduled vigorous exercise.

5___ I am energetic in my usual chores, hobbies and leisure time activities, and I also participate in regularly scheduled vigorous exercise

6___ Other (please specify)_____

15. What are your typical daily physical activities?
(Include both occupational & leisure activities)

_____

_____

_____

_____

16. For each of the following periods of your life, if any, please indicate how often
(Column 1) and for how long each time (Column 2) you engaged in regular exercise:

AGE	Column 1 days/week	Column 2 minutes/day
a) your childhood (through age 12)	1___ 1 day/wk or less 2___ 2 or more days/wk	1___ less than 30 min/day 2___ at least 30 min/day
what kinds of activities? _____		
b) your teens (13-19)	1___ 1 day/wk or less 2___ 2 or more days/wk	1___ less than 30 min/day 2___ at least 30 min/day
what kinds of activities? _____		
c) your earlier adulthood (20-39)	1___ 1 day/wk or less 2___ 2 or more days/wk	1___ less than 30 min/day 2___ at least 30 min/day
what kinds of activities? _____		
d) your mid adulthood (40-59)	1___ 1 day/wk or less 2___ 2 or more days/wk	1___ less than 30 min/day 2___ at least 30 min/day
what kinds of activities? _____		
e) your later adulthood (over 60)	1___ 1 day/wk or less 2___ 2 or more days/wk	1___ less than 30 min/day 2___ at least 30 min/day
what kinds of activities? _____		
f) past two years specifically	1___ 1 day/wk or less 2___ 2 or more days/wk	1___ less than 30 min/day 2___ at least 30 min/day
what kinds of activities? _____		

16a. If you have ever exercised regularly, during which decade of your life did you begin?
(teens, twenties etc.)

_____

Nutrition

17. Thinking back over the years. for each life stage indicate. how often would you say you drank at least one glass of milk or ate at least one cup of yogurt:

a) your childhood:
(through age 12)

1___ with most meals (at least 3 times/day)
2___ frequently (at least once daily)
3___ sometimes (a few times a week)
4___ rarely or never (less than once/week)

b) your teens:
(13-19)

1___ with most meals (at least 3 times/day)
2___ frequently (at least once daily)
3___ sometimes (a few times a week)
4___ rarely or never (less than once/week)

c) your earlier adulthood
(20-39)

1___ with most meals (at least 3 times/day)
2___ frequently (at least once daily)
3___ sometimes (a few times a week)
4___ rarely or never (less than once/week)

d) your mid adulthood
(40-59)

1___ with most meals (at least 3 times/day)
2___ frequently (at least once daily)
3___ sometimes (a few times a week)
4___ rarely or never (less than once/week)

e) your later adulthood
(over 60)

1___ with most meals (at least 3 times/day)
2___ frequently (at least once daily)
3___ sometimes (a few times a week)
4___ rarely or never (less than once/week)

f) past two years
specifically

1___ with most meals (at least 3 times/day)
2___ frequently (at least once daily)
3___ sometimes (a few times a week)
4___ rarely or never (less than once/week)

18. Food Frequency

Think about the past year. During that year, about how often would you say you have eaten each of the following foods. Place an "X" in **only one column** for each food category.

FOOD	More than once/day	Once/day	3-4 times per week	Once/week	Less than once/week
Milk					
Yogurt					
Frozen Yogurt					
Ice Cream Ice Milk					
Hard Cheeses*					
Milk Puddings					
Canned Salmon					
Dark green leafy vegetables**					
Legumes***					

*   Hard cheeses: Cheddar. Swiss. American: alone or in a mixed dish

**   Dark green leafy vegetables: **broccoli. spinach. kale. dark green lettuce varieties** (not iceberg)

*** Legumes: All kinds of **dried beans and lentils** (not green beans)

19. Beverage Frequency

Listed below are several types of beverages people drink. Think about a typical day
and indicate all of the following eating times you usually drink each kind of beverage.
For example, if you ususally drink coffee for breakfast and snack, place an "X" in the
"Coffee/Tea" row in the "Breakfast" and "Snack" columns:

	Breakfast	AM Snack	Lunch	PM Snack	Supper	Snack
Water						
Juice						
Milk						
Coffee/Tea						
Other: SodaPop, KoolAid Wine, etc.						

20. Have you ever had an upset stomach, gas, or diarrhea after consuming milk, yogurt
or ice cream?   1___ No

2___ Yes

20a. If yes, please explain:_____

21. Did you encourage your daughter to consume milk and milk products during
childhood and adolescence?

1___ Yes, strongly

2___ Moderately

3___ No, not at all

22. Do you think you and your daughter have similar attitudes towards health and
nutrition?

1___ Yes, about most issues

2___ We agree on some issues but not others

3___ No, we disagree on most issues

Factors associated with bone health

23. I have a history of:
1____ rheumathoid arthritis
2____ an overactive thyroid gland
3____ an overactive parathyroid gland
4____ alcoholism
5____ chronic liver disease
6____ multiple myeloma
7____ blood tumor. leukemia
8____ stomach ulcers

24. I have been treated with or am you currently being treated with:
1____ cortisone or similar drugs
2____ lithium **for over one year**
3____ chemotherapy for cancer
4____ tamoxifin as a treatment for breast cancer

25. Have you ever taken or do you currently take:
1____ thyroid hormone pills
2____ phenobarbitol or dilantin **for over a year**
3____ Maalox or Mylanta antacids **(frequently)**
4____ furosamide (Lasix) **for over a year**
5____ cyclosporin A (Sandimmune)
6____ anabolic steroids

26.	I have a close relative with osteoporosis.	1____ Yes	2____ No
27.	Some of my stomach has been surgically removed.	1____ Yes	2____ No
28.	I have lost more than 1 inch in height.	1____ Yes	2____ No
29.	I have received an organ transplant (kidney etc.).	1____ Yes	2____ No
30.	I have had trouble with anorexia nervosa or bulimia.	1____ Yes	2____ No
31.	How many children have you given birth to?	_____	
32.	What was the date of your last menstrual period?	_____	

Menstruation

33. I lost my period for a year or more before it came back.   1___ Yes   2___ No
34. I have had a history of skipped periods.   1___ Yes   2___ No
35. My menstrual period did not begin until after age 16.   1___ Yes   2___ No
36. I have a medical history of endomitriosis.   1___ Yes   2___ No
37. I lost my periods when I was exercising heavily.   1___ Yes   2___ No
38. I have had both ovaries surgically removed.   1___ Yes   2___ No
39. I have breast fed a baby for one month or more.   1___ Yes   2___ No
40. I went through menopause before age 50.   1___ Yes   2___ No
41. I have gone through menopause.   1___ Yes   2___ No
42. I have received estrogen treatment after menopause   1___ Yes   2___ No

PAST HISTORY (Check if yes)

43. Have you **ever** had?

1___ High blood pressure

2___ Osteoporosis

3___ Bone fracture

4___ Back injury

5___ Diabetes

6___ Operations (specify)_____

6___ Heart trouble (specify)_____

7___ Other musculoskeletal injury or
       problems (specify)_____

PRESENT SYMPTOMS REVIEW (Check if yes)

44. Have you **recently** had?

1___ Chest pain

2___ Shortness of breath

3___ Heart palpitations

4___ Cough on exertion

5___ Coughing blood

6___ Back pain

7___ Painful, stiff or swollen joints

8___ other (specify)_____

_____

Additional data (optional)

45. Which best describes your ethnic identity?

1___ Caucasian

2___ African-American

3___ Asian-American

4___ Hispanic

5___ Native American

6___ other (please specify)_____

46. What is the highest level of education you have completed?

1___ Less than high school diploma

2___ High school diploma

3___ Some college but not a degree

4___ Bachelor's degree

5___ Master's degree

6___ Doctorate

7___ Professional training/certification

*MULTIDIMENSIONAL HEALTH LOCUS OF CONTROL SCALES

		Strongly Disagree	Moderately Disagree	Slightly Disagree	Slightly Agree	Moderately Agree	Strongly Agree
1.	If I get sick, it is my own behavior which determines how soon I get well again.	1	2	3	4	5	6
2.	No matter what I do, if I am going to get sick, I will get sick.	1	2	3	4	5	6
3.	Having a regular contact with my physician is the best way for me to avoid illness.	1	2	3	4	5	6
4.	Most things that affect my health happen to me by accident.	1	2	3	4	5	6
5.	Whenever I don't feel well, I should consult a medically trained professional.	1	2	3	4	5	6
6.	I am in control of my health.	1	2	3	4	5	6
7.	My family has a lot to do with my becoming sick or staying healthy.	1	2	3	4	5	6
8.	When I get sick I am to blame.	1	2	3	4	5	6
9.	Luck plays a big part in determining how soon I will recover from an illness.	1	2	3	4	5	6
10.	Health professionals control my health.	1	2	3	4	5	6
11.	My good health is largely a matter of good fortune.	1	2	3	4	5	6
12.	The main thing which affects my health is what I myself do.	1	2	3	4	5	6
13.	If I take care of myself, I can avoid illness.	1	2	3	4	5	6
14.	When I recover from an illness, it's usually because other people (for example, doctors, nurses family, friends) have been taking good care of me.	1	2	3	4	5	6
15.	No matter what I do, I'm likely to get sick.	1	2	3	4	5	6
16.	If it's meant to be, I will stay healthy.	1	2	3	4	5	6
17.	If I take the right actions, I can stay healthy.	1	2	3	4	5	6
18.	Regarding my health, I can only do what my doctor tells me to do.	1	2	3	4	5	6

* Wallston KA, Wallston BS, and DeVellis R. Health Education Monographs 6:160, 1978.

APPENDIX E

Questionnaire Daughters (Question 21 and 22)

(questionnaires for mothers and daughters are identical except questions 21 and 22)

19. Beverage Frequency

Listed below are several types of beverages people drink. Think about a typical day and indicate all of the following eating times you usually drink each kind of beverage. For example, if you ususally drink coffee for breakfast and snack, place an "X" in the "Coffee/Tea" row in the "Breakfast" and "Snack" columns:

	Breakfast	AM Snack	Lunch	PM Snacl	Supper	Snack
Water						
Juice						
Milk						
Coffee/Tea						
Other: SodaPop, KoolAid Wine, etc.						

20. Have you ever had an upset stomach, gas, or diarrhea after consuming milk, yogurt or ice cream?

    1___ No

    2___ Yes

20a. If yes, please explain:_____

21. Did your mother encourage you to consume milk and milk products during childhood and adolescence?

    1___ Yes, strongly

    2___ Moderately

    3___ No, not at all

22. Do you think you and your mother have similar attitudes towards health and nutrition?

    1___ Yes, about most issues

    2___ We agree on some issues but not others

    3___ No, we disagree on most issues