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RATES OF BONE LOSS IN POSTMENOPAUSAL WOMEN:

RELATIONSHIP TO

CALCIUM INTAKE, CALCIUM ABSORPTION, SERUM ESTROGEN, BODY MASS AND PHYSICAL ACTIVITY

By

PATRICIA HOOVER PEARSON, M.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Doctor of Philosophy

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RATES OF BONE LOSS IN POSTMENOPAUSAL WOMEN

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Hamilton, Ontario

TITLE: Rates of Bone Loss in Postmenopausal Women: Relationship to Calcium Intake, Calcium Absorption, Serum Estrogen, Body Mass and Physical Activity

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ABSTRACT

A prospective 2-year study was designed to test the hypothesis that 3 core factors influence the postmenopausal decline in bone mineral: adequate supply of calcium to the skeleton, endogenous estrogen production by lean and fat tissue mass, and mechanical stress imparted by physical activity and body mass.

The rate of change in bone mass (ΔBMD) was established for 61 postmenopausal women from semi-annual measurements of bone mineral density (BMD) at the proximal femur and lumbar spine using dual energy X-ray absorptiometry (DXA). Whole body BMD and body composition were also assessed annually using DXA. Serum estradiol was determined at baseline by radioimmunoassay. Calcium intake was evaluated using a food frequency questionnaire. Calcium absorption was measured using a single isotope technique. Grip strength was measured using a Jamar hand dynamometer. Aerobic fitness was determined using a submaximal 1-mile walk test. Habitual daily activity was assessed using a portable accelerometer.

At baseline, the strongest associations were between BMD and body mass values. These explained 22 to 25% of the variance in BMD. Estradiol was an independent predictor of BMD of the whole body. No physical activity variable was independently predictive of BMD. Of the dietary variables, only tea consumption was independently predictive of BMD at the femoral and whole body sites.

With age, bone loss was attenuated. At the lumbar spine, ΔBMD was also positively influenced by lean mass, weight gain, protein intake and increased calcium intake. The contribution of estradiol was borderline ($p = 0.06$). Weight gain was similarly

influential at the femur. There was no positive influence of any physical activity measure on ΔBMD at any site.

Lean mass and weight gain had the greatest positive influence on BMD and ΔBMD. A hormonal rather than mechanical explanation was favoured. Trabecular bone may also be responsive to dietary perturbations.

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CHAPTER 1

INTRODUCTION

1.1 The Burden of Osteoporosis

Osteoporosis has been defined as "a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" (Consensus development conference 1991). The World Health Organization has set the diagnostic criterion for osteoporosis at a value for bone mineral content or density more than 2.5 standard deviations below the young adult mean value (Kanis et al. 1994).

As the number of elderly people in western society increases, osteoporosis is anticipated to place increasing burdens upon society in terms of financial cost, morbidity, and mortality (Cooper 1993; Fogelman and Ryan 1991; Tilyard 1993). The most commonly reported osteoporotic fractures are those of the distal forearm, the proximal femur, and the vertebrae, although fractures at numerous other sites have demonstrated an increased incidence in association with low bone mass (Seeley et al. 1991a). Costs related to hip fracture impose the greatest burden upon society, both in terms of costs related to institutionalized care, as well as in excess mortality and morbidity (Chrischilles et al. 1994; Cooper 1993; Fogelman and Ryan 1991; Norris 1992).

Women are at greater risk of osteoporotic sequelae than men, with twice the risk of vertebral fracture (Kanis and McCloskey 1992), and an even greater excess risk of hip fracture (Cooper 1993; Gallagher et al. 1980a). In Canada, 18,909 hospitalizations in 1992-93 were attributed to fractures of the femur in women age 65 or older. For men in the same age group, 5778 hospitalizations were the result of femoral fracture (Statistics

Canada 1995). Estimated lifetime hip fracture risk is 17.5% in women and 6.0% in men (Melton et al. 1992). Lifetime fracture risk for clinically diagnosed vertebral fracture is greater, estimated at 39.7% for women, and may be higher considering that half of radiologically diagnosed vertebral fractures may be asymptomatic (Kanis and McCloskey 1992).

1.2 Prediction of Bone Strength and Fracture Risk

It has been established in both animal and human cadaver studies that bone strength is related to bone mass (Peel and Eastell 1993). In cortical and trabecular specimens, compressive strength declines exponentially with decreasing volumetric density (Carter and Hayes 1976). Bone mineral content assessed by densitometry has also demonstrated positive correlations with failure load in isolated rat femurs, and human cadaver forearms and vertebrae (Eriksson et al. 1989; Myers et al. 1991; Nordsletten et al. 1994). In intact specimens geometric properties or architecture may also influence the behavior of bone under load (Cordey et al. 1992; Gordon et al. 1996; Myers et al. 1991).

Cohort and prospective studies in adult populations have demonstrated the utility of densitometric assessment of bone mass in predicting fracture risk at either appendicular or axial sites (Cummings et al. 1990; Gardsell et al. 1989; Hui et al. 1989; Seeley et al. 1991b; Wasnich et al. 1985; Wasnich et al. 1989), although for the hip, site-specific bone densitometry may predict fracture better than other measurements (Cummings et al. 1993).

Bone densitometry does not distinguish perfectly between individuals who do or do not sustain fracture (Riggs et al. 1981). Other factors including age, previous fracture, medication use, the ability to see obstacles, and postural instability have also been reported as independent predictors of fracture risk although these may vary with the population

studied (Cummings et al. 1990; Cummings et al. 1993; Hui et al. 1989; Nguyen et al 1993). It is generally accepted, however, that assessment of bone mass provides a useful prediction of fracture risk and that there is approximately a 1½ to 2 fold increase in fracture risk for every 1 standard deviation decline in bone mineral density or bone mineral content from the young adult peak (Miller et al. 1996).

1.3 The Contribution of Peak Bone Mass vs. Bone Loss to Low Bone Mass in the Elderly

It has been estimated that the population variance in young adult peak bone mass and subsequent rates of bone loss may make equal contributions to bone mass measured 15 to 20 years postmenopause (Hui et al. 1990). By improving peak mass or reducing postmenopausal loss, fracture risk could be reduced. Heritability estimates suggest that peak bone mass has a limited capacity for manipulation by environmental factors, in the order of 20 to 40% (Johnston and Slemenda 1993). Estimates of heritability decline with age however (Kelly et al. 1995) perhaps indicating that environmental factors are more operative in determining changes in bone mass during adulthood. Rates of loss have demonstrated several fold differences between individuals (Christiansen et al. 1987; Hansen et al. 1991). Ultimately, the clinical value of attempts to reduce osteoporosis by intervening during either periods of bone accretion or bone loss, may depend in large part upon population compliance, ease of administration, cost, and effectiveness of available intervention strategies.

The true intraindividual variability of rate of bone loss has not been well established. There is some evidence to support the existence of subgroups of fast and slow losers (Christiansen et al. 1987; Riis et al. 1986b), and there have been some reports of increased incidence of fracture among those identified as fast losers (Hansen et al. 1991). Conversely, lack of an increase in population variance with age (Nordin 1990)

suggests that individuals may not demonstrate consistently high or low rates of loss over prolonged periods. Hansen et al. (1991) found that rates of loss determined by repeated densitometry over a two year period explained only 50% of the variance in rates of loss, determined by the difference in bone mass measured initially and twelve years later. Recently Pouilles et al. (1996) assessed rates of loss by repeated densitometry measurement in two consecutive 22 month periods and found that most individuals did not maintain their rank of high, intermediate, or slow losers.

Such evidence for instability in individual rates of bone loss supports the theory that there exist intrinsic or extrinsic precipitating factors which may be amenable to manipulation. Numerous studies have attempted to identify these factors.

1.4 The Contributions of Age and the Menopause to Bone Loss

Bone density has been reported to peak in females by the end of the second or third decade (Aloia 1994) or later (Halioua and Anderson 1989; Halioua and Anderson 1990), and may vary according to skeletal site (Bonjour et al. 1991). The onset of bone loss also appears to vary according to skeletal site, beginning earlier in more trabecular regions such as the lumbar spine, although some disagreement exists regarding the exact onset of this loss (Gotfredsen et al. 1987; Krolner and Nielsen 1982; Mazess et al. 1987; Prior et al. 1996; Riggs et al. 1986a).

Cross sectional studies have reported various patterns of loss at the lumbar spine. Riggs et al. (1981) reported a simple linear decline of approximately 0.6% per year in lumbar bone mineral density from age 20 to 80, in 105 women. Krolner and Nielson (1982) a year later, reported decline in bone mineral content at the spine beginning slightly later in the mid thirties, then following a gradual rate of decline estimated at 1.4% per year to old age. A small subgroup followed longitudinally demonstrated an accelerated loss in

the first 5 years postmenopause. Guesens et al. (1986) analyzed the rate of decline in spinal bone mineral density in 220 women age 20 to 85. Examined by decade, spinal loss began from the third decade onward, accelerating after menopause. The linear decline in postmenopausal women was reported as 1.6% per year. More recently, Barquero et al. (1992) also examined lumbar bone mineral loss by decade, reporting a similar average rate of loss for the 50 to 60 age period, but minimal loss after age 70.

Others have reported similar average losses at the lumbar spine in cross sectional studies but, finding non-linear functions to give better fits to the data, have further characterized the pattern of postmenopausal bone loss. Cann et al. (1985) assessed loss of spinal trabecular bone using QCT from youth to old age. Postmenopausal loss was linearly estimated at 1.2%/year, but the authors best fit the data using a cubic function. They described a period of accelerated loss in midlife, and an apparent slowing of loss in the elderly. Studies by Nilas et al. (1988) and Ortolani et al. (1991) examined bone mineral density in women from young adulthood to the ninth decade (141 and 234 women respectively). Using a menopause-adjusted age in postmenopausal women, they were able to demonstrate a rapid period of loss occurring at the menopause that declined in an exponential fashion. Elders et al. (1988) concentrated solely on the early postmenopausal years, studying a group of 286 Dutch women aged 46 to 55. They confirmed a non-linear loss, which gradually declined with the number of years since menopause.

Longitudinal studies have supported cross-sectional findings of an accelerated loss of bone mass at the lumbar spine around the time of menopause and a lower rate of loss in later life (Krolner and Nielsen 1982; Riggs et al. 1986b). Riggs (1986) reported a mean postmenopausal loss of 0.97% per year, but the curve describing the data indicates the greatest rate of loss occurring at about 50 years of age, with a zero rate (i.e. no loss) by about 80 years of age. Jones et al. (1994) followed 385 women age 60 and over with a

mean scan interval of 2.5 years and detected no significant loss from the lumbar spine in this group. Ferrari et al. (1995) in an 18 month study of an elderly group of 64 women and 8 men (mean age 73 years) reported the rate of loss from the lumbar spine averaged 0.47% per year. The declining reduction in bone loss at the lumbar spine with advancing age may be in part attributed to osteoarthritic changes and other extraosseous calcifications (Miller et al. 1996). Patients with severe osteoarthritic changes are frequently excluded from study populations, but even osteophytosis of mild or moderate degrees has been demonstrated to artificially increase bone density measurements by up to 20% (Masud et al. 1993).

At other regions of the skeleton the pattern of postmenopausal changes in bone mass are similar to those observed at the spine. Both cross sectional and longitudinal studies have demonstrated accelerated loss from the forearm (Nilas et al. 1988; Nordin 1990; Ortolani et al. 1991), hip (Mazess et al. 1987), and whole body (Gotfredsen et al. 1987) at the time of menopause. The pattern of loss at these sites with advancing age is less clear. Loss at a constant percent of initial bone mass has been reported in the radius at both distal and proximal sites (Hannan et al. 1992; Mazess et al. 1987; Riggs et al. 1986b). At variance with these results are those of Davis et al. (1989) who reported a declining absolute and percent rate of loss at both sites in a population of over 600 Japanese-American women, and a recent cross sectional study by Travers-Gustafson et al. (1996) reporting an increasing percent loss per decade at the proximal radius. Ethnic and lifestyle differences may partly explain the discrepancies. At the proximal femur, increasing loss (absolute and percent), with age, has been described (Jones et al. 1994). Average aging rates of loss at all these sites have been reported at ½ to 1% per annum (Geusens et al. 1986; Gotfredsen et al. 1987; Jones et al. 1994; Riggs et al. 1981).

Nordin (1990) examined the relative contributions of the rapid early postmenopausal decline and the continued decline with age, to the total bone loss. Bone density was examined at the distal radius in 588 women age 33 to 75 years and 1 to 28 years postmenopause. By regressing mineral density on age and the natural logarithm of the number of years since menopause, estimates of the relative importance of each component were derived. Age was confirmed as an independent predictor of bone density, making an equal contribution to total loss experienced by 20 years postmenopause. Linear age-related loss was calculated as 1.1% per year from age 54.

The rapid period of loss in the peri- and early postmenopausal years is attributed to withdrawal of the influence of estrogens. A similar pattern of loss is seen following oophorectomy (Richelson et al. 1984), and this component of loss can be largely attenuated by estrogen replacement therapy (HRT). A number of years after natural or surgical menopause, or cessation of HRT, bone loss continues. Slow bone loss has also been reported with long term (> 5 years) continued HRT (Lindsay 1995). This has been suggested as support for aging bone loss as a separate entity controlled by non-estrogenic factors (Mazess 1982). It is possible that non-hormonal factors may be operative in independently regulating bone loss in the estrogen deficient state, or of interacting with low residual estrogen levels in postmenopausal women.

The following chapters will address the contributions of some environmental and lifestyle factors, as well as the role of residual circulating estrogen levels, to bone mineral density and rates of bone loss in postmenopausal women. The purpose is to provide the necessary background information for the design of a study of the relations between such factors and the rate of bone loss in postmenopausal women.

CHAPTER 2

ESTROGEN

2.1. The Menopause and Bone Loss

It is widely accepted that estrogen deficiency has a major role in the development of osteoporosis in women (Arnaud 1993; Fox et al. 1993; Hillard and Stevenson 1991; Lindsay et al. 1993). In the early 1940s Fuller Albright recognized a relationship between the menopause and osteoporosis (Albright et al. 1941). Since that time, the development of methods to non-invasively quantify bone mineral has enabled further documentation of the temporal association between the decline in ovarian function and reduction in bone mass. As discussed in the previous chapter, there are numerous reports of the accelerated loss of bone mass that occurs following natural menopause.

Bone mass is also reduced in other instances of disturbed ovarian function: exercise-induced amenorrhea (Drinkwater et al. 1984; Marcus et al. 1985), amenorrhea during prolonged dietary restriction (Rigotti et al. 1984), surgical menopause (oophorectomy) (Ohta et al. 1992), ovarian dysgenesis (Davies et al. 1990), or pharmacological ovarian suppression in the treatment of endometriosis (Davies et al. 1990). These conditions support an etiological role for ovarian dysfunction in the loss of bone mass.

2.2. Steroid Changes at the Time of Menopause

At the time of menopause, the secretion of estrogen and other steroids of ovarian origin changes dramatically, as a result of loss of ovarian follicles. Within a year of the

complete cessation of the menses at menopause, estradiol levels decline from 70-2900 pmol/l to approximately 50 pmol/l (Lipsett 1986; Thomas 1993). The other major ovarian estrogen, estrone, also declines but to a lesser degree, from premenopausal levels of 160-750 pmol/l to 130 pmol/l postmenopausally. Comparison of serum levels of estrone and estradiol levels in naturally postmenopausal women to those with surgically induced menopause demonstrate that the postmenopausal ovary contributes little to serum levels (Lipsett 1986). Another major ovarian hormone, progesterone, mainly secreted by the ruptured follicle (corpus luteum) following ovulation, also is drastically reduced following menopause.

The ovary is not lifeless with menopause, however. Catheterization of ovarian veins has suggested that at least in some women, perhaps 10%, a significant contribution of estradiol to circulating levels may be made by one or both ovaries (Longcope et al. 1980). The ovary also continues to produce androgens; androstenedione, testosterone, and dehydroepiandrosterone (DHEA), and makes a major contribution to serum levels of testosterone (Longcope et al. 1980). Androgens are also produced by the adrenal cortex which makes the major contribution to serum levels for androstenedione and DHEA. At menopause, androstenedione, a precursor of estrogens, declines to levels almost half those previously, whereas levels of DHEA and testosterone demonstrate little change.

The major source of circulating estrogens in the postmenopausal woman appears to be the peripheral conversion of androstenedione to estrone (Grodin et al. 1973) by aromatization in tissues such as fat, muscle, liver, kidney, and brain (Longcope et al. 1978). The efficiency of this conversion may increase with age (Ferenczy 1994; Longcope and Baker 1993) and excess body weight (Frumar et al. 1980; MacDonald et al. 1978). Estradiol is principally derived from estrone and to a minor extent from testosterone (Judd et al. 1982; Longcope et al. 1969). Both absolute and relative concentrations of estradiol

and estrone are reduced in comparison to premenopausal levels such that estrone becomes the major circulating estrogen. Estrone is less potent than estradiol (Fotherby 1998), although differences in potency among estrogens may vary according to the biological endpoint measured. For this reason, the relative contributions of estrone or estradiol to a particular physiological response may be difficult to determine.

2.3. "Replacing" Ovarian Hormones After Menopause Improves Bone Mass

Although a number of ovarian hormones have demonstrated a potential for osteogenic effects in some circumstances, the major ovarian product, estrogen appears to predominate in its effects at the time of menopause.

The most convincing evidence for this is provided by the results of treatment of postmenopausal or oophorectomized women with exogenous estrogens. Bone loss can be prevented or reduced in a dose-dependent manner by estrogen replacement therapy (Hasling et al. 1994; Hillard et al. 1994; Lindsay et al. 1976). Improvements in bone density occur at clinically important sites such as the hip and spine, and improvements have been shown to continue into the third year of intervention (The Writing Group for the PEPI Trial 1996). The bone sparing effect is limited to the duration of hormone administration, with rapid loss ensuing thereafter (Christiansen et al. 1981). Therapeutic doses are modest. Using a transdermal route of administration, doses that raise serum estradiol to relatively low physiological levels (110-180 pmol/l or equivalent to early to midfollicular phase levels in premenopausal women) effectively prevent bone loss (Lufkin et al. 1992). Similar serum estradiol levels are achieved with oral preparations of conjugated equine estrogens at a dose of 0.625 mg per day (Levrant and Barnes 1994). Some studies have demonstrated that even lower doses may also be effective (Ettinger et al. 1987).

In untreated postmenopausal women, natural estrogens continue to be present in the circulation at low levels up to approximately 100 pmol/l, near those achieved by some treatment regimes. It may be suggested that individual differences in these levels could contribute to the variability in postmenopausal bone mass and rates of aging bone loss.

The therapeutic levels of estrogens required for the preservation of bone mass may be contrasted somewhat to those doses of androgens or progesterones used for the same effect. Androgens such as methyltestosterone or synthetic derivatives of testosterone have been demonstrated to prevent bone loss (Chestnut et al. 1983; Johansen et al. 1989). An excess of endogenous androgens (2.8 nmol/l testosterone; normal range < 2.0 nmol/l) in hirsute young women may also preserve bone mass in spite of estrogen deficiency i.e. serum estradiol levels less than 70 pmol/l (Dixon et al. 1989).

Because normal postmenopausal serum testosterone levels are little changed from premenopausal levels (Johnston et al. 1985), and because the normal estrogenic antagonism to androgenic influence is reduced at menopause, it has been suggested that the postmenopausal woman is already in a relatively hyperandrogenic state (Cutler and Garcia 1984b). Some treatment preparations may raise serum levels of total testosterone even further from less than 0.7 nmol/l up to 7 nmol/l, levels recognized to be associated with masculinization (Cutler and Garcia 1984a; Naftolin 1994). During androgen treatment, voice changes and facial hair growth have been observed (Plouffe, Jr. and Cohen 1994).

Natural and synthetic progesterones have also been used in the treatment of low bone mass, but their use in isolation in postmenopausal women has been limited. Gallagher et al. (1991) compared the progestin, medroxyprogesterone acetate (MPA, 20 mg daily), conjugated estrogens (CEE, 0.6 mg daily) and combination therapy (10 mg MPA and 0.3 mg CEE daily) with respect to effectiveness in preventing or reducing bone

loss at cortical and trabecular skeletal sites. MPA appeared to retard bone loss at cortical sites, but was less effective in slowing trabecular bone loss than was estrogen. Combined treatment appeared as effective as the higher dose of estrogen alone, which may indicate a synergistic effect (Gallagher et al. 1991). Others have found, however, that progestins such as medroxyprogesterone acetate (MPA) and micronized progesterone (MP) in conjunction with estrogen failed to significantly impact BMD above that achieved by estrogen alone (Adachi et al. 1997; The Writing Group for the PEPI Trial 1996). Low doses of progestins (10 mg intermittent MPA or 2.5 mg continuous MPA) in conjunction with estrogen reduce the incidence of endometrial hyperplasia in non-hysterectomized women, although higher doses (20 mg intermittent MPA) may have the undesirable effect of reducing high density lipoproteins thereby increasing cardiovascular risk (Adachi et al. 1997).

With hormone replacement, circulating concentrations of steroid hormones, particularly estrogens, may approximate premenopausal ranges. However, in untreated women, the relevance of low endogenous levels and their interindividual differences remains to be established.

2.4. *Subtle Changes in Ovarian Function Are Associated with Changes in Bone Mass*

Some evidence for the response of bone to subtle changes in ovarian hormones is gathered from the perimenopausal years. The natural menopause is associated with a gradual decline in ovarian function. During the transition from normal cyclicity to the complete cessation of the menses, which may last up to 7 years (Chang et al. 1994), estrogen levels may be reduced (Sherman and Korenman 1975; Cutler and Garcia 1984b), whereas ovarian androgens remain stable (Chang et al. 1994). Individual estradiol levels may vary widely, however, sometimes in excess of those associated with normal cyclicity

(Chang et al. 1994; Sherman and Korenman 1975) and the intervals between episodes of menstruation may be short or excessively long. Declining bone mass has been reported during this time of transition, perhaps due to changes in total yearly estrogen exposure, although the potential exists for the involvement of other ovarian hormones.

Reductions in the ovarian secretion of progesterone occur during inadequate or shortened luteal phases of the menstrual cycle or in association with frank anovulation (Prior et al. 1990; Sherman and Korenman 1975). Prior et al. (1996) have reported trabecular bone loss at the spine over a five year period in premenopausal women who demonstrated relatively short luteal phases (luteal index) during the first year of the study. No significant association was found between estradiol levels and rate of bone loss, but estradiol was not sampled during the midcycle peak (ovulation). Sherman and Korenman (1975), using daily serum samples, demonstrated that both follicular and luteal peaks of serum estradiol may be reduced in women with short or inadequate luteal phases. Johnston et al. (1985) reported significant declines in spinal bone density among premenopausal women who demonstrated irregular cycle lengths and reduced plasma levels of estrone and estradiol. The same group subsequently reported bone loss at the proximal and distal radius in 20 perimenopausal women with irregular cycles, elevated FSH, and decreased serum estradiol (Slemenda et al. 1987). Unfortunately, progesterone levels were not determined in these studies.

It has been suggested that maintenance of peak bone density in adulthood may require adequate levels of both estrogens and progesterones (Prior et al. 1990). Although these observations in the perimenopausal years fail to clarify the relative etiological role of progesterones and estrogens to changes in bone mass, the physiological impact of subtle changes is supported.

2.5. Endogenous Estrogen and Bone Mass in the Postmenopausal Woman

Postmenopausal women continue to demonstrate low circulating levels of the estrogens, estrone and estradiol. The precise relationship of serum levels of these hormones to bone mass and bone loss remains to be established.

A cross sectional study by Cauley et al. (1988) examined the relationship of lifetime calcium intake, serum estrone (E_1), androstenedione, and testosterone with radial bone density in 176 postmenopausal women. The mean number of years since menopause was 8.76. A modest but significant correlation was found between E_1 and bone density (r = 0.27) which remained after controlling for age and body mass index. When sex hormone measurements were categorized by quartile, women in the lowest quartile of E_1 (<5.93 pmol/l), demonstrated significantly lower bone density values than the remaining women, which may indicate a threshold effect. There also appeared to be an interaction of estrogen levels with lifetime calcium intake in determining bone density. Women with low E_1 and low reported milk intakes in childhood, adolescence and the present, demonstrated bone density values more than 2 standard deviations below the mean of the study group. Because bone density values were not expressed in standard units for this study, comparison with population normal values is not possible. The study population may also not be entirely representative of the general postmenopausal population, as E_1 levels appeared to be very low in this group of women (mean 8.1 pmol/l).

Rozenberg et al. (1990) examined serum levels of sex steroids and bone mineral density at the radius and spine in a cross section of Belgian women (n= 617) age 40 to old age, none of whom had received hormone replacement therapy. In this study androstenedione (A), estradiol (E_2), and DHEA-S were all negatively and significantly associated with age. The age range in this study did, however, span the menopause, which may explain this association for A and E_2. E_2, A, and DHEA-S were also positively

correlated with bone mineral content. Partial correlation coefficients, controlled for age, continued to demonstrate significant, albeit weak, associations between E_2 and lumbar bone mineral content ($r = 0.11$), and between DHEA-S and bone mineral at both radial and lumbar sites ($r = 0.13$ and $r = 0.09$ respectively). The fact that E_2 explained additional variance in BMC above that explained by age alone, may have resulted from the temporal association between the rapid decline in estrogens at the menopause and the substantial decline in bone mineral content (about 10% at the lumbar spine) between the ages of 55 and 60 in this subject population. This would not explain the persistence of an association between DHEA-S and cortical bone mass. BMC was not significantly associated with E_1 or testosterone (T). Although BMC was categorized in 5-year intervals, sex hormone levels were not reported and their temporal patterns of change could not be determined for this study.

Rae et al. (1991) examined radial BMC in two groups of women; 35 women who were 1 to 9 years postmenopausal, and 53 women 10 to 40 years postmenopausal. In the younger group, BMC was negatively associated with age and years since menopause. No association of BMC with age or years since menopause was found in the older group, but urinary estrone excretion (estrone glucuronide, E_1-G) demonstrated a positive association with BMC. E_1-G was not correlated with age or years since menopause, but demonstrated a positive association with body weight and body fat mass determined from skin-fold thickness. Body weight was positively associated with BMC in the older group. This relationship was similar in the younger group, but because of the smaller number of subjects, failed to reach statistical significance. It was curious that BMC failed to demonstrate an association with age in the older group. The actual age span of the group was not presented and a wide range in body size (also not presented), in conjunction with a small range in age, may have obscured this relationship. Also, because levels of E_1 and

total BMC are both influenced by body size, the independent contribution of E_1 to bone density is difficult to determine.

Slemenda et al. (1987) measured serum concentrations of E_1, E_2, T, A, and pituitary gonadotropins in 84 peri- and postmenopausal women aged 42 to 58 years. Bone mass at the proximal and distal radius was assessed over a 3 year period to determine rates of bone loss. For the whole population, serum concentrations of E_1 and E_2 demonstrated the strongest correlations with rate of change in bone mass. When data was examined for only postmenopausal women and the range of estrogen values was smaller, E_1 continued to demonstrate a significant positive correlation with change in distal radius bone mass ($r = 0.30$). The relationships with E_2 were no longer significant (data not presented) perhaps due to difficulty in measuring low levels of hormone. Lower limits of detection for the assays were not reported. There may also have been greater uncertainty in determining rates of bone loss as many of the women in this subgroup were not followed for a 3 year period (follow-up 1 to 3 years).

Van Beresteijn et al. (1992) measured concentrations of sex steroids, indices of body mass, calcium intakes and cortical bone mass and bone loss in 51 postmenopausal women (mean time since menopause of 10 years). Although simple correlations of sex steroids with bone mass and bone loss were not presented, partial correlations (controlling for body mass index) demonstrated a significant association between serum E_2 and change in bone mineral density in women with calcium intakes less than 800 mg per day ($n = 22$). Because estrogens influence calcium absorption, it might be hypothesized that women in this study with low estrogen levels were unable to demonstrate adequate adaptation to low calcium intakes, thereby exacerbating a negative calcium balance and inducing a greater rate of bone loss.

In a 4 year longitudinal study, Mole et al. (1992) measured the rate of change in bone mineral at the proximal and distal forearm, urinary metabolites of estrone and estradiol, and the markers of bone resorption, pyridinoline and deoxy-pyridinoline, in 68 postmenopausal women. The authors did not present the univariate correlations of the relationships of BMC with the estrogen metabolites. In multiple regression analysis for women 6 to 40 years since menopause (YSM), estrone glucuronide entered the regression model to explain the age matched *z*-score for initial bone mass or density. Estradiol glucuronide entered the regression model as a significant predictor of rate of bone loss at the distal forearm in younger women (1-5 YSM), and at the proximal forearm in older women (>5 YSM, 6 to 40 YSM). A positive correlation (data not shown) was reported between estrogen excretion and fat mass.

In the preceding studies, summarized in table 2.1 (pg.21), several authors reported positive associations between estrogen levels and body weight or fat mass in postmenopausal women. In cross sectional studies, if bone mass is reported as bone mineral content, which is highly dependent on body size, a positive association between estrogen and bone mass may be observed which may be spurious in nature. Although few longitudinal studies have examined the relationship of residual estrogen levels to change in bone mass, in older postmenopausal women there appears to be an interaction of calcium intake and residual estrogen levels.

2.6 The Influence of Estrogen on Calcium Conservation

A relationship between estrogen status and calcium balance has been recognized since the early 1940's (Albright et al. 1941). Estrogen therapy has been noted to improve calcium balance (Heaney et al. 1978; Heaney et al. 1989; Thalassinos et al. 1982) by increasing calcium absorption as well as reducing urinary calcium excretion (Heaney et al.

1978). The positive shift in calcium balance has been estimated to be of the order of 24 mg per day (Heaney 1990), an amount capable of offsetting an annual bone loss of approximately 1% per year.

This effect may be mediated through parathyroid hormone and the vitamin D endocrine system which together regulate calcium absorption and excretion. Gallagher et al. (1980b) reported an increase in serum 1,25-$(OH)_2D$ and serum immunoreactive parathyroid hormone (iPTH) in a small group of osteoporotic women treated for 6 months with 1.2 to 2.5 mg per day conjugated equine estrogens (CEE). Fractional calcium absorption also improved significantly in the treated group and this increase demonstrated a strong correlation with the increase in serum 1,25-$(OH)_2D$ ($r = 0.89$; $p < 0.001$). Correlations between absolute levels of hormone and calcium absorption were lower ($r = 0.33$) and failed to reach statistical significance due to the small sample size.

Elevations in serum parathyroid hormone levels positively influence calcium balance by reducing urinary losses and improving intestinal calcium absorption. Increases in PTH are also associated with increased bone resorption. Estrogen may impart some skeletal resistance to PTH (Lindsay 1993) although the relationships of remodeling activity to parathyroid levels in postmenopausal and aging bone loss are far from clear.

Bone remodeling is increased in early postmenopausal women (Parfitt 1988). PTH has been reported to be increased in some osteoporotic women, but not all (Gallagher et al. 1980a; Gallagher et al. 1980b). Serum iPTH is often normal or low in postmenopausal women (Riggs et al. 1973) in spite of, or perhaps as a result of increased resorption in the early postmenopausal phase. It has been suggested that estrogen deficiency renders the skeleton more sensitive to the effects of circulating PTH and that estrogen replacement decreases this sensitivity (Arnaud 1993; Cosman et al. 1991; Ernst et al. 1989). Estrogen therapy has a suppressive effect on remodeling (Heaney et al. 1978), possibly by reducing

activation frequency and therefore the total number of remodeling sites (Melsen and Steiniche 1993).

2.7 Estrogen Receptors and the Cellular Response

The precise mechanisms whereby estrogen may exert a direct influence upon bone remain to be determined. Estrogen receptors have been detected in osteosarcoma and osteoblast-like cells in cell culture (Eriksen et al. 1988; Komm et al. 1988). In vitro, responses to estrogen have included increases in mRNA for Type I procollagen (Ernst et al. 1989) and for transforming growth factor-β (TGF-β) (Komm et al. 1988). TGF-β increases the production of non-collagenous proteins by osteoblasts and can inhibit osteoclast action (Oursler et al. 1993). Other cytokines may also be involved in mediating the skeletal effects of estrogen. The administration of estrogen or antibodies for interleukin-6 (IL-6) to ovariectomized mice reduces the number of osteoclast-like cells in *ex vivo* cultures of bone marrow (Jilke et al. 1992). Estrogen receptors have been detected in avian osteoclasts (Oursler et al. 1991) although the significance of a direct effect of endogenous estrogens on human osteoclast function is controversial (Lindsay 1996).

Systemically, circulating levels of interleukin-1 (IL-1) increase following ovariectomy. Estrogen reduces the production of IL-1 by human monocytes. These changes in IL-1 are temporally associated with changes in indices of bone turnover (Pacifici et al. 1991).

Although there does not appear to be general agreement concerning the precise mechanisms, it appears that estrogen may exert both direct and indirect effects on the skeleton and these may be mediated through complex interactions of a number of cytokines (Manolagas et al. 1993; Martin 1993).

In summary, estrogens appear capable of influencing calcium economy and may directly influence bone turnover. These actions may be mediated by local and systemically circulating cytokines in a manner not yet fully determined. In postmenopausal women, the influence upon calcium economy may become important in situations of dietary deficiency and subsequently affect skeletal homeostasis. The calcium requirement will be discussed in a later chapter.

Studies in untreated postmenopausal women suggest that circulating levels of estrogen may be sufficient to significantly influence the skeleton. Therefore it is appropriate to include an assessment of residual estrogen levels (such as serum estradiol or estrone) when evaluating bone mass in prospective studies of postmenopausal subjects. Estrogen levels may be influenced in postmenopausal women by body mass to a greater extent than by age. Body mass may also influence the skeleton by other mechanisms. The influence of body mass will be explored in the following chapter.

Table 2.1. The association of bone mass with endogenous estrogens in peri- and postmenopausal women; X-sectional and prospective studies.

Source	*Study Type*	*N*	*Independent Variables*	*Results*	*Comments*
1987 Slemenda	Prospective 3 year	84 peri- and postmenopausal (Ages 42-58 yrs, divided into early and late peri- and postmenopausal)	- E_1, E_2, T, A, hormone production rates, clearance, and conversion rates of androgens to estrogens - BMD (proximal and distal radius), osteocalcin - diet, smoking	E_1 and E_2 were significantly higher in the early perimenopausal group than other groups Whole Group: E_1 with ΔBMD-p, -d, $r = 0.24, 0.36$, $p < 0.05$ E_2 with ΔBMD-p, -d, $r = 0.26, 0.33$, $p < 0.05$ T with ΔBMD-d, $r = 0.23$, $p < 0.05$ A with ΔBMD-p, -d, $r = 0.16, 0.09$, NS	Among only postmenopausal women, only E_1 and distal radial ΔBMC were significantly correlated $r = 0.30$, $p < 0.05$. No differences in clearance or aromatization across groups.
1988 Cauley et al.	X-Sectional	176 postmenopausal (Age: 58 ± 4 yrs) (9 ± 6 YSM)	- E_1, A, T - bone density (CT scan) at midradius - current calcium intake and milk intake as a child, adolescent, adult; physical activity	E_1 and bone density, $r = 0.27$, $p < 0.001$ T and bone density, $r = -0.10$, NS A and bone density, $r = 0.01$, NS Significantly lower bone density in lowest quartile of E_1 E_1, calcium intake and age independent predictors in multiple regression analysis	Very low levels of E_1 (mean 8.1 pmol/l; normal range = 160-750 pmol/l) Apparent interaction of low calcium and low E_1 Importance of lifetime calcium intake suggested
1990 Rozenberg et al.	X-Sectional	617 peri- and postmenopausal (Age 40+; mean 63 ± 7 yrs)	- A, E_1, E_2, T, DHEA-S - BMC-r (midradius) and BMC-l (spine)	Partial r, controlled for age DHEA-S and BMC-r, partial $r = 0.13$, $p<0.01$ DHEA-S and BMC-l, partial $r = 0.09$, $p<0.05$ E_2 and BMC-l, partial $r = 0.11$, $p<0.05$	E_2 remained a significant predictor of BMC-l, along with age, in multiple regression analysis; DHEAS and age predicted BMC-r
1991 Rae et al.	X-Sectional	1) 35 (1-9 YSM) 2) 53 (10-40 YSM)	- E_1-G - BMC-p and BMC-d (proximal and distal radius)	1) BMC and E_1-G, r = 0.20-0.22, NS BMC and E_2-G, r = 0.13-0.16, NS 2) BMC-p, -d and E_1-G, $r = 0.33$, $p < 0.05$ BMC-d and E_2-G, $r = 0.10$-0.19, NS	BMC negatively associated with age and YSM only in the younger group; E_1-G positively associated with weight and skin-fold thickenss
1992 Mole et al.	Prospective 3.8 year	68 postmenopausal (Ages 50-76) 1) 1-5 YSM 2) 6-40 YSM	- E_1-G, E_2-G, height, BMI - BMD-p and -d (proximal and distal forearm), Pyd, Dpd	E_1-G a predictor of initial BMD-p and -d in multiple regression 1) E_2-G predictive of ΔBMD-d 2) E_2-G predictive of ΔBMD-p	E_1-G and E_2-G demonstrated positive associations with fat mass
1992 Van Beresteijn et al.	Prospective 10 year	51 postmenopausal (Ages 58-66 yrs & 7-12 YSM at finale)	- E_2, T, P, A, DHEA, - BMD(midradius), ΔBMD - diet, BMI	E_2 with ΔBMD, partial r = 0.44, $p < 0.05$ (controlled for body mass)*	*Relationship significant <u>only</u> for women with calcium intakes < 800 mg/day

-p = proximal, -d = distal, ns = not significant, Dpd = deoxy-pyridinoline crosslinks, Pyd = pyridinoline crosslinks

CHAPTER 3

THE INFLUENCE OF BODY SIZE, BODY WEIGHT, AND BODY COMPOSITION ON BONE MASS AND DENSITY

3.1 Role of the Skeleton - Size of the Skeleton - Fracture Risk

One of the fundamental roles of the skeleton is support. Size of the skeleton is inexorably linked to stature, and presumably to the mass of tissues supported and the forces sustained during normal daily activities. The mechanisms which determine this linkage are less well understood. Particularly, after the cessation of normal bodily growth and development, a functional link between bone size (which is relatively stable) and body mass (which may be relatively changeable) is less obvious. However, if the size and strength of the bone becomes inadequate to support the body tissues, fracture results.

Bone strength is determined by material and structural properties. If we assume that in most instances the material properties of bone mineral are relatively stable, then the ability of bone to withstand external forces, without failure, must be determined by properties such as the total mass of bone mineral, or its distribution expressed as porosity or density of bony elements (mass within a given volume), or the architectural arrangement of structural elements (orientation to the line of force, and cross-sectional distribution or geometry). Although any of these properties may be linked to body mass, this discussion will focus on bone mass and density.

3.2 The Association of Body Mass with BMD

Epidemiological studies have identified that women who experience osteoporotic fractures tend to weigh less than women who don't fracture (Aloia et al. 1985; Daniell 1976; Hassager and Christiansen 1989; Lau et al. 1996; Paganini-Hill et al. 1991; Wickham et al. 1989). Similarly, body weight has been repeatedly shown to be an important determinant of BMD in both pre- and postmenopausal women and in men (Compston et al. 1992a; Edelstein and Barrett-Connor 1993; Lau et al. 1996; Ortolani et al. 1993; Reid et al. 1992b; Ribot et al. 1992). Ribot et al. (1992) examined the strengths of reproductive and anthropometric predictors of low vertebral bone mass in a large group of peri- and postmenopausal women. Risk of low BMD was almost doubled for each standard deviation decline in body weight (about 8.8 kg). Mazess et al. (1990) reported a similar relationship between weight and lumbar BMD in men. Vertebral BMD was reduced by 4% for each 10 kg decrement in body weight.

Unadjusted correlations between body weight and BMD are typically in the moderate range. Lau et al. (1996) reported a correlation of 0.50 between weight and lumbar BMD for an elderly population of Chinese women, and slightly higher correlations with the femoral neck and intertrochanteric region of the hip. Similarly, Reid et al. (1992a) reported a correlation of 0.48 between body weight and lumbar BMD in 140 postmenopausal women, although in this population, correlations between weight and femoral neck BMD were slightly lower. In a second study of 68 premenopausal women, the correlation between body weight and lumbar BMD was 0.69 (Reid et al. 1992b). Among predominantly postmenopausal women of the Rancho Bernardo trial, Edelstein and Barrett-Conner (1993) found that body weight explained from 4 to 17% of the variance in BMD, depending on site, after adjustment for age and other covariates

including exercise and hormone replacement therapy. Body weight was a more powerful predictor of BMD at the femur and spine (weight bearing sites) than at the radius.

Obesity, a health risk for numerous diseases, appears to confer some degree of protection against osteoporosis. Dawson-Hughes et al. (1987) found that obese postmenopausal women had significantly higher BMD at the radius, spine and proximal femur, than normal-weight women. The linear regression coefficients reported in the study indicated that each 10% increase above ideal body weight was accompanied by an increase in BMD of 1.5 to 4% depending on site. The smallest influence of body weight was observed at the radius, and the largest at Ward's triangle suggesting that trabecular sites may be preferentially affected. At the spine, the change in BMD was 3.6% for each 10% increase above ideal body weight. Extrapolation of these figures suggests a halving of the risk of vertebral fracture for women with a body weight 25 to 30% above ideal.

3.3 The Influence of Current Body Mass on Bone Mass and Bone Loss

It appears there is substantial support for a role of excess body weight in the attainment or preservation of bone mass, and reduction of fracture risk for adults of both sexes. It has been suggested that the influence of weight on bone mass and density occurs predominantly during the growing years, i.e. to influence peak mass (Compston et al. 1992a). Holbrook and Barrett-Conner (1993), however, have reported that current body mass index (BMI) and weight gain in adulthood had the greatest association with age-adjusted BMD at the radius, total hip and lumbar spine, in both men and women of an adult retirement community. There was no association between body weight at age 18 and current BMD. This suggests that current or recent body weight may influence bone maintenance or rate of bone loss.

The influence of body mass on rate of bone loss has been directly addressed in a few longitudinal studies. Christiansen et al. (1987), in a 2-year study of 178 early postmenopausal women, found that body fat mass was significantly higher in women who lost bone at a rate of less than 3% per year (slow losers) than those women who lost bone at a rate greater than 3% per year (fast losers). Van Beresteijn et al. (1992) also found that initial body mass index was positively related to cortical BMD at the radius and negatively related to the rate of loss of BMD over a 10-year period (r = 0.42 and -0.45 respectively).

3.4 Theory of the Relationship of Low Bone Mass with Body Size and Composition

At the individual level, low bone mass may result from the attainment of inadequate peak mass or from excessive bone loss. Low body mass may be linked to low bone mass because a smaller body requires a smaller supportive structure to withstand gravitational forces. Also, during physical activity, smaller muscles would exert a lesser force and thereby induce less strain within both weight-bearing and non weight-bearing bones. Finally, assuming that a low absolute mass of soft tissue could diminish the peripheral conversion of androgens to estrone, smaller lighter women might be disadvantaged hormonally, particularly after the menopause when the contribution of estrogens from the ovaries is greatly reduced (Grodin et al. 1973; Longcope et al. 1986). The dominance of any one of these mechanisms, gravitational, mechanical, or hormonal, should be reflected in the strengths of the relationships of body weight, lean body mass (as a surrogate for muscle size) or body fat mass, with bone mass and density.

3.5 Lean Mass versus Fat Mass: Which Plays the More Influential Role?

The independent roles of lean and fat mass on BMD have been investigated by a number of researchers. Lau et al. (1996) found that unadjusted correlations between fat mass and BMD of the lumbar spine, femoral neck or intertrochanteric region of the femur, were very similar in magnitude to the correlations between body weight and BMD at these sites, and ranged from 0.46 to 0.58. Correlations between lean body mass and BMD although still highly statistically significant, were somewhat lower (ranging from 0.41 to 0.45). Reid et al. (1992a) similarly found correlations between either body fat or body weight and BMD of the lumbar spine, total body and femoral neck to be similar (ranging from 0.36 to 0.54), but correlations between lean mass and BMD to be much lower and lacking statistical significance (ranging from 0.18 to 0.20).

The relative importance of body fat to BMD may be greater in postmenopausal women than premenopausal women and men. Aloia et al. (1995) found that in women aged 24 to 79, the variability in total body calcium measured by neutron activation, was largely explained by differences in lean body mass. However, in postmenopausal women, fat mass continued to explain a small but significant percent of the variability of the data. Edelstein and Barrett-Conner (1993) found that, in older men, lean mass was more strongly related to BMD than fat mass, at the proximal femur, radius, and lumbar spine. In women, fat mass demonstrated the strongest relationship with BMD at the total hip and femoral neck. Total body weight was, however, the best predictor of BMD at all sites, for both sexes. Similarly, in younger adults, Reid and colleagues also reported a stronger relationship between fat mass and BMD for women than for men (Reid et al. 1992b). In young athletic women, however, the same investigators found that percent lean body mass was a significant predictor of BMD at the femoral neck, a weight bearing site (Reid et al. 1995). Sowers et al. (1992) also found that in young women, those who were high in

both fat and lean mass had the highest femoral neck BMD. These results may be interpreted to suggest that in estrogen deficient postmenopausal women, a hormonal influence due to conversion of androgens to estrogens in adipose tissue, controls bone mass. Conversely, in younger women and men who are more muscular, more active, and exposed to a different hormonal environment, mechanical influences on the skeleton may predominate.

A hormonal link between body mass and bone mass may not, however, be exclusively dependent on body fat stores. As discussed in the previous chapter, higher body mass, particularly in postmenopausal women, is associated with increased plasma estrogens due to the aromatization of adrenal androgens to estrogens in peripheral muscle, fat, and other tissues. Although the literature favours body fat as the predominant peripheral site of aromatization, Longcope et al. (1978) suggest that this may not always be so. For men, a greater proportion of aromatization may occur in muscle rather than fat due to its greater daily blood flow. This assumes equal rates of androgen to estrogen conversion, at the tissue level, in both fat and muscle, regardless of the type of muscle or the location of the fat depot. Data from cadaver studies have found muscle to comprise approximately 50% (42 to 60%) of adipose-free tissue weight (Brodie 1988). In obese individuals, however, body fat may represent 30% or more of total body mass. Muscle mass is also known to decrease with age, whereas fat increases (Aloia et al. 1995). Fat stores could therefore be an important site of peripheral estrogen production in older obese populations. The decrease in sex hormone binding globulins in obesity may also permit greater access of the precursor androgens to aromatizing tissues, and access of the estrogen product to target tissues.

There is somewhat inconsistent support for the role of estrogens as an operative link between body fat and bone mass. Estrogen production is influenced by body fatness.

In obese individuals, the conversion of androgens to estrogens is increased. MacDonald et al. (1978) found that the conversion of (4-^{14}C) androstenedione to labeled E_1 (as the urinary metabolite estrone glucuronide) was significantly correlated with both body weight and excess body weight in 50 postmenopausal women ($r = 0.69$ and $r = 0.68$ respectively, $p < 0.001$). Jensen et al. (1985) reported positive correlations of absolute levels of circulating estrogens with fat mass (estimated from height, weight and age) although the magnitude of the associations was lower than those identified by MacDonald et al. ($r = 0.14$ to 0.31 with E_1 and E_2 respectively).

Although estrogen levels are well recognized for their positive effects on bone mass, fat-derived estrogens may exert a clinically important effect during the postmenopausal period. This effect may be easiest to detect in the early postmenopausal period when the decline in bone mass is greatest. Riis et al. (1986a) noted that among a group of early postmenopausal women, those with slow rates of bone loss (< 3 % per year over 2 years) had greater body fat mass and higher levels of E_1 and E_2 than women with high rates of bone loss (> 3 % per year). Correlations between body mass and estrogen levels were not reported. Mole et al. (1992) found urinary metabolites of E_2 together with BMI to be <u>independent</u> predictors of rate of bone loss at the distal forearm for early postmenopausal women (< 6 years post menopause). In a 10-year longitudinal study of cortical bone loss at the radius in early postmenopausal women, van Beresteijn et al. (1992) found serum E_2 to be positively correlated with BMI, but not related to baseline BMD or change in BMD, except in a subgroup of women with calcium intakes less than 800 mg per day. The weakness of association of BMD with hormonal status in this study may be due to the timing of the sampling, which was done at the conclusion of the 10-year follow-up. Because bone loss occurs at the greatest rate during the early postmenopausal years, this time period would have the greatest influence upon 10-year bone loss. Had

measurements of E_2 been performed at intake, they may have demonstrated a stronger correlation with the rate of change in BMD. Also, assuming late menopausal E_2 levels to be very low, sensitivity and precision of the hormone assay would become critical issues.

In a cross-sectional study of slightly older women (average 9 years since menopause), Cauley et al. (1988) reported a low positive correlation (r = 0.27) between serum E_1 and cortical forearm density. Interestingly, the relationship was not appreciably altered after controlling for degree of obesity (BMI). In contrast, Riis et al. (1986b) reported no difference in levels of E_1 and E_2 between two groups of older women with and without osteoporotic fractures. Thus is seems that a number of cross-sectional and longitudinal studies support positive but perhaps partly independent effects of both obesity and postmenopausal estrogens on bone mass.

3.5 Problems of Colinearity and Scale Artifact

Colinearity

Unfortunately, examination of the relative importance of total body mass, lean mass, and fat mass to bone mass and density is problematic due to the degree of co-linearity between these anthropometric characteristics. In older women body weight may be more highly dependent on fat mass than on stature or muscularity. For example, Lau et al. (1996) reported a correlation between body weight and fat mass of 0.93, whereas the correlation between lean mass and weight was lower at 0.76, and the association of weight with height was only 0.47. Edelstein and Barrett-Conner (1993) reported similar correlation coefficients of 0.94, 0.81, and 0.35 for total weight with fat mass, lean mass, and height respectively for older women. For men, however, the relationship between fat mass and body weight was lower (r = 0.86) and similar in magnitude to the correlations between weight and lean body mass. It may be suggested that for postmenopausal women

with low levels of habitual physical activity, mechanical forces may operate largely through gravitational influences on total soft tissue mass, and could therefore be positively and strongly associated with obesity rather than muscularity.

Scale Effect

As previously described, bone mass and bone density (measured by common densitometry techniques) are strongly associated with indices of body size, lean tissue mass and fat tissue mass. These relationships, and the strengths of the relationships, may be partly explained by co-dependence of these variables on body size (scale effect). Increases in stature result in increases in skeletal size (bone mass) and increases in soft tissue mass, particularly lean mass (Edelstein and Barrett-Connor 1993; Lau et al. 1996). Also, because density (BMD), measured by dual photon absorptiometry, is not volumetric density but rather areal density (a 2-dimensional expression; g/cm^2), it too is influenced by skeletal size. In terms of clinical importance, however, it is desirable to investigate those characteristics of bone which affect its competency and are concurrently responsive to changes in muscularity or fatness, rather than those characteristics which are only determined by development of a larger skeleton.

Scale effect may be reduced statistically by a number of techniques. By expressing fat mass as obesity or relative fat mass (percent fat mass or percent body weight above ideal), the dependence of this measure of body composition on size is largely eliminated, assuming that neither tall nor short individuals have a greater tendency toward being classified obese by the chosen method. By using this type of correction, however, a true relationship between bone and any factor related to absolute mass of adipose, or muscular size, may be obscured. For example, obese individuals not only have an increased mass of fat tissue relative to normal weight individuals, but also a tendency toward an increase in

lean tissue as well. This phenomenon is substantiated by demonstration of an increase in basal metabolic rate (which is dependent on lean mass) in obese individuals (Segal and Pi-Sunyer 1989). If both fat and lean tissue are expressed as percentages of total soft tissue mass, as an individual gets fatter, % fat mass increases and % lean decreases despite the absolute increase in the mass of both. Fatter persons might therefore demonstrate a positive relationship between % fat mass and BMD, but a negative association between % lean and BMD. It would still be possible, however, for BMD to be dependent, both in magnitude and in direction, on changes in the absolute mass of lean tissue. Therefore, when examining the relationships of BMD with body composition, it may be advisable to consider absolute, as well as relative, expressions of fatness or leaness, in order to avoid misinterpretation of results. Generally, as previously discussed, moderate correlations between indices of obesity and BMD suggest a relationship between fat mass and bone that is largely independent of stature.

A second method of eliminating co-dependency of bone and soft tissue on body size, is to correct the bone mineral measurements for bone size. The mass of bone mineral or ash weight is highly dependent on size and has been shown to demonstrate a strong relationship to lean mass and muscle weight (Aloia et al. 1995; Doyle et al. 1970). Bone density, corrected to height and width of the projected scan area (BMD, g/cm^2), is also related to lean tissue mass as described previously, but less so (Ito et al. 1994). Volumetric bone density may be estimated mathematically from the results of areal densitometry scans and some additional measured body parameter which is used to correct for the missing third dimension, i.e. depth. Using a number of different methods devised to estimate bone volume, Reid et al. (1994b) examined the relationships between body composition and volumetric bone density for 140 postmenopausal women. Calculated in

this manner, lumbar bone density was no longer related to lean body mass, but continued to demonstrate significant positive associations with body fat mass.

Another measurement technique, quantitative computed tomography (QCT) directly measures the structural density of bone and, expressed as mass per unit volume, gm/cm^3, is independent of skeletal size. Density measured in this manner is influenced by cortical porosity and density of trabecular elements, and is correlated with bone strength (Louis et al. 1995). Berning et al. (1993) found that height, weight and BMI were unrelated to lumbar trabecular bone density, measured by QCT, in a cross-sectional study of normal-weight early postmenopausal women. In postmenopausal Japanese women, Ito et al. (1994) reported significant positive correlations between volumetric density of the vertebral cortex and body weight. Muscle size, assessed as cross-sectional area, was related to bone density in younger women and men, but not in postmenopausal women. Overall, the results of studies investigating the determinants of true or estimated volumetric density support a size-independent influence of fat mass in postmenopausal women, and lean mass in young women and men.

Longitudinal studies are a useful and necessary step in the process of distinguishing association from causation. In addition, examination of the direction and magnitude of change in one variable relative to change in another also largely eliminates scale artifacts.

A swift response of bone to current body mass is suggested by a few studies that demonstrate a change in BMD concurrent with short term weight loss or gain. In a 2-year study of postmenopausal women, Reid et al. (1994a) found that not only was rate of change in both cortical and trabecular BMD related to body fat mass, rate of change in body weight or fat mass was an independent predictor of rate of change in BMD of the whole body, lumbar spine and trochanter. A change of approximately 10 kg of body fat was associated with a 3% change in whole body BMD, a quantitative relationship similar

to that reported between body weight and BMD in cross-sectional studies. These findings are consistent with those of Compston et al. (1992b) who monitored total body BMD in response to a drastic 10 week diet, in a small group of overweight women. In this short period, there was an average decline in total body BMD of 2% for each 10 kg of weight loss. Follow-up 10 months later indicated a rebound of both body weight and BMD to baseline values. In a sense, this sort of short term change in BMD is surprising, considering the 3-6 month interval required to complete a single cycle of bone turnover, and may be due to enhanced osteoclastic recruitment and a sudden increase in the total number of remodeling sites. If so, the changes in BMD associated with this synchronized increase in bone turnover may not persist. The short term nature of the data does not permit examination of this theory. Compston suggests that BMD may be influenced by adjustments in body weight, in this postmenopausal population, as a result of increases or decreases in endogenous estrogen levels brought about by changes in total body fat stores. Measurements of serum E_1 and E_2 were not, however, performed in this group.

Interestingly, Ramsdale and Bassey (1994), also reported a decrease in total body BMD coincident with planned weight loss, in a group of premenopausal women. Over the six month interval, the magnitude of the bone loss (1.4% of total body BMD, per 10 kg loss in body weight) was similar to that observed in the postmenopausal women described above. For premenopausal eumenorrheic women, the contribution of body fat stores to the total circulating estrogen pool would be relatively small when compared with the major contribution of this source of estrogens to the total pool in postmenopausal women. It appears less likely, therefore, that a weight-loss induced reduction in plasma estrogens is the predominant factor behind the observed bone loss.

For both of these studies, the magnitude of the correlation between change in body weight and change in BMD , as well as the consistency and immediacy of the change raise

the possibility of systematic measurement error. This possibility was addressed by Compston in a series of *in vitro* experiments (1992b). Scanning an aluminum phantom with a Lunar model bone densitometer beneath varying thicknesses of fat and water, Compston reported that the presence of fat, and changes in depth of fat, did indeed cause changes in the measurement of bone mass. In the presence of increasing thicknesses of fat, mean projected bone area and BMC values increased, and BMD values decreased. The direction of these changes in BMD were, in fact, opposite to the direction of the changes in BMD associated with weight loss. The authors therefore concluded that bone loss which appears to accompany weight loss is a true phenomenon, and is perhaps underestimated. A criticism of the in vitro experiments, may be that the distribution of fat and bone in a plastic holding tank is not representative of the *in vivo* situation, and therefore the results may not be representative of those observed during a scan of the whole body. Highlighting a potential inconsistency in the direction of machine-derived error when measurements are made under different circumstances, Haarbo et al. (1991), found that increasing the proportion of alcohol to water (a surrogate of increases in fat proportion) in an 18 x 30 cm tank containing bones, caused a decline in BMC rather than an increase. Unfortunately, the effect on BMD was not reported, nor the scan mode used for the experiment. In light of some of these uncertainties, it would prove valuable to confirm the relationships between change in bone mass or density and change in body mass, using other methodologies.

3.6 Summary

Bone mass and density (g/cm^2), measured by densitometry, are strongly related to body size and composition. Data corrected for the effects of body size or skeletal size continue to demonstrate independent associations with indices of weight, obesity, or

muscle mass. In postmenopausal women, fat mass and obesity demonstrate the most consistent associations with bone density and appear to influence rate of bone loss. The effect appears to be immediate and responsive to adult changes in body weight. Maintenance of body weight may, therefore, be an important factor in the risk of osteoporosis and should be taken into consideration when examining the longitudinal effects of other interventions or treatments. As discussed, the relationship between body weight and bone may be related to hormonal or mechanical factors, each of which may need to be explored and documented independently, and the relative contributions of fat and muscle mass to these factors may vary depending on the age and activity level of the individual. We therefore chose to include assessments of total body composition (fat and lean mass) in the proposed investigation of bone loss in postmenopausal women. Further discussion concerning the relationship between mechanical influences and bone mass in the adult will be presented in the following chapter.

CHAPTER 4

MECHANICAL INFLUENCES UPON THE SKELETON: THE ROLE OF PHYSICAL ACTIVITY

The skeleton as a structural entity serves an essential role to the human body. It is protective and provides the framework upon which the levering action of skeletal muscles can act to cause movement. It would seem logical then, that bone, as a dynamic tissue, might respond or adapt to the external mechanical forces placed upon it.

The nature of forces and circumstances necessary to precipitate structural change might differ during the various developmental stages from infancy to adulthood. The extent and nature of the skeletal response to a particular stimulus might also vary according to the stage of life. In the following discussion, the primary focus will be upon the adult skeletal response.

4.1 Disuse

The most dramatic responses of bone to mechanical influences are demonstrated in response to immobilization, disuse, or withdrawal of gravitational forces. These changes have been described in both animal and human models.

Lanyon, Rubin, and Baust (1986) found that the cross-sectional area of adult turkey ulnas was reduced by 17%, over a 6 week period of immobilization. This was above and beyond a 15% reduction in cross-sectional area of the contralateral ulna induced by a calcium deficient diet. The loss attributed to immobilization could be partially attenuated by extremely brief periods (36 cycles; 100s per day) of artificial

loading. This suggests that bone can respond to the addition or removal of a local loading stimulus, independent of or in addition to systemic influences. In a similar study of disuse involving the rooster, Rubin and Lanyon (1984), reported additional histological evidence of local changes following disuse. Non-loaded ulnas demonstrated an increased number of remodeling sites, a reduction in cortical thickness due to endosteal resorption, and increased intracortical porosity. In this model bone loss proceeded at a rate of 1 to 2% per week (Rubin and Lanyon 1987).

Similar rates of loss have been reported in humans subjected to bedrest (Inoue et al. 1993; Krolner and Toft 1983; Lappe 1994; Smith and Gilligan 1991). Imposed bedrest has also been demonstrated to induce a negative calcium balance (Hantman et al. 1973; Schneider and McDonald 1984) not corrected by oral calcium supplementation. Bone loss during bedrest has been shown to be greater at weight bearing sites (Whedon 1984) suggesting that the structure adapts to a level of stress encountered in usual daily activity; greater reductions from normal stresses resulting in greater losses.

Theories and mathematical models (Frost 1990; Turner 1992) have been developed to explain the response of individual bones to the magnitudes and directions of the common peak strains encountered at each site. The osteogenic influence of a load appears to be influenced by the magnitude of strain, strain rate, intermittency of the stimulus, and variability or uniqueness of the direction of peak strain (Rubin and Lanyon 1984; Rubin and Lanyon 1987).

Withdrawal of mechanical stress has demonstrable effects as noted above. In some situations re-establishment of "normal" forces can completely or partially restore lost bone (Inoue et al. 1993; LeBlanc and Schneider 1991). The question remains, however, if the ongoing application of external stresses could induce a further increase in bone mass. Rubin and Lanyon's experiments on isolated turkey ulnas hold promise. They found that

osteogenesis could be induced by the application of externally applied forces producing strains of physiological intensity within the range of that measured by strain gauges in normal wings (Rubin and Lanyon 1984; Rubin and Lanyon 1987).

4.2 Osteogenic Effects in Response to External Loading

Perhaps the simplest way to determine the potential effects of physiological loading *in vivo* is to examine skeletal differences between highly active and inactive populations. Numerous cross-sectional studies have compared athletes with non-athletes and described greater bone mass among individuals in competitive weight-bearing sports.

A club of committed older runners (average 9.2 years of running) was reported to have a 40% increase in lumbar trabecular bone density, relative to community controls (Lane et al. 1986; Michel et al. 1989), and a positive correlation of change in bone density with change in training status (Michel et al. 1991). Unfortunately, no assessment of lifetime activity pattern was reported, nor if declining health or age were responsible for change in training status.

Heinonen (1995) reported significantly greater (6 to 19%) bone mineral density values at the femur and lumbar spine in young adult athletes from three different sports who had been training a minimum of 5 years, than either those of physically active or sedentary volunteers. Interestingly, no significant differences were noted between active and non-active controls. It was also noted that athletes whose training did not specifically load the arms had lower BMD at the distal radius than controls.

Fehling et al. (1995) noted that collegiate female volleyball players and gymnasts had higher BMD measurements for the whole body, lumbar spine, and proximal femur than swimmers and a group of non-active controls. Gymnasts had higher regional arm density than other groups. Interestingly, swimmers did not differ from inactive controls in

spite of the fact that Fehling's data indicates that swimmers participated in at least 60 minutes of weight training, 3 times a week, throughout the year. An inadequate training stimulus was suggested as the reason for lack of difference between the swimmers and controls. Small sample size may have also played a part (n = 7 swimmers; n = 17 controls).

Difficulties arise when attempting to attribute the skeletal difference between athletes and non-athletes to a causative effect of mechanical loading histories, partially because of the potential for such factors as preselection bias. Athletes with a genetic predisposition to certain body builds and higher bone densities may suffer fewer injuries and experience greater success in sports that exert strong demands on the musculoskeletal system. This may artificially elevate the differences observed between athletes and non-athletes, or between various sports. Individuals engaged in high levels of sport-specific physical activity, either on a professional or non-professional basis, may also exhibit a tendency toward greater general sport participation throughout life. The greatest influences on bone density may then have occurred during the time of skeletal accretion, during growth.

Observations of consistent regional differences in bone density in athletes subjected to long term loading stimuli unique to a particular sport, strongly support a direct influence of mechanical forces on those areas liable to experience the highest mechanical strains. For example, although intense exercise and reduced body mass in female athletes may be associated with hypogonadism, which in turn can adversely affect bone mass (Lloyd et al. 1988), exercise may regionally modify this effect. Amenorrheic or oligomenorrheic ballet dancers demonstrate normal or increased bone density at the femur, but reduced bone density in the ribs, arms, and skull (Young et al. 1994).

Regional differences may sometimes be difficult to identify. MacDougall et al. (1992) studied regional differences in bone density among young male runners and non-runners. Bone density values increased with training mileage, up to a point, then decreased to levels similar to non-runners. The cross-sectional area of the tibia and fibula increased without apparent plateau, perhaps as an alternative adaptive measure to disperse compressive forces. Michel et al. (1991) reported that extreme training was associated with lower bone density values in some athletes. Lumbar spine QCT values in older runners, at the extremes of training, were lower than runners with more modest training schedules. It is not known if changes in cortical thickness could have occurred as a compensatory mechanism, or alternatively if true lumbar losses represented a "steal" to supply excess mineral demands for osteogenesis in the lower extremities. Discrepancies between studies regarding the apparent effects of exercise on osteogenic responses may be attributed to inadequacies in measurement techniques, potential adverse effects of excessive loading, or unidentified systemic influences (aging effects, hormonal status, or diet inadequate for extreme training stresses on the musculoskeletal system) that may reduce or reverse local responses.

If true differences exist between the bone densities of athletes and non-athletes or within the skeletons of athletes, the importance of current versus past activity pattern must be clarified. Perhaps of equal importance to our aging society, however, is whether bone density can be influenced by the small differences in habitual activity patterns which exist within the general population.

4.3 Habitual Activity Relationships To Bone Mass

Among non-athletes, cross-sectional studies have also identified significant relationships between the level of participation in regular recreational exercise and BMD

at weight bearing sites (Stevenson et al. 1989). In studies spanning wide age ranges, however, it must be noted that activity or fitness may be expected to decline with age and this may occur at a rate similar to or greater than bone density (Cottreau et al. 1995). Both participation in exercise and bone density may be expected to decline with age, and may demonstrate a fortuitous positive correlation.

Stevenson et al. (1989) found that in men aged 28 to 51, regular participation in recreational exercise, but not daily walking mileage, was associated with increases in lumbar spine and femoral neck BMD. Positive correlations were also found between bone density values and grip strength, back extensor strength, and quadriceps strength. Differences in levels of recreational exercise, strength, or BMD by age were not, however, addressed.

In a study of 216 British women covering an age span of 21 to 82 years, the number of hours per week spent in weight bearing activity failed to correlate significantly with bone mass at lumbar spine or femoral neck sites, when controlled for the effects of age (Rutherford and Jones 1992). Interestingly, isolated strength of the quadriceps muscles did contribute significantly, along with age, to bone mass at all sites. This measure of strength was, however, positively correlated with height and could potentially be acting as a surrogate for skeletal size.

Increased activity levels, past or present, have been linked to decreases in hip fracture incidence. Jaglal et al. (1993) found that a higher recent or past activity level was associated with a favourable odds ratios for hip fracture of 0.61 and 0.66 (recent and past), respectively. The percent of respondents reporting high recent activity levels that also reported high past levels was not given. Astrom et al. (1987) also examined different aspects of past activity for 49 women with hip fractures and 49 controls matched for age and social status. Differences in type of work activity and sports participation failed to

reach statistical significance, but the number of labour saving amenities in the house, and the presence of household help were significantly higher in the fracture group, suggesting that habitual daily activities may contribute significantly to total daily activity for women in a manner that positively stresses the skeleton or reduces falling.

A reduction in fracture risk (RR 0.3 or 0.4) was associated with past non-occupational outdoor activity (>1hr/week) in a large sample of European women in the MEDOS study (Cano et al. 1993). Wickham et al. (1989) also found current outdoor activity (intensity not specified) to be associated with lower odds ratios for hip fracture in elderly British men and women. Both mobility and lower outdoor activity scores may have reflected general health restrictions rather than activity choices or past activity patterns.

In a 7 year prospective study of 8600 postmenopausal women and 5049 men (Paganini-Hill et al. 1991), age-adjusted relative risks for hip fracture were reduced almost equally for both sexes when exercise, reported in a health questionnaire, was equal to or greater than 1 hour per day (RR = 0.6 and 0.5 for men and women respectively) Outdoor exercise specifically was identified with decreased risk of 0.7 and 0.4 for women and men respectively.

Some population studies have also addressed the association between current and past activity and measurements of bone mass. Greendale et al. (1995), as part of the Rancho Bernardo Study, found **both** current and past exercise to correlate positively with bone density of the femur.

When interpreting the data, mechanical loading appears to exert a positive effect on bone mass and to reduce fracture risk. Other factors may, however, be operative in these associations. Outdoor activity exposes an individual to sunlight with the potential of influencing bone mass by increasing dermal production of vitamin D. Active individuals

may also reduce their risk of hip fracture by maintaining better muscular strength, agility, and balance.

4.4 Exercise Intervention: Effects on Bone Mass

In order to be confident that a population will benefit from a change in lifestyle, a response of bone mass to a controlled change in activity should be clearly evident. This is particularly important when recommending changes in activity pattern in a target group, such as postmenopausal women. Ideally, it should be possible to prescribe the appropriate type, intensity, duration, and frequency of exercise for a desired skeletal response.

Exercise intervention studies may be roughly divided into two types. The first type of study attempts to increase weight bearing stimuli through the lower extremities using activities such as walking, running or exercise classes of an aerobic nature. The second type increases bone loading with resistive training using high loads and few repetitions. Studies utilizing each of these methods will be briefly summarized below.

Study Interventions of a Predominantly Aerobic Nature

Walking, jogging, and running programs in postmenopausal women have demonstrated varying degrees of success (refer to summary of aerobic training studies in table 4.1, page 51). Cavanaugh and Cann (1988) compared a small group of early postmenopausal women who walked for 15-40 minutes, 3 times weekly, to sedentary controls. Spinal trabecular bone density as measured by QCT was not significantly different between the groups after 52 weeks. Rates of loss were similar in both groups at 5.6% per year for walkers and 4.0 % for non-walkers.

Dalsky (1988) studied a group of 50 to 70 year old female volunteers. Seventeen women trained 3 times a week for 9 months with a program of walking and jogging at 60

to 70% of $\dot{V}O_{2max}$. Stair climbing, cycling, rowing, and upper extremity weight training were also added to the routine for variety. Eleven of the women continued to train for a total of 22 months. Fourteen women served as controls. After 9 months, BMD of the lumbar spine was significantly increased in the exercise group (+5.2%) compared to a decline in controls (-1.4%). After 22 months the respective changes relative to baseline were +6.1% compared to -1.1%, indicating that most of the change had occurred during the initial intervention period. Fifteen women were followed for 13 months after the cessation of training. In this detraining group BMD values declined (-4.8%) toward baseline. Grove and Londeree (1992) also reported positive results of the influence of aerobic exercise on lumbar BMD. They compared high impact with low impact exercise regimes in a very small group of women who were an average of 4 years postmenopausal. Differences were significant between a 2% increase in lumbar BMD over the 1 year study period compared with a 6% decrease in the control group (a decline larger than expected). There were no significant differences between the low impact and high impact exercise regimes. In both of these studies, floor exercises, or seated resistive exercises were included in a supplemental way to the aerobic routine. Although details of these exercises were not specified, it may be suggested that the forces exerted on the lumbar spine by the muscular work accomplished in the flexed posture may have acted as an intermittent loading stimulus on the vertebral bodies.

Bloomfield et al. (1993) found stationary cycling to be a positive stimulus at the spine but not the femur in 7 late postmenopausal women. Compared with sedentary controls, women who cycled 3 times per week for 8 months at 60-80% of their maximum heart rate, positively influenced lumbar BMD (+3.55% vs. -2.44%, exercise vs. control). Once again, loads experienced at the lumbar spine during flexed sitting postures (forward leaning) may be increased relative to loads encountered during standing (Andersson et al.

1974). The opposite may occur at the femur where the loads may be reduced during sitting activities relative to loads imposed during comparable muscular activity while weight bearing.

Martin and Notelovitz (1993) followed postmenopausal women for a 12 month period of treadmill training at 70 to 85% $\dot{V}O_{2max}$, 3 times per week for either 30 or 45 minute sessions. No differences in BMD of the distal radius or lumbar spine were detected between exercise groups and a non-exercise control group. A subgroup of recently postmenopausal women demonstrated a smaller loss in lumbar BMD than controls (-1.67% vs. -3.36% respectively), but the numbers of subjects were small for these subgroups (n = 7 and n = 11), and it was not reported if the mean time since menopause was similar between the two groups. Similarly, Prince et al. (1995) after two years, found no change at the lumbar spine in response to a walking and weight bearing exercise routine in a large group of Australian women. A small effect of exercise was noted at the femoral neck (a significant difference between groups but not from baseline) but not at any other femoral or tibial site.

Bassey and Ramsdale (1995) attempted to improve the osteogenic stimulus from weight bearing activity by introducing a heel drop activity designed to impart high strain rates and loads through the lower extremity on a daily basis. The increase in loads at the heel and at the femoral neck during this exercise were estimated by testing with floor force plates, and with strain gauges engineered into hip prostheses of two non-study patients. Ground reaction forces were found to be 2 to 3 times body weight. After one year, changes in BMD at the lumbar spine, proximal femur and distal radius were not significantly different from a group of women who performed weekly low-impact exercises and daily stretches. The only significant changes in BMD from baseline, in either group, were significant losses at the proximal and distal radius in the exercise group.

The osteogenic influences of changes in weight-bearing ambulatory activity in postmenopausal women appear small or absent. It has been suggested that walking and similar activities exert strains on the bones that are not sufficiently novel in terms of direction, and perhaps not sufficiently high or rapid to elicit an osteogenic response (Bassey 1995). Lanyon has demonstrated in animal studies that relatively few repetitions of a high, rapid and novel (in terms of direction) load may be the best stimulus to elicit positive bone remodeling (Lanyon 1996).

Study Interventions Using Weight Training

Studies of the effects of resistive exercise routines on forearm BMD have demonstrated some promising results (refer to summary of weight training studies in table 4.2). Simkin et al. (1987) exercised the upper extremities of 14 postmenopausal women aged 53 to 74 years, 3 times per week over a 5 month period with a variety of exercises intended to induce compressive, tensile, and torsional strains. Bone mineral content and density (BMC/bone width) at the distal radius were determined by single photon absorptiometry (SPA) and a Compton scattering technique was used to measure distal radius trabecular bone density. No differences were detected in this short time span by SPA, perhaps as a result of the poor reproducibility of the method reported from this institution. Significant changes (a 3.8% increase vs. a 1.9% loss in the control group) were, however, detected by the Compton method. Although the changes are large for the 5 month intervention period, it is suggested that trabecular density may demonstrate a more rapid response to mechanical stimuli than cortical and trabecular BMD combined.

Smith et al. (1989) studied a group of 142 women with a mean age of 50.8 years. Almost half of the subjects were postmenopausal. Eighty women exercised three times per week for 45 minutes over a four year period. An aerobic program in the first year was

followed by the addition of specific upper extremity strengthening exercises in subsequent years. Both pre- and postmenopausal women in the exercise group demonstrated significantly higher BMC and BMD measures in the forearm than non-exercisers. Postmenopausal women who participated in exercise had lower rates of loss than non-exercisers for most measurements at the radius and ulna. Unfortunately the data were not presented in a manner that would allow comparisons of the rates of change over the 4 year time span. It is not clear, therefore, if the apparent effect of exercise on rate of loss was a transient phenomena or persisted for the entire study period. Also, because the intended intervention of interest appears to have been the aerobic component of the program, the exact nature of the upper extremity strengthening regime is not detailed. The true osteogenic stimulus is therefore uncertain.

Creating preferential loading to optimally stress the lumbar spine is a challenging task. Heikkinen et al. (1991) attempted this in 78 healthy recently postmenopausal women in an effort to reduce regional bone loss. Estrogen replete and deplete groups were assessed to determine the permissive role of estrogen in mediating the effects of exercise. Women were randomly allocated to either of two hormone replacement therapy groups, or a control group. Half of each group was allocated to either an exercise or non-exercise group. Each week, subjects in the exercise groups participated in a one-hour guided and two-hour independent program of exercises involving weights and bending. No increase in lumbar BMD could be demonstrated above that induced by hormone replacement therapy. It may be argued that further increases were unlikely, due to limitations of remodeling changes, but a similar lack of effect in response to exercise was also observed in the control group. Interestingly, no loss was noted in the control group over the one year period. Lack of supervision of much of the exercise routine and hence compliance may also have hindered this study. Smidt et al. (1992) also found no effect of a

progressive resistive exercise program for the trunk muscles in preserving lumbar BMD in postmenopausal women. The prescription for each exercise was determined as 70% of the maximal moment of force (Nm) measured on a dosimeter, and the imposed resistance progressed throughout the year of testing. An increase of 20 to 30% in strength confirmed compliance. In spite of this, both groups experienced a decline in BMD at the lumbar spine and proximal femur over the year of intervention, and there was no difference between the groups.

There have been some attempts to incorporate the use of free weights and weight training systems such as Nautilus, to examine the effects of more generalized resistive training routines on BMD at a number of sites. Notelovitz et al. (1991) examined the effects of a year of a circuit training regimen in conjunction with HRT, on bone loss from the mid radius, whole body and whole spine (assessed from subregions of the whole body scan) in a small group of women. Compared with HRT-only controls, those that exercised increased bone mass significantly at the spine and radius. The increases were, however, excessive (+8% and +4% respectively), casting some doubt on the validity of the findings.

Peterson et al. (1991) examined bone mineral changes at a number of sites in a mixed group of pre- and postmenopausal women. Menopausal status was not detailed. Sedentary controls were compared with women who chose to supplement a previously attended dance class with an upper body weight routine, and a third group who attended the dance class only. At the end of one year, there were no significant differences between the groups for change in BMD at the lumbar spine, proximal femur, radius, or humerus. An effect on BMD of the dance routine may not be expected because this did not represent a change in activity at the onset of the study, and there was no indication of a progression in intensity of this component. Similarly, the weight routine focused solely on the upper extremities, so may be unlikely to exert significant effects on the lumbar spine or

femur. In the upper extremities, although the free weight routine was accompanied by increases in strength, this was not accompanied by increases in BMD. The increase in strength over the year was modest, averaging 7% among muscle groups tested, and the activity may have been insufficient to induce a change in bone mass.

Recently, McCartney et al. (1996) followed elderly women randomized to exercise or sedentary control groups for two years. The exercise group participated in a bi-weekly progressive exercise routine of weight training for the upper and lower extremities. No increases were noted in BMD of the whole body or lumbar spine in the exercise group, although the control group demonstrated significant increases. The weight training routine may not have adequately stressed the lumbar spine area, and unfortunately, BMD at the hip and radius was not examined. The reason for the increases in the BMD of the control group is uncertain, although in this age group, osteoarthritic changes may have played a part.

The studies described above suggest that some resistive exercise programs may exert positive site specific effects on BMD. Where present, however, the positive results are relatively modest, particularly when compared with differences observed between sedentary and athletic groups in cross-sectional studies, or with the rapid losses that have been described in response to disuse. Inadequate training stimulus may be involved. Difficulties arise in determining the true *in vivo* loads experienced at different skeletal sites during different types of exercises, and long-term compliance in an adult population can also be a great hindrance to testing the effects of time consuming exercise routines.

Many adult exercise intervention programs often include only healthy and hence reasonably active women. Within this group, it may be difficult to achieve a sufficiently high activity level to induce an osteogenic response. Frost would describe this as the minimal effective strain to induce remodeling. This theoretical threshold may differ

immensely from the minimal effective strain to prevent bone loss. We cannot be certain at what point an individual may reduce habitual activity to the point of encountering the minimal effective strain for bone loss. It is possible that not only age, but hormonal and genetic factors may alter this threshold, placing some individuals at higher risk of exacerbating bone losses by relative disuse. Regionally, the forearm appears to respond well to site-specific exercise, even (or particularly) in elderly women, perhaps due to lower habitual strains encountered in daily activities, and therefore a greater opportunity for large increases in externally applied forces during structured exercise.

Table 4.1. The effect of physical activity on bone mineral mass in postmenopausal women; studies with a primary aerobic component.

Source	*Mesurement Site*	*N (per group)*	*Exercise*	*TIme*	*Outcome*	*Change*	*Comments*
1988 Cavanaugh and Cann	lumbar spine	1) 8 exerc 2) 9 contr	walking 60-85% VO_{2max}	52 wk 3x/wk	QCT	ns	early postmenop.
1988 Dalskey et al	lumbar spine	1) 17 exerc 2) 18 contr ages 55-70	walk, jog, treadmill; stairs, cycle, row, bench press	9 m or 22 m 3x/wk	DPA	+5.2% exerc +6.1% exerc -1.4% contr	not randomized; detraining also examined; some on estrogen
1992 Grove and Londeree	lumbar spine	1) 5 high impact 2) 5 low impact 3) 5 contr	high impact or low impact aerobics plus sit-ups, hip abduction etc. as warm-up and cool-down	1 yr 3x/wk	DPA	1) + 2% 2) 0% 3) - 6%	group size very small; excessive bone loss in control group
1993 Bloomfield et al	lumbar spine, femoral neck	1) 7 exerc 2) 7 contr	stationary cycling at 60-80% of maximum heart rate	8 m 3x/wk	DPA	Lumbar +3.55% exerc -2.44% contr Femur: ns	ns change in VO_{2max} due to low statistical power; not randomized; no current HRT
1993 Martin and Notelovitz	lumbar spine, prox and distal radius (midshaft and distal)	1) 20 exerc 2) 16 exerc 3) 19 contr	treadmill 70-80% VO_{2max}	1 yr 3x/wk	DPA SPA	ns change over time or between groups	improved VO_{2max}; lesser bone loss in women < 6YSM but few women
1995 Bassey and Ramsdale	lumbar spine (AP and lateral), prox femur, and distal radius	1) 20 exerc 2) 24 contr	1) heel drops and high impact aerobics 2) low impact and stretching exercises	1 yr daily	DXA	ns between groups; except women > 6 yrs postmenopause	loss in BMD only observed at the distal radius; maintenance at other sites
1995 Prince et al	lumbar spine, prox femur, and distal tibia	1) 42 exerc 2) 84 calcium 3) 42 contr	2 hours unspecified supervised weight bearing exercise (in a class) plus recommended 2 hr/wk walking 60% peak HR	2 yr 4x/wk	DXA	Femoral Neck +0.28% exerc -0.18% calcium -0.67% placebo	randomized design; only 39% compliance with exercise; changes not different from baseline; all apparent gains within first 6 months of treatment

exerc = exercise, contr = control, prox = proximal, ns = not significant

Table 4.2. The effect of physical activity on bone mineral mass in postmenopausal women; weight training studies.

Source	*Site*	*n*	*Exercise*	*Time*	*Outcome*	*Change*	*Comments*
1987 Simkin et al	distal forearm	1) 14 exerc 2) 26 contr	loading distal forearm	5 m 3x/wk	Compt.scat.; SPA BMC	+3.8% exerc - 1.9% cont	poor precision of SPA BMC
1989 Smith et al	radius,ulna (1/3 site), and humerus	1) 80 exerc 2) 62 contr	dance, walk, jog; light weights (not specified) introduced after one year	4 yr 3x/wk	SPA	right radius -0.65%exerc -1.67%contr	pre- and postmenopausal; reduced loss at radius and ulna; BMD for controls obtained at a different time
1991 Heikkinen et al	lumbar spine	6 groups - 35 exerc (3 groups) - 35 non (3 groups)	weight training for lumbar and lower extremities; home walk, jog	1 yr 1x/wk wts; 2x/wk aerobi c	DXA	ns exerc vs. non-exerc; improved with HRT	two different regimes of HRT, plus no HRT, half of each group exercising; no loss in control group
1991 Notelovitz et al	whole body and whole spine from subregions	1) 9 exerc +HRT 2) 11 HRT only	circuit training utilizing Nautilus; 8RM; low back, leg extension, pullover, torso arm, calf raises negative chin, hip and back, triceps, biceps, leg curl	1 yr 3x/wk	DPA	<u>Whole body</u> exerc +2.1% HRT 0.6% <u>Spine</u> exerc +8.3% HR 1.5% <u>Mid radius</u> exerc +4.1% HRT +0.3%	surgical menopause; mean age 43.3 yrs; larger than expected increase in lumbar spine BMD in exercise group
1991 Peterson et al	lumbar spine, femur,prox and distal radius, and humerus	1) 18 exerc 2) 17 exerc 3) 19 contr	1) upper body free weights + dance 2) dance 3) sedentary	1 yr 3x/wk	SPA and DPA	ns change in BMD	pre- and postmenop, not examined separately; home weight program; all previously enrolled in dance component
1992 Smidt et al	lumbar spine, and prox femur	1) 22 exerc 2) 27 contr	sit-up, double leg raise, prone extension at 70% of max	1 yr 3- 4x/wk	DPA	ns any site; loss in both groups	strength gains 21-30%
1996 McCartney et al	lumbar spine, and whole body	1) 57 exerc 2) 56 contr	weight training: unilateral military press, leg press, ankle plantarfl and dorsifl, bench press, biceps curls, modified abdominal curls	2 yr 2x/wk	DPA	<u>Whole Body</u> +3.6% contr <u>Lumbar</u> +3.8% contr	significant 2 yr increases in BMD only in control group; exercise group demonstrated increased in muscle area and performance on exercise tests

exerc = exercise, contr = control, prox = proximal, ns = not significant, RM = repetition maximum

CHAPTER 5

CALCIUM AND CALCIUM ABSORPTION

Approximately 99% of body calcium is stored in the skeleton predominantly as crystals of calcium phosphate or hydroxyapatite $[3Ca_3(PO_4)_2 \cdot (OH)_2]$. Calcium thus comprises 27% of bone mineral (Oser 1965), and the requirement for a positive calcium balance throughout skeletal growth is self-evident. Yet, it seems the relationship between calcium intake and bone mass remains controversial (Cumming 1990; Hegsted 1994; Kanis and Passmore 1989; Nordin and Heaney 1990; Prince 1993).

5.1 World-Wide Data on Fracture

Part of this controversy stems from the fact that the direction of the relationship between world-wide calcium intakes and hip fracture rates appears to be counter-intuitive. In fact, the data suggest that countries such as Hong Kong and Singapore, with habitually low intakes of calcium, experience lower rates of hip fracture than many countries such as the United States, Britain or Sweden, with relatively high calcium intakes (Chalmers and Ho 1970; Hegsted 1986). The data might be interpreted to suggest that poor calcium intake somehow protects against osteoporotic fracture (Hegsted 1994). Bone mass, however, tends not to be increased in all nations with low rates of hip fracture, and it has been reported that the prevalence of vertebral compression fractures is not reduced in Chinese women relative to women of North America. The apparent protection from hip fracture may be the result of differences in bone architecture and geometry, or in prevalence of falls.

5.2 Calcium Intake and Fracture

Within populations or ethnic groups, there is support for a positive relationship between calcium intake and bone mass and an inverse relationship between intake and fracture rate. Matkovic et al. (1979) found a higher peak cortical bone mass at the metacarpal site, and lower prevalence of hip fracture, in a region of Yugoslavia where calcium intakes were high (average 876 mg per day for women) when compared with a region with low calcium intakes (average 394 mg per day for women). There were, however, additional differences in total energy and nutrient intake that may have contributed to the observed differences in fracture rate. Lau et al. (1988) found that the odds of fracture were almost doubled for Hong Kong women who consumed less than 83 mg of calcium per day compared with women who consumed 244 mg per day or greater. The mean intake level of dietary calcium for this population was less than 200 mg per day. Other aspects of nutrition were not addressed.

Hip fracture patients may exhibit multiple nutritional inadequacies that increase their risk not only of future fracture but of other clinical complications and premature death. Delmi et al. (1990) found that administration of an oral supplement, providing 20 g protein, 525 mg calcium, and additional nutrients to hip fracture patients during hospitalization improved outcomes, reduced hospital stays, and lowered 6-month mortality by 74%. High calcium intake, when reported by the elderly in cross-sectional studies, may signify better nutrition and better health. Alternatively calcium supplementation may in some way influence falling, improve muscular reaction time or exert some other physiological influence independent of an effect on bone mass.

A positive influence of total caloric intake was not supported by the results of a 14-year prospective study of the incidence of hip fracture in a Southern Californian

retirement community (Holbrook et al. 1988). In women, increased caloric intake was associated with increased incidence of fracture. Hip fracture was reduced, however, among those adults with a baseline calcium intake in the upper tertile (greater than 765 mg per 1000 kcal; 24-h recall method) compared with those in the lowest tertile (less than 470 mg per 1000 kcal). Interestingly, calcium intake (and the intake of numerous other nutrients) was reported to be highly correlated with caloric intake (data not shown). This suggests that fracture rate may have been higher in those with greater absolute intakes of dietary calcium. Alternatively, higher caloric intake may have been an indication of higher physical exertion and greater risk taking activity.

Chapuy et al. (1992) found that calcium and vitamin D supplementation (1.2 g and 800 IU respectively) reduced the risk of hip fracture in nursing home residents by 43% and the incidence of nonvertebral fractures by 32%. Baseline calcium intake was in the order of 500 mg per day. However, because milk products are not supplemented with vitamin D in France, the vitamin D status of these women may have been low, and the separate influence of dietary calcium is uncertain.

Chevalley et al. (1994) found that 18 months of calcium supplementation reduced bone loss at the femoral shaft and reduced the incidence of vertebral fracture in vitamin-D replete elderly. Over the 18-month intervention, the detection of new vertebral fractures was lower in adults who received calcium (and had no history of hip fracture) than in placebo-treated individuals. Among patients with previous hip fracture, all of whom received calcium, the incidence of vertebral fracture was highest (144/100,000), in spite of a significant increase in lumbar BMD. This suggests that the additional fracture risk associated with history of previous fracture is, in part, independent of the risk due to low bone mass. The study design did not include a placebo-treated hip fracture group, which might then have made possible a comparison of fracture statistics according to calcium

intake for this high-risk subpopulation of elderly. For the entire study, although fracture rates differed among groups, the actual numbers of new vertebral fracture cases were low (4 to 8 per group).

5.3 Calcium Intake and Bone Mass: Cross-Sectional Studies in Adults

The relationships detected between calcium intake and BMD or rate of bone loss in peri- and postmenopausal women are somewhat less impressive than the fracture data. Again this is somewhat counter-intuitive if we expect that the main influence of calcium intake is on skeletal mass. It may be difficult to detect relationships between bone mass and calcium due to imprecise measurement of dietary intake or bone mass, or because the stages of life which are chosen to explore the relationships are dominated by other major physiological events.

A recent analysis of the skeletal status of a North Argentina population in a region with a habitually low calcium intake (mean 356 mg per day) found that men and women of this region had higher bone mass at the proximal femur than did reference populations drawn from other regions of Argentina (Spindler et al. 1995). It was suggested that habitually low calcium intakes could not therefore be considered detrimental to bone mass. Unfortunately, the average calcium intakes of the Argentinean reference populations were not reported, and the sample size of the target population was relatively small (n = 78) considering the age range studied (ages 20 to 80).

Data from the Rancho Bernardo trial demonstrated in a group of older women (ages 60 to 79) that milk consumption during adulthood (ages 20 to 50, or 50+), was more strongly related to BMD of the proximal femur than was milk consumption during skeletal growth (ages 12 to 18) (Soroko et al. 1994). The study did not examine the effects of consistency of intake, or the influence of changing from high to low or from low

to high milk consumption during life. The relationship of BMD with milk consumption after age 50 was generally slightly weaker than with milk consumption from age 20 to 50. This may be because declining milk consumption with age results in a greater contribution of calcium supplementation to total calcium intake. This would result in an apparent reduction in the strength of the relation between BMD and calcium from diet (milk) alone. In support of this explanation, a recent study by Ulrich et al. (1996) reported positive associations between calcium supplement use after age 60, and total body or peripheral BMD in older women (mean age 72). A significant relationship between milk consumption and BMD was not detected in these women, or in their premenopausal daughters. Supplement use was described as a major source of calcium for the older women. The types of commonly-used supplements and habit of intake (i.e. with meals or fasting at bedtime) were not detailed. At odds with these findings are those of Bauer et al. (1993). They found a very weak association between current unsupplemented dietary calcium intake (past year) and BMD of the distal radius in women 65 years of age or older. The use of calcium supplements, or total intake including supplements, was not significantly associated with BMD, however. Again, the types and habitual pattern of supplement use are unknown. If supplements were poorly absorbed, this would reduce the association between bone mass and total calcium intake.

Recently, Uusi-Rasi et al. (1998) reported that whole body BMC was higher in a group of older women (age 60 to 65 years) with a mean calcium intake of 1475 mg per day than women with calcium intakes of 638 mg per day. The cross-sectional moment of inertia and section modulus of the radial shaft were also significantly higher in older women with high calcium intakes. In 2 groups of younger women (ages 25 to 30 and ages 40 to 45) these differences were not observed. No effect of calcium intake was noted at the distal radius or femoral neck among any age group. Because consistency of dietary

habit (regular milk consumption as a child) was reported by most women (98%) with high calcium intakes, the authors concluded that lifetime sufficiency of calcium intake could improve long-term competence of the skeleton. The authors also reported, however, that the vast majority (94%) of the women in the low calcium group also reported regular milk consumption as children. The results could therefore be interpreted to suggest that older women may influence their skeleton negatively by reducing their calcium consumption, regardless of early intake. The effects of recent versus lifetime calcium intakes cannot be determined by this study. For example, there are no data to examine the effect of changing from low calcium intake in childhood to high intake in adulthood.

A cross-sectional study by Andon et al. (1991) reported positive correlations between calcium intake in older women (mean age 65) and BMD of the lumbar spine ($r = 0.19$, $p < 0.03$). Dietary calcium and body weight were independent predictors of BMD in stepwise (forward) multiple regression analysis, and women with calcium intakes above the mean (average 878 mg per day) had BMD values 8% greater than those with calcium intakes below the mean (average 402 mg per day). The division by calcium intake was, however, decided post hoc. There may, therefore, have been other important differences between these subgroups.

5.4 Calcium Intake and Bone Acquisition

As with a number of factors, calcium has the potential of influencing the risk of osteoporosis by impacting upon either peak acquisition or bone loss. It has long been recognized that deficiencies in calcium intake may influence skeletal development and mineralization in non-primate mammals and non-human primates (Jowsey and Raisz 1968; Pettifor et al. 1984). In man, malnutrition is known to influence skeletal size (Matkovic et

al. 1993), but the influence of a fine interplay of low to moderate intakes of various nutrients is less certain.

A direct influence of nutrient intake on growth is suggested in early infancy. Premature infants do not demonstrate equivalent gains in bone mass in the early weeks after birth, that term infants apparently achieve in utero (Kleerekoper et al. 1981). This deficit, however, may be attributable to a number of factors including the increased energy cost associated with respiration, poor gastric motility and inadequate total nutrient supply, and the influence of corticosteroid medications used during the weaning process from respirator support. The growth deficit appears to be largely reconciled in infancy, although it may persist in excess of 6 months. In childhood and adolescence, nutritional deprivation may also retard growth and skeletal acquisition, but multiple nutritional deficiencies are involved. Restoration of appropriate nutrient intake may permit a certain amount of "catch-up" growth (Matkovic et al. 1993), although the final impact on genetic potential is uncertain.

In normal children, Ruiz et al. (1995) found calcium intake to be an independent predictor of vertebral BMD before puberty, and that most children (84 to 95%) with vertebral or femoral densities more than one standard deviation below the age-adjusted norm, had calcium intakes of less than 1000 mg per day. Among children with bone densities within one standard deviation of the age-adjusted norm, or above the norm, only 60% demonstrated calcium intakes below 1000 mg/d. In the Netherlands, however, VandenBergh et al. (1995) failed to detect a relationship between calcium intake and bone mineral content of the midphalanx in primary school children with average daily calcium intakes exceeding 1200 mg (interquartile range 900 - 1400 mg per day). Slemenda et al. (1994) similarly found no relationship between calcium intake determined by yearly diet diaries and changes in BMD determined over 3 years, at the radius, femur, and spine in

children and young adolescents. In young Finish adolescents and women, Valimaki et al. (1994) demonstrated a trend toward an increase in femoral BMD with increasing intake of calcium. Women consuming greater than 800 mg per day of calcium demonstrated femoral neck BMD values 4.7% higher than those consuming less than 800 mg per day. There was no apparent additional benefit at very high levels of intake (> 1200 mg per day) however. Calcium intake was not an independent predictor of BMD in multiple regression analysis, after inclusion of age, weight, and exercise. This may be due to the relatively small sample size (n = 153 women), due to threshold behaviour (a non-linear response) of BMD to calcium intake, or because subgroups divided according to calcium intake were not matched for the other characteristics of weight and age which were determinants in multiple regression analysis.

In a 3-year placebo-controlled intervention trial of well-nourished monozygotic twin pairs, Johnston et al. (1992) found that supplementation with 1000 mg calcium per day increased bone mineral density in children who were prepubertal. There were no differences in weight gain or longitudinal growth between supplemented and non-supplemented twins. Similarly, among Chinese children with habitually low intakes of dietary calcium, Lee et al. (1994) found calcium supplementation to positively influence gain in bone mass at the radial shaft, but have no influence on gain of body weight or height. Bonjour et al. (1997) found that a 1-year program of calcium supplementation in prepubertal girls resulted in incremental increases in BMD at radial and femoral cortical sites, but not at the lumbar spine. Height but not weight, was also increased in the supplemented group. The greatest benefits were seen in girls with initial calcium intakes below the median intake of 855 mg per day (mean 650 mg versus 1143 mg).

After puberty, calcium intake may influence skeletal consolidation and the maintenance of peak bone mass prior to the onset of menopause. Picard et al. (1988) used

a food frequency questionnaire and 3-day food records to investigate the dietary histories and current dietary habits of 183 French-Canadian premenopausal women (mean age 44). Dietary intake of calcium averaged 576 ± 383 mg per day. Calcium intake from age 20 demonstrated modest but significant univariate correlations with bone mass at the distal 1/3 radius and lumbar spine (r = 0.18 and 0.24 respectively). Calcium intake also remained a significant predictor of lumbar BMC and BMD, along with body weight and height, in multiple regression analyses. Exercise habit, smoking, alcohol use, caffeine, parity and contraceptive use were not related to bone mass.

5.5 Calcium Intake and Bone Loss after the Menopause

Ultimately fracture risk depends not only on the peak bone mass, but on the subsequent bone loss. In adults, current and recent dietary habits may be more strongly related to the rate and magnitude of bone loss rather than acquisition. This would explain the stronger relationships of BMD with recent and current diet as opposed to childhood diet, in cross-sectional studies of older adults. Support for this theory from prospective studies has, however, tended to be unconvincing.

Stevenson et al. (1988) failed to detect a relationship between calcium intake and baseline total body calcium, or BMD of the lumbar spine, distal or proximal radius in 59 recently postmenopausal women (< 5 years since menopause). Similarly there was no relationship between calcium intake and 1-year change in BMD. This was an intervention trial involving treatment with calcitonin or estradiol, versus placebo. Interestingly, there was a lack of effect of the two treatment regimes, which is unusual. This may indicate that the computed tomography technique employed (reported precision of 2%) was unable to detect the small changes in bone likely to occur within a 1-year period.

Jones et al. (1994) failed to detect a relationship between dietary calcium intake and BMD of the femoral neck or lumbar spine in a 2.5-year study of the rates of change in BMD in older men and women (mean age 71 years). In women, there appeared to be a negative effect on BMD of the interaction between increasing age and increasing dietary calcium. The apparent deleterious effects of high calcium intakes on BMD in these older women may simply be due to the phenomenon of increased supplement use by women who have identified themselves to be at high risk for osteoporosis.

The results of intervention trials using calcium supplementation, have demonstrated more favourable effects on bone loss, but their effectiveness appears to be stronger with increasing time since menopause (Cumming 1990). At the menopause obligatory calcium loss increases, as evidenced by higher urinary calcium excretion (Nordin and Polley 1987), and loss of bone mineral occurs. During the early postmenopausal period, increases in dietary calcium appear incapable of preventing bone loss. Nilas et al. (1984) supplemented early postmenopausal women with 500 mg calcium daily for 2 years. Bone loss at the distal radius continued at a rate of 1.6 to 2% per year in spite of supplemented dietary calcium intakes ranging from 1000 to 2000 mg. calcium daily, and there were no differences in rate of bone loss according to final supplemented intake. Unfortunately, there was no control group against which to compare these rates of loss. In a study of the effectiveness of 2 doses of estrogen and calcium supplementation, Ettinger et al. (1987) found that calcium alone was unable to prevent cortical bone loss at the metacarpal or trabecular bone loss at the spine in 41 recently menopausal women. Compared with controls, 2-year loss was slightly reduced at the proximal radius.

Although calcium supplementation may not entirely eliminate bone loss, it may be capable of reducing the rate of bone loss (Horsman et al. 1977; Riis et al. 1987). In a double-blind, two-year controlled study of a small group of Danish women, Riis et al.

(1987) examined the effects of calcium supplementation and HRT on BMD. Bone loss was found to be reduced at cortical but not trabecular sites in response to calcium supplementation in the order of 2,000 mg per day. Interestingly, biochemical indices of bone turnover were not reduced at 24 months in those taking calcium. Calcium absorption (estimated by a single-isotope method) was reduced at 12 months, but not after 24 months of treatment. Together these may suggest long-term non-compliance with treatment. Baseline calcium intake was not determined for this group.

In a randomized controlled trial, Elders et al. (1991) found that bone loss from the lumbar spine was reduced in both perimenopausal and postmenopausal women supplemented with 1000 or 2000 mg of calcium daily for 2 years. Baseline calcium intake was initially high (mean 1150 mg per day), yet bone loss was not entirely eliminated in the early postmenopausal group. Most of the reduction in bone loss occurred within the first year of study. This may again suggest a waning of compliance, or may be explained by filling of the remodeling space due to the initial suppression of bone turnover (Parfitt 1988).

In a placebo-controlled study, Dawson-Hughes et al. (1990) found that for early postmenopausal women (mean 3 years since menopause), 2-year bone loss was not statistically different between placebo and calcium supplemented groups, although there was a trend toward a reduction in radial bone loss in supplemented groups. For women greater than 5 years post menopause (mean 13 years), however, calcium supplementation significantly retarded bone loss at the spine, femoral neck and radius, in women whose initial calcium intake was below 400 mg per day (mean 274 mg per day). Women with baseline intakes greater than 400 mg per day already demonstrated a low rate of bone loss at the spine and no significant loss at the femur or radius. This was not altered by supplementation. Women with high and low intakes did not differ in baseline BMD or

body mass index. Although physical activity was measured, differences between the low versus high calcium intake groups were not reported. In this study, calcium citrate-malate was more effective in reducing bone loss than calcium carbonate. Subjects were instructed to ingest the supplements at bedtime, however, rather than with a meal. This may have limited absorption of calcium carbonate in some women.

In a 2-year placebo-controlled trial, Prince et al. (1991) studied the effects of an exercise regimen, exercise plus 1 gram daily calcium supplementation, and exercise plus HRT in a group of osteopenic postmenopausal women (mean age 56 years; 1 to 10 years since menopause). Exercise plus HRT was the most effective in preventing bone loss or increasing bone mass at both proximal and distal forearm and lumbar sites, but exercise plus calcium supplementation also reduced bone loss significantly from the distal forearm when compared with exercise alone or no-treatment controls. Although the exercise routine could be considered a confounder, the program was modest in intensity and may have suffered from poor compliance. Only one weekly session was supervised, and only 56% of the subjects demonstrated a good attendance at these sessions. The results support the effectiveness of calcium supplementation, either as an exclusive influence, or in combination with moderate exercise. In this study, the relationship between number of years since menopause and rate of change in BMD at the distal forearm was positive (either a greater increase in BMD or a slower rate of loss) in the exercise-estrogen and exercise-calcium groups . The authors suggested that this implied greater effectiveness of the estrogen and calcium interventions in older women. Rate of bone loss is expected to decline with years since menopause, however, and the authors did not present the determinants (and their coefficients) of the rate of change in BMD for the exercise-only and placebo groups to permit comparison of the influence of this factor. There is no indication that the results were examined by subgroup according to years since

menopause, or that results were examined for non-linear trends or interactions that might suggest a change in behavior of the intervention according to years since menopause.

The pattern of skeletal response to supplementation may be illustrated by two recent trials. Reid et al. (1993) reported positive results of a 2-year program of supplementation in a placebo-controlled trial of a group of women who were a minimum of 3 years postmenopausal (mean 9 years). Compared with controls, bone loss in the treatment group was reduced at the whole body, lumbar spine and proximal femur (Ward's triangle). In a 2-year extension of the trial, rates of bone loss continued to be reduced for the whole body. At the lumbar spine and proximal femur, however, after the initial reduction in the first half of the study, loss continued approximately parallel to that of the control group. The incidence of new vertebral fracture was also reduced in the treated group. Very similar results have been reported in a newly completed 4-year placebo-controlled trial from the Mayo Clinic (Riggs et al. 1998b). By 1 year, supplemented and placebo groups differed significantly in the change in lumbar BMD from baseline. This between-group difference narrowed by trial completion. Between-group differences in proximal femur BMD appeared to peak by 18 months, then remain stable throughout the remainder of the study (suggesting bone was lost in the treatment group at a rate equivalent to the placebo group). The between-group differences in total body BMD were reported by the authors as increasing between year 1 and year 4, which gives the impression of a sustained reduction in the rate of bone loss from the whole body. Further examination of the illustrative figure in the report, however, reveals that the between-group differences in total body BMD appeared to peak at year 2, and were then maintained (equivalent rate of bone loss for treatment and control groups) for the final 2 years.

The results of intervention trials support a positive acute effect of calcium supplementation in older women, but are unable to establish its long term efficacy. The recent studies may indicate that trabecular bone responds to supplementation in a manner that is rapid but poorly sustained, whereas the response of more cortical regions is delayed and perhaps more protracted. This may explain some of the discrepancies among studies, several (Elders et al. 1991; Ettinger et al. 1987; Riis et al. 1987) but not all (Prince et al. 1991) of which detected a more obvious effect of calcium supplementation at cortical bone sites and a waning of effect or no effect at trabecular sites. Trabecular sites may be more easily perturbed by short term variations in a number of factors including hormonal levels, physical activity, or body weight, or diet, making it more difficult to detect trends due to diet over a 1 or 2 year period.

There has been some criticism that the response of bone in supplementation trials may be largely a phenomenon of a transient remodeling response to pharmacological intervention (Kanis and Passmore 1989; van Beresteijn 1994) and, as suggested by recent trials, may not be maintained. These trials, although supporting the potential use of supplements in treatment, may not be representative of the role that dietary habit plays in long term bone health. Others have argued that prospective studies examining the relationship between dietary habit and bone loss have little hope of demonstrating any true effect of calcium deficiency due to limitations in the assessment of dietary intake as well as neglect of the influences of population variability in calcium absorption and obligatory calcium losses (Avioli and Heaney 1991; Heaney 1993a). Neglect of these influences on calcium balance may result in misclassification of individuals whose calcium sufficiency is determined purely on the basis of dietary intake (Heaney 1993b). Additionally, due to the characteristic behaviour of normal calcium absorption, the relationship between calcium intake and bone loss may not be linear. Awareness and adjustment for these factors

should improve the ability to detect a true relationship between dietary calcium and change in bone mass.

5.6 Calcium Balance/Calcium as a Threshold Nutrient

Calcium supplementation may be most effective in individuals with low baseline intakes because it behaves as a threshold nutrient. A threshold nutrient demonstrates a positive correlation with some physiological outcome when its levels are below a pre-defined level (of intake). Above that level, little relationship is observed between nutrient intake and physiological outcome. This may imply a plateauing of a positive skeletal response to the nutrient, or an attenuation of a negative skeletal response to nutrient insufficiency (i.e. resolution of deficiency). The question of whether calcium behaves as a threshold nutrient at low versus high intakes may be difficult to determine without lengthy prospective studies to demonstrate that high intakes generate a sustained positive or neutral skeletal response, and large numbers to determine with fair accuracy the average intake at which balance becomes negative.

The intervention study by Dawson-Hughes (1990), as well as some of the international data, suggest that the influence of calcium is most evident at relatively low calcium intakes. This may suggest a threshold effect. Under such circumstances, the body could adjust to the wide range of calcium intakes observed in western society and demonstrate little or no skeletal response because calcium balance remains neutral. At very low intakes, perhaps 400 mg per day or less, the body is unable to adapt, calcium balance becomes negative, and bone loss corresponds to the degree of deficiency.

Adult skeletal bone loss in postmenopausal women implies a negative calcium balance (net loss greater than intake). It has been suggested that calcium balance for this population occurs at fairly high levels of intake i.e. 800 mg or greater (Heaney et al.

1978). Under these circumstances, calcium intake should be correlated with changes in bone mass over the wide range of intakes commonly observed in most western societies. The response to supplementation should also be positive in most normal postmenopausal women. The validity of data from traditional balance studies has been questioned, however, because of the complexity of accurately assessing fecal losses (Heaney et al. 1977; Isaksson and Sjogren 1967). The errors generated may not be random, but may tend to yield positive results more readily at high intakes (Hegsted 1994). Calcium balance studies have also been criticized because they are unable to predict adaptive responses to changes in usual levels of intake (Hegsted 1994). The potential role that long term adaptation plays is also largely ignored in intervention trials.

An additional physiological factor which may interfere with the detection of a correlation between intake and bone loss is the body's ability to adjust its digestive absorptive efficiency. As calcium intake rises, absorptive efficiency declines (Heaney et al. 1990b; Lutwak 1969), and any response to supplementation should be quantitatively less.

5.7 Calcium Absorption

Calcium absorption occurs by two routes. The first, passive diffusion, occurs when calcium concentration in the intestinal lumen is greater than the concentration of diffusible calcium in plasma, approximately 1.5 mM (Ireland and Fordtran 1973). The second route is the active transfer of calcium through the enterocyte, regulated by 1,25-$(OH)_2$ vitamin D in response to parathyroid hormone and ultimately serum ionized calcium. This is presumed to be mediated in part by the induction of calcium-binding protein (calbindin-D_{28k}) synthesis. Increases in cytosolic levels of the protein may facilitate calcium diffusion from the brush border to the basolateral membrane (Dunn et al. 1995). At the basolateral membrane, Ca^{2+} ATP-dependent pumps are involved in

extrusion of the Ca^{2+} ion against an electrochemical gradient (nanamolar concentrations inside the cell; 1.2 millimolar concentrations outside the cell). The density of these pumps may also be increased under circumstances such as chronically low calcium intake or elevated PTH; circumstances that are normally recognized to increase intestinal absorption (Wasserman et al. 1992).

The active portion of absorption is saturable at high intakes. Because of the regulation of this active component, the fraction or percentage of calcium absorbed from the intestinal lumen tends to decline as habitual dietary intake increases, and conversely increase at very low levels of calcium intake (Birge et al. 1969; Ireland and Fordtran 1973). The relationship between calcium intake and calcium absorbed is therefore curvilinear in shape (Heaney et al. 1975; Ireland and Fordtran 1973; Nordin et al. 1979)

Different portions of the digestive apparatus demonstrate varying degrees of absorptive capability. The rate of absorption is greatest in the duodenum; 3X, or if corrected for length, 9X greater than the remainder of the gut. However, the jejunum and ileum may be more responsive to regulation in response to changes in PTH (Birge et al. 1969), and some calcium may be absorbed from the large intestine. The rate of calcium absorption as determined by kinetic studies of oral calcium tracers, is highest during the first hour following ingestion. The majority of the isotope is absorbed, yielding maximal serum concentrations, within 2½ hours in normal subjects (Birge et al. 1969).

5.8 Factors Influencing Intestinal Calcium Absorption

There are two types of influences on calcium absorption; those that acutely influence the amount of calcium absorbed from a meal or supplement due to interactions in the gut, and those that influence absorptive mechanisms in an adaptive response that contributes to calcium homeostasis.

Acute Influences

The measured intestinal calcium absorption in normal adults has been reported to range from approximately 14% to 50% depending on the calcium carrier and calcium load used in the test (Heaney et al. 1990b; Mautalen et al. 1969). Although with increased calcium load, the fraction absorbed decreases, the total amount absorbed increases due to a combination of active and passive absorptive processes (Heaney et al. 1990b; Recker and Heaney 1985). Calcium preparations vary in their solubility, particularly in environments that are of neutral pH or low acidity (Heaney et al. 1990a; Sheikh et al. 1987). Low gastric acidity, achlorhydria (gastric pH 7 or greater), occurs in about 10% or more of elderly (Recker 1985). In this population, preparations such as calcium carbonate, may be poorly absorbed if taken in a fasting state. Taken with meals, however, these differences do not appear to interfere greatly with absorption (Bo-Linn et al. 1984; Recker 1985)

Calcium availability may also be influenced by other nutrients within a particular food or other nutrients within a meal. The calcium in spinach, in the form of calcium oxalate, is poorly absorbed. Other factors, such as the carbohydrate or fat contents of the meal may have some influence (Kelly et al. 1984). It has been suggested that lactose improves absorption, but this has not been substantiated in all instances. High fiber diets may reduce absorption by up to 20% (Knox et al. 1991), a factor which may have relevance in light of recent nutritional trends. It has been estimated that increasing daily fiber from 10 g per day to 20 g per day, might cause a reduction in calcium absorption of 6 to 10% (Heaney 1993b).

Chronic Influences

Calcium absorption is directly influenced by the levels of 1,25-$(OH)_2$ vitamin D due to its influence on transport of calcium across the enterocyte and extrusion at the basolateral membrane. Vitamin D acts as part of the calcium homeostatic mechanism, responding to serum parathyroid levels which in turn are responsive to serum calcium levels. Pathological conditions which interfere with any portion of this pathway, such as occurs in renal failure, result in lowered calcium absorption. Evidence that this is a chronic rather than acute regulatory system stems from the results of kinetic studies which find no response of absorption within 4 to 5 hours of injection of parathyroid hormone, although in chronic hyperparathyroidism, absorption is increased (Birge et al. 1969).

Calcium absorption declines with age, and the ability to adapt to chronically low calcium intakes may decline with age (Avioli et al. 1965; Bullamore et al. 1970; Gallagher et al. 1979; Ireland and Fordtran 1973). This may be due to a number of mechanisms including vitamin D insufficiency, declining renal function, or resistance to the actions of vitamin D at the enterocyte (Francis et al. 1983; Francis et al. 1984). A decline in absorption, estimated in the order of 2 to 3%, has also been demonstrated to occur at the menopause (Heaney et al. 1989) and may be related to the decline in estrogen levels. Treatment with estrogen therapy has been shown to increase calcium absorption as measured by a dual isotope technique (Gallagher et al. 1980b). In the same study, serum 1,25-$(OH)_2$ vitamin D levels were also increased in response to treatment. Animal studies have demonstrated a reduced concentration of 1,25-$(OH)_2$ D receptor in aged rats compared with young rats (Horst et al. 1990)

Some (Gallagher et al. 1979) but not all (Francis et al. 1984; Lutwak 1969; Nordin et al. 1968) studies have found calcium absorption to be reduced in osteoporotic patients, relative to normal subjects.

5.9 Calcium Excretion/Secretion/Dermal Loss

Calcium balance is determined not only by intake and absorption, but also by the extent of calcium losses through intestinal, renal and dermal routes.

Obligatory calcium losses in urine are estimated at about 150 mg per 24 hours (Nordin et al. 1979) although lower excretions have been reported (Spencer et al. 1964). This value is determined by the plasma concentration of filterable calcium (approximately 5.5 mg/dl, 1.4 mmol/l), glomerular filtration rate (about 100 ml/min; about 8,000 mg filtered calcium per day), and the limited capacity of the kidney for calcium reabsorption (about 97.5%) (Breslau 1996). Although most calcium is reabsorbed in the proximal tubules, the regulated component of absorption largely involves the 5 to 10% of calcium reabsorbed in the distal tubules.

Obligatory calcium losses also occur at the intestine. Endogenous fecal calcium is the fecal calcium attributable to failure to reabsorb calcium that is secreted in digestive juices or passively diffuses into the intestinal lumen. This calcium is lost even at zero dietary intakes. Estimates of this loss range from 80 to 110 mg per day (Nordin et al. 1979) or greater (Heaney and Skillman 1964).

Together obligatory urinary and fecal calcium losses are about 250 mg per day, and this determines the minimum value for true calcium absorption to maintain balance. At an absorptive efficiency of 40%, this would establish minimum dietary intake required to ensure calcium balance, at just over 600 mg per day. This value has not, however, taken into consideration possible calcium loss through other systems.

Dermal losses (sweat and desquamated epithelium) have been estimated at 15 to 25 mg per day (sedentary) (Gitelman and Lutwak 1963; Lentner et al. 1975). Inclusion of losses in hair, nails and other secretory losses (menstrual, respiratory, ocular) may increase

this figure to 60 mg per day (Charles et al. 1983). This estimated loss has been determined from the difference between 2 radioisotopic retention curves of intravenously administered ^{47}Ca; one determined by the method of whole body counting and another simultaneously calculated from the cumulated daily excretions of urine and feces over a 7 day period. This method may overestimate losses due to errors resulting from incomplete urine and fecal collections, or reduced detection of the retained isotope due to redistribution to deeper portions of the body (i.e. spine). It is therefore difficult to accurately determine the magnitude of this component of calcium loss, although it is generally understood that variations in environment such as climate (temperature) or intense activity may impact the extent of these losses (Charles et al. 1983).

5.10 Dietary Influences on Intestinal Secretion and Renal Excretion

Phosphorus

Endogenous fecal calcium loss increases slightly at higher calcium intakes (Nordin et al. 1979) and may be increased under certain dietary conditions such as increased intakes of dietary fiber or phosphorus. In the case of phosphorus, because high dietary phosphorus is associated with increases in PTH and subsequently reduced urinary excretion of calcium, minor variations in phosphorus intake may have little short term influence on overall calcium balance (Hasling et al. 1992; Heaney and Recker 1982).

The secondary hyperparathyroidism which may accompany chronically high phosphorus intakes, particularly in the presence of low calcium intakes, may have a deleterious effect on bone mass. Primates fed low calcium, normal phosphorus diets show evidence of increased bone resorption, whereas those fed diets low in both nutrients demonstrated a mineralization defect but no evidence of increased bone turnover (Pettifor et al. 1984). Chronically high phosphorus intakes may also suppress production of plasma

1,25-$(OH)_2$ vitamin D levels by suppression of renal 1-alpha hydroxylase enzyme in the kidney (Portale et al. 1986). In adult humans, however, a strong link between dietary phosphorus and bone mass or bone loss has not been demonstrated in population studies.

Protein

Protein intake has been repeatedly linked with urinary calcium excretion. A doubling of protein consumption results in about a 50% increase in urinary calcium (Kerstetter and Allen 1994). The increased urinary excretion appears to be attributable to both an increase (up to 20%) in glomerular filtration rate (GFR) as well as reduced reabsorption in the distal renal tubule (Hegsted and Linkswiler 1981). Current theory suggests these changes are a response to acids generated in the metabolism of sulfur containing amino acids, although other mechanisms may also be involved (Draper 1990; Kerstetter and Allen 1994). Renal reabsorption may be hindered as weak complexes are formed between calcium and inorganic sulfate in the renal filtrate. In plasma, acute metabolic acidosis has been recognized to reduce the binding of serum calcium to albumin and thus increase serum ionized calcium (Broadus 1993). An increase in ionized calcium at the expense of protein-bound calcium would increase the ultrafilterable component of calcium in plasma and could increase the usual obligatory renal losses that result from imperfect reabsorption in the renal tubules. Increasing serum ionized calcium also has the potential to stimulate the parathyroid-renal (PTH-1,25$(OH)_2$D) axis to maintain homeostasis. Inhibition of PTH would result in reduced reclamation of calcium from the distal renal tubule. It has also been suggested that calcium may be liberated from bone along with phosphate to permit the participation of phosphate in the buffering of an acid load (Wachman and Bernstein 1968). The excess serum calcium would be subsequently eliminated in the urine. A high phosphorus intake may blunt the hypercalciuric response to

high dietary protein due to its influences on PTH, but may not fully restore calcium balance because higher endogenous fecal calcium losses occur in response to higher phosphate intakes (Heaney and Recker 1982).

Epidemiologically, nations with high intakes of animal proteins tend to demonstrate a higher incidence of hip fracture over the age of 50 (Abelow et al. 1992). Although cross-cultural data may be considered inaccurate due to reporting biases and the confounding effects of numerous cultural differences, reasonable agreement is demonstrated between the epidemiological data and results of studies of calcium balance and calcium excretion. In a summary of 16 human studies, Kerstetter and Allen (1994) were able to demonstrate a linear relationship between protein intake and urinary loss of calcium. In young adult women, a negative relationship has been demonstrated between dietary intake of protein and bone mass or density of the distal forearm (Metz et al. 1993). Opposing results have been reported for young Japanese women. Hirota et al. (1992) found protein intake to be positively related to forearm BMD. Protein intake was below the population RDA of 60 gm per day. BMD was also associated with better dietary habits and higher caloric intake. Similarly, in the frail elderly, protein intakes may not show the expected relationship with bone mass or rates of fracture. This raises the possibility that low protein intake in adulthood could directly increase fracture risk. It may also serve as a marker for poor nutrition, poor self care or poor health generally.

Caffeine

High caffeine intake may also be associated with increased urinary calcium and intestinal calcium excretion (Heaney and Recker 1982), but an impact of caffeine consumption on bone mass or fracture incidence has not been definitively established. Harris and Dawson-Hughes (1994) demonstrated accelerated bone loss in a subgroup of

postmenopausal women with high caffeine intakes (3 or more cups of coffee or 7.5 cups of tea) and dietary calcium intakes below the mean for the study (744 mg per day). This subgroup was significantly lower in self reported physical activity, however. There was also no evidence of a dose effect for caffeine intake at lower levels.

5.11 Summary

Although there appear to be numerous acute and chronic influences on calcium availability and balance, it may be useful when examining the calcium status of older women to estimate both calcium intake and absorption. Calcium intake should be estimated across a time span which reflects dietary habits which have influenced recent bone turnover and will help identify those at risk of accelerated bone loss due to deficiency. Simultaneous dietary assessment of other influences on calcium balance, such as protein and phosphorus intakes, may also assist in the identification of those women with generally poor dietary habits. Calcium absorption should be assessed because it determines the amount of calcium made available to the skeleton. An assessment of intestinal calcium absorption also reflects the current adaptive state of the organism to the quantity of bioavailable calcium in the usual diet. In older individuals it may help identify those with inadequate adaptive abilities.

Adjustment of dietary calcium intake for absorptive efficiency should improve the ability to detect weak relationships between this nutrient and either bone mass or bone loss, and identify those at risk for accelerated bone loss due to calcium insufficiency. Due to the limitations in sample size imposed due to the scope of the proposed study, we felt it imperative to maximize our detection of a bone-calcium relationship by including a measurement of fractional absorption.

CHAPTER 6

VITAMIN D

Vitamin D_3, also called activated 7-dehydrocholesterol or cholecalciferol, is an antirachitic vitamin produced in the skin by the actions of ultraviolet light (UVB) on a cholesterol precursor, 7-dehydrocholesterol (7-DHC, provitamin D_3). It requires further metabolic conversion before exerting biological activity. The metabolic product 25-hydroxycholecalciferol [calcidiol or 25-(OH)D_3] is the major circulating metabolite (McKenna and Freaney 1995). Its daughter metabolite, biologically active 1,25-dihydroxycholecalciferol [calcitriol or 1,25-$(OH)_2D_3$], is involved in calcium homeostasis, maintaining adequate serum levels by acting on the intestine to increase absorptive efficiency, and on bone to liberate calcium through increased resorption.

6.1 Cutaneous Production of Vitamin D_3

Stores of 7-dehydrocholesterol are present in the epidermis and dermis. When exposed to sunlight, 7-DHC is converted to previtamin D_3 by energy wavelengths between 290 and 315 nm. Thermal energy then induces isomerization of previtamin D_3 to vitamin D_3.

Most conversion of 7-DHC to previtamin D_3 occurs in the epidermis. Both dermal concentrations of 7-DHC and the capacity to produce previtamin D_3 decline with aging (MacLaughlin and Holick 1985). Vitamin D production is limited by other factors besides age. Use of sunscreens may block vitamin D production, as does glass or Plexiglas. In northern latitudes such as Boston or Edmonton, sunlight is unable to promote dermal production of vitamin D during winter months (Holick 1995). At the latitude of southern

Ontario (Hamilton, approximately 43 degrees north) this period of inadequacy lasts from November through February. During this time, the body relies on fat stores of the vitamin and dietary sources to fulfill the vitamin requirement. Seasonal variations in serum concentrations of 25-(OH)D have been well documented (Woitge et al. 1998)

6.2 Dietary Sources of Vitamin D

Dietary sources of vitamin D_3 (cholecalciferol) include liver oils of various fish (including cod liver oil), butter and egg yolk. Vitamin D_2 (ergocalciferol) is derived from plant and yeast sources, and is used to fortify some food products, such as milk, in North America. Although slightly different in chemical structure, vitamins D_2 and D_3 function similarly in terms of biological potency (Holick et al. 1998). Food fortification would appear then to be a simple solution to inadequate solar exposure and cutaneous production of this vitamin. There is evidence, however, that standards of fortification have not always guaranteed consistency of vitamin content in North American milk products. A 1992 testing of milk samples and infant formulas revealed that a large proportion of both products failed to contain the vitamin content claimed on the label. Vitamin D (absence of subscript refers to either vitamin D_2 or D_3) concentrations tended to be lowest in low fat milks and were undetectable in some samples (Holick et al. 1992). Inadequate fortification increases the potential for vitamin D insufficiency, particularly in housebound or institutionalized elderly with limited solar exposure.

6.3 Metabolism

Vitamin D_3 produced in the skin, and vitamin D_2 entering from the intestine, are transported in the circulation bound to vitamin-D-binding protein, an alpha-globulin. In the liver, vitamin D is hydroxylated on carbon 25 to produce 25-(OH)D (absence of

subscript refers to D_2 or D_3 metabolites). Because this enzymatic conversion is not tightly regulated, levels of 25-(OH)D fluctuate in response to sunlight exposure and dietary intake, and are considered an indicator of vitamin D sufficiency (Holick et al. 1998; McKenna and Freaney 1995). Although 25-(OH)D is capable of binding to the vitamin D receptor in various tissues, its potency is several orders of magnitude less than that of the metabolite 1,25-$(OH)_2D$ (Lawson 1980). The second hydroxylation that results in the production of the biologically more active product, 1,25-$(OH)_2D$, occurs in the kidney. It is at this site that action of a mitochondrial cytochrome P450-linked enzyme, 1α-hydroxylase, is tightly regulated. Although α-hydroxylation may occur in other tissues, evidence for a dominant renal role comes from the observation that anephretic animals have extremely low levels of this metabolite (DeLuca and Schnoes 1983).

6.4 Mechanism of Action

The mechanism of action of 1,25-$(OH)_2D$ is largely that of a steroid hormone. Being lipid soluble, it passes easily through cell membranes and binds to a nuclear receptor in its target cells, although it may also have non-nuclear regulatory effects in some tissues (Nemere 1996). The vitamin D receptor (VDR) has been the subject of considerable recent attention. The vitamin D receptor is a nuclear localized phosphoprotein with an N-terminal zinc finger DNA binding domain, and C-terminal domains for binding hormone and for binding the retinoid X receptor with which it forms a heterodimer (Haussler et al. 1995). Polymorphisms (alleles) of the VDR have been recognized, and these variations may contribute to the heritable component of the population variability in bone turnover and bone density. How VDR polymorphisms influence the cellular response to 1,25-$(OH)_2D$ is at this point uncertain.

In Australian studies, receptor alleles identified by specific endonucleases appear to have functional significance, influencing circulating osteocalcin levels (Morrison et al. 1992) and bone density (Morrison et al. 1994). A North American study confirmed that rates of bone loss were greater in some VDR genotypes (Krall et al. 1995). The variance between genotypes was attenuated in women taking calcium supplementation, however, perhaps due to greater absolute absorption of calcium at the gut through passive diffusion rather than vitamin D-dependent mechanisms.

At the intestine, 1,25-$(OH)_2D$ increases calcium absorption by increasing synthesis of calcium transport proteins in the cell cytosol as well as calcium pumps on the cell surface (Wasserman et al. 1992). In response to enhanced resorption, increases in serum ionized calcium then inhibit the synthesis and release of PTH, as well as decrease the production of additional 1,25-$(OH)_2D$ by reducing 1α-hydroxylase activity in the kidney. Through this negative feedback regulation, 1,25-$(OH)_2D$ is critically involved in maintaining calcium homeostasis.

In bone, it appears the influence of 1,25-$(OH)_2D$ could favour either formation or resorption. 1,25-$(OH)_2D$ increases production of Type I collagen and other matrix proteins by osteoblasts, enhancing bone formation (Anderson 1996). 1,25-$(OH)_2D$ and PTH act in concert to recruit monocytic stem cells to become osteoclasts, ultimately increasing bone resorption (DeLuca and Schnoes 1983; Holick et al. 1998). Vitamin D may promote bone mineralization through maintenance of an adequate calcium-phosphorus product. Infusions of calcium have been demonstrated to be effective in the prevention and treatment of the skeletal effects of vitamin D deficiency, and treatment of genetic target tissue resistance to 1,25-$(OH)_2D$ (vitamin D-dependent rickets type II) (Balsan et al. 1986; Holick 1996)

6.5 Effects of Vitamin D Deficiency

The influence of vitamin D on skeletal integrity can be realized through observation of the deleterious effects of deficiency. The effects of deficiency have been well described. In children, inadequate vitamin D results in rickets, a disorder characterized by a chaotic array of hypertrophic chondrocytes at the growth plates. There is expansion of the epiphyseal plates with inadequate mineralization of both cartilage and bone.

In the adult, when longitudinal bone growth has ceased, vitamin D deficiency results in osteomalacia, a condition characterized by an increase in unmineralized osteoid at sites of normal remodeling. The clinical picture is one of diminished radiographic density, focal areas of radiographic translucency known as Looser's zones, insufficiency fractures, bone pain (Chalmers et al. 1967), and proximal muscle weakness (Schott and Wills 1976).

The pathophysiological events surrounding deficiency appear to be largely a response to inadequate intestinal calcium absorption due to insufficient activity of the active metabolite, 1,25-$(OH)_2$D. The resulting homeostatic response required to maintain adequate serum calcium, contributes to skeletal pathology.

In response to a decline in serum calcium, the synthesis and secretion of PTH is elevated; a mechanism to restore and maintain calcium homeostasis by actions at the kidney and on bone. In bone, PTH causes an increase in the number and aggressiveness of osteoclasts, which may contribute to age-related bone loss and a clinical picture of osteoporosis (Holick 1996). PTH actions at the kidney, while conserving calcium, cause an increased phosphorus diuresis and a lowering of serum phosphorus. This may contribute to a low calcium-phosphorus product and inadequate mineralization of existing osteoid (Holick 1996).

Biochemical changes typical of osteomalacia are a lowering of plasma calcium and phosphorus, and an elevation in plasma alkaline phosphatase. Because of diminished intestinal calcium absorption and the calcium conserving influences of PTH at the kidney, urinary calcium excretion is also generally reduced, with values for 24-hour urinary calcium ranging from 0.25 to 3.0 mmol (normal range 2.0 to 7.5 mmol per 24 hours) (Chalmers et al. 1967). Elevated serum intact PTH may also be a useful but not exclusive early marker of disease, if accompanied by other biochemical or radiographic features of osteomalacia (Bingham and Fitzpatrick 1995; McKenna and Freaney 1995). Diagnosis is confirmed by bone biopsy in which there is histological evidence of excess osteoid in undecalcified sections of trabecular bone.

Osteomalacia has been recognized as a periodic complication of intestinal malabsorption syndromes, specific pathology of the renal tubules or chronic renal failure (Chalmers et al. 1967). Symptomatic osteomalacia is commonly associated with serum 25-(OH)D concentrations under 12.5 nmol/l (Bouillon et al. 1987), although hypovitaminosis D has been defined as a serum level below 25 nmol/l (McKenna 1992). In the elderly it has been suggested that low levels of 25-(OH)D (hypovitaminosis D) may result in secondary hyperparathyroidism and perhaps asymptomatic osteomalacia which may complicate existing osteoporosis or be mistaken for it (Chalmers et al. 1967; Holick 1996). It is important therefore to confirm that there exists a reasonable association between serum 25-(OH)D and bone mass or clinical pathology commonly attributed to age-related osteoporosis.

6.6 Seasonality and the Risk of Hypovitaminosis D in the Elderly

A seasonal variation in serum concentrations of 25-(OH)D has been observed repeatedly in North American and European populations (Lester et al. 1977; Scharla et al.

1996; Stamp and Round 1974). This speaks strongly for the role of sunlight exposure in ensuring vitamin D sufficiency. In those who are sunlight deprived, or in cultures where sunlight exposure is avoided, seasonal rhythms may be reduced and serum calcidiol levels may be correlated with estimates of dietary intake. The reverse may also be true. Krall et al. (1989) found that dietary intakes above 220 IU of vitamin D per day by postmenopausal women were sufficient to obscure the seasonal variations in 25-(OH)D levels that were obvious in women with lower dietary intakes. In this healthy ambulatory group of women (mean age 58; range 43-71), the contribution of stored vitamin D resulting from summer solar exposure to wintertime 25-(OH)D levels is, however, uncertain. Declining kidney function with age may also necessitate higher intakes in the elderly.

Community dwelling elderly in North America demonstrate 25-(OH)vitamin D levels similar to their European counterparts during the winter season, but are at a slight advantage in the spring and fall. The winter nadir is more pronounced in the elderly than in young adults, and 25-(OH)D levels in this group may be suboptimal in both North America and Europe, in spite of the supplementation of milk with ergocalciferol on this continent (McKenna 1992) and the supplementation of butter and oils with ergocalciferol in the Netherlands (van der Wielen et al. 1995). In North America, an estimated 25% of elderly have hypovitaminosis D (serum 25-(OH)D levels below 25 nmol/l) in the winter.

Although there has been general concern for the vitamin D status of institutionalized elderly due to the potential for limited sunlight exposure and poor eating habits (Chapuy et al. 1996; Corless et al. 1975; Pun et al. 1990), hypovitaminosis D has not been universally noted in this important subpopulation of older adults. Disparate results may be attributable to differences in dietary intake in the study populations, or differences in control groups. Kinyamu et al., (1997) found that 25-(OH)D levels did not

differ between young, elderly, and sunlight deprived institutionalized elderly in Omaha, NB. Dietary intake of vitamin D-containing foods was actually higher in the institutionalized women than the other groups, however, and equivalent to the RDA of 200 IU per day. The study was conducted during the winter months which would tend to minimize any differences in 25-(OH)D attributable to sunlight exposure, but maximize the detection of deficiency. The authors did note that in spite of apparently adequate 25-(OH)D concentrations, 1,25-$(OH)_2$D levels were lowest in the institutionalized women and calcium absorption was decreased. This may be the result of declining renal function i.e. inadequate renal response to PTH. Serum concentrations of PTH increased with age, being significantly higher in institutionalized elderly, and were also negatively correlated with serum 25-(OH)D levels.

In a group of 391 men and women over age 65, Dawson-Hughes et al. (1997) found that when 25-(OH)D fell below 110 nmol/l, PTH was inversely correlated with 25-(OH)D, suggesting that bone turnover may be increased when vitamin D status falls below a threshold level that is much higher than that required to avoid symptomatic osteomalacia. Differences between the sexes did not exist during the winter, but did during the summer suggesting that older women are less likely to take advantage of exposure to summer sun. Wintertime travel (south), however, was able to attenuate the wintertime decline in 25-(OH)D concentrations.

In a very recent North American study by Kinyamu et al. (1998), serum PTH levels in a group of 245 women over age 65, were found to correlate inversely with calcium intake derived from milk sources but not with calcium intake derived from non-milk sources. Serum calcidiol also correlated positively with dietary calcium ($r = 0.33$, $p < 0.001$), particularly calcium from milk sources ($r = 0.35$). In multiple regression analysis, serum PTH was best predicted by serum 25-(OH)D, which was best predicted by calcium

intake from milk sources. These findings suggest that in the presence of adequate dietary intake of calcium (mean 666 mg per day in this group), that milk as a dietary source of vitamin D may be an important regulator of serum PTH. Hypovitaminosis D, defined in this study as serum 25-(OH)D levels < 30 nmol/l (12 ng/ml), was observed in 4% of women not taking supplements.

Hypovitaminosis D may be more common in populations with low habitual calcium intakes (Clements et al. 1987). Adaptation by an increase in serum PTH and subsequent elevation in 1,25-$(OH)_2$D levels may result in more rapid clearance of 25-(OH)D from the blood due to activation of oxidative enzymes such as 24,25-hydroxylase in the liver and other peripheral tissues (Clements et al. 1992). Thus 25-(OH)D is converted to metabolically inert metabolites and eliminated. It is possible, however, that other aspects of poor nutrition, particularly low protein, may induce this sequence of events. Kerstetter et al. (1997) fed diets equivalent in energy intake and a variety of nutrients including calcium, but varying in protein content, to 16 young adult women. Although urinary calcium excretion was increased in response to the high protein diet, the low protein diet induced elevations in serum PTH and 1,25-$(OH)_2$D.

These findings suggest that there is a risk for declining vitamin D status in the elderly, and that this as well as other nutritional inadequacies may perturb the vitamin D-endocrine axis. There is also evidence to suggest that with advancing age or osteoporosis, some individuals may experience maladaptive alterations in the normal homeostatic response to changes in serum calcium.

6.7 Alterations in Calcium Homeostatic Mechanisms in the Elderly

Several authors have noted alterations in the vitamin D-endocrine axis with age that suggest a decline in the ability of the body to adapt to a low calcium challenge.

Gallagher et al. (1979) failed to detect differences in calcium intakes or serum 25-(OH)D levels between age-matched normals and osteoporotic patients (n = 20 and 27 respectively). Among osteoporotic patients, however, serum iPTH (amino terminus 1-84; n = 10) was elevated and serum 1,25-$(OH)_2D$ levels were significantly reduced (n = 12), suggesting inappropriate control of 1α-hydroxylation in the kidney. Conversely, Slovik et al. (1981) noted no difference in baseline 1,25-$(OH)_2D$ levels, between elderly osteoporotics and young controls, but reported a blunted increase in 1,25-$(OH)_2D$ in the older group in response to 24-h PTH infusion. Tsai et al. (1984) failed to note a decline in 25-(OH)D levels with age or osteoporosis in a small sample of pre- and postmenopausal women (n = 8 to 10 per group). Serum iPTH was higher and 1,25-$(OH)_2D$ was lower in the older groups, however. Also, the response to a 24-h infusion of PTH was blunted, declining with age and glomerular filtration rate. This suggests that declining renal function may be an important determinant of abnormal vitamin D metabolism in older adults.

Prince et al. (1997) reported differences in homeostatic responses to a 4-day low calcium diet (170 mg per day plus an ingested calcium binder) among a small group of osteoporotic women (n = 17) and age-matched controls. At baseline, osteoporotic women had higher plasma levels of PTH compared with age-matched normal subjects, and had higher values for the bone resorption indices, hydroxyproline and deoxypyridinoline. Serum 25-(OH)D was lower in osteoporotics (185 versus 218 nmol/l) than normals, but was not deficient and the difference did not reach statistical significance. In response to a low calcium diet, osteoporotic women demonstrated a blunted increase in both PTH and 1,25-$(OH)_2D$, as well as the fractional absorption of strontium (a surrogate measurement for intestinal calcium absorption). Measurements of 1,25-$(OH)_2D$ were not continued past the 4-day time point to determine if calcitriol levels and intestinal absorption would

also eventually adapt to dietary intake in the osteoporotic women. Interestingly, the rise in 1,25-$(OH)_2D$ levels was correlated with the baseline 25-(OH)D levels in both osteoporotics and controls ($r = 0.45$; $p = 0.007$) suggesting that substrate availability may continue to play an important role in the homeostatic response to calcium deficiency in this group. The results are also in general agreement with theories that support relative hyperparathyroidism as a contributing factor to senile osteoporosis, and with studies of intestinal calcium absorption that report the capacity to adapt to low calcium diets by increasing absorptive efficiency decreases with advancing age (Gallagher et al. 1979) and estrogen deprivation (Heaney et al. 1989). A weakness of the study is the fact that no time was given for equilibration to the test diet following the measurement of baseline values, and prior to the measurement of the response to the test diet. The measured responses therefore reflect an equilibration period. In the early few days this period may also be influenced by individual differences in intestinal transit time (which determines the proportion of the intestinal contents which reflect either the pre-test or test diet). The true response to steady state low calcium diet is therefore uncertain.

6.8 Evidence for Hypovitaminosis D in Fracture Patients

A progressive increase in serum PTH and decline in serum 25-(OH)D levels with age has been noted in most recent cross-sectional studies of pre- and postmenopausal women (Khosla et al. 1997). Raised PTH and low concentrations of vitamin D metabolites have also been noted in elderly hip fracture patients compared to non-fracture controls (Boonen et al. 1997; Dawson-Hughes et al. 1997). Hip fracture patients also frequently demonstrate signs of general nutritional inadequacy (lower BMI and lower serum albumin), however (Boonen et al. 1997). 1,25$(OH)_2D$ levels, corrected for vitamin D binding protein in this population, may fall within the normal range. Other studies

support a link between hip fracture and vitamin D insufficiency, however. Hip fracture rates across the United States demonstrate a seasonal periodicity (winter peak, summer nadir) which is independent of latitude, age or sex (Jacobsen et al. 1991). Lack of a dependency on latitude suggests that ice and snow play a relatively minor role in these seasonal variations. Although vitamin D levels in young adults and the elderly are typically lower in Europe than North America, seasonal variations occur in both continents and at both northerly and southerly latitudes (McKenna 1992; van der Wielen et al. 1995).

Hip fracture is considered one of the most debilitating consequences of osteoporosis, yet the frequency of osteomalacia in hip fracture patients is higher than in the normal population. In the United Kingdom, Aaron et al. (1974), identified osteomalacia in 37% of iliac crest biopsies from 134 hip fracture cases. The frequency of excess osteoid (greater than 24% of surface) and absent calcification fronts in biopsy specimens was greatest from February through April and lowest from August to October. Osteoid surfaces are, however, increased in instances of increased bone turnover, such as primary or secondary hyperparathyroidism, and in the absence of other discriminating features, some misclassification may occur. Because PTH levels may be elevated in vitamin D deficiency, however, these histological uncertainties may not require abandonment of hypovitaminosis D as a putative etiological factor.

A relationship between vitamin D status and other osteoporotic fractures has been described. In a case control study, Aloia et al. (1985) noted reduced 25-(OH)D and 1,25-$(OH)_2D$ levels in 58 women with vertebral crush fractures.

6.9 Vitamin D Status and Bone Mass

Significant correlations between 25-(OH)D levels and bone mass have not always been detected. Sowers et al. (1986) failed to detect a relationship between calcidiol levels

and bone density at the midradius in a population-based study of 20 to 80 year old women, although a relationship may have been obscured by the estrogen-related drop in bone mass at the menopause. Also, 25-(OH)D levels were measured only during the summer months, perhaps limiting the ability to correctly identify those women who might demonstrate deficiency during the winter. No instances of deficiency were identified in this group of 417 women, although a decline with age was identified.

Villareal et al. (1997) noted that women screened at an osteoporosis clinic who had serum 25-(OH)D levels less than 38 nmol/l had higher serum PTH and lower vertebral bone density as assessed by QCT. There was no similar correlation observed among women with 25-(OH)D in the normal range.

In a British study of 138 pre- and postmenopausal women, aged 45 to 65, BMD of the lumbar spine, femoral neck and femoral trochanter were examined for their relationships to serum PTH and 25-(OH)D (Khaw et al. 1992). Partial correlations were adjusted for age and body mass index. BMD, at all sites, was negatively associated with PTH (partial $r = -0.15$ to -0.18) and positively associated with 25-(OH)D (partial $r = 0.18$ to 0.22). When examined across the quartiles of BMD, the relation between bone density and PTH or 25-(OH)D demonstrated dose-response characteristics. This suggests that the influence of vitamin D status on BMD may be operative across a broad range of values (3.97 to 58.16 nmol/l).

In a population based study of 415 men and women between the ages of 50 and 80, Scharla et al. (1996) reported an inverse relationship between 25-(OH)D levels and urinary excretion of deoxypyridinoline crosslinks (a marker of bone resorption) in women ($r = -0.32$, $p < 0.01$ and $r = -0.24$, $p < 0.02$, summer and winter respectively). Positive correlations were detected between 25-(OH)D and BMD of the total proximal femur and femoral neck, in women. After correction for age and body mass index, correlations

remained statistically significant only during the summer months at the femoral neck ($r = 0.30$, $p < 0.01$). There was no association between 25-(OH)D and spinal BMD. Data were not analyzed separately for individuals with very low 25-(OH)D levels, and no adjustments were made for menopausal status.

In a group of 53 women an average of 12 years since menopause, Albanese et al. (1996) found that age and serum PTH were predictors of lumbar BMD in multiple regression analysis. Although serum 25-(OH)D did not enter the model as an independent predictor, it demonstrated the expected negative correlation with serum PTH ($r = -0.30$, $p = 0.05$). Again this is supportive, albeit not conclusive, evidence of a contribution of vitamin D status to bone mass in older adults.

6.10 Effect of Vitamin D Supplementation on Bone Loss

Daily oral 1,25-$(OH)_2D$ (calcitriol) has proven beneficial in controlled trials for the treatment of established osteoporosis by several (Aloia et al. 1988; Gallagher and Godgar 1990; Tilyard et al. 1992) but not all (Ott and Chesnut 1989) investigators. In a small 2-year controlled trial, 12 postmenopausal women with vertebral fracture received 0.8 μg per day dose of calcitriol and were compared with 15 women who received placebo (Aloia et al. 1988). Total body calcium, distal radius BMC, lumbar spine BMD, as well as the aluminum equivalence of the second to fourth middle phalanges were measured. At the radius, whole body, and phalanges (all cortical sites) there were small gains in the calcitriol treated group (0.56 to 1.26 % increase) and small losses in the placebo-treated group (0.73 to 1.39 % decline), resulting in significant differences between the groups. At the lumbar spine (considered a trabecular site), the differences were more pronounced. Calcitriol-treated women maintained their bone density, whereas the placebo-treated group experienced a loss in excess of 4%.

In a similar trial, using a lower average dose of calcitriol (0.43 µg per day), Ott and Chesnut (1989) failed to detect significant differences in the 2-year change in total body calcium, radius cortical bone density, or bone mass (BMC divided by vertebral height) of the lumbar spine. This lack of effect may be attributable to the low dose of calcitriol, or perhaps insufficient sensitivity of the measurement technique at the lumbar site.

Gallagher and Goldgar (1990) compared 0.62 µg per day calcitriol with placebo in a 2-year double-blind study of 40 women with non-traumatic vertebral fracture. Women also received a multivitamin supplement containing 400 IU vitamin D. Bone biopsies (tetracycline labeled) and histological studies excluded women with osteomalacia. Whole body BMD and lumbar BMD (from whole body scans) improved in the calcitriol group but decreased in the placebo group. Incidence of new vertebral fractures did not differ between the two groups likely due to the small number of events. Urinary hydroxyproline levels were reduced in calcitriol-treated patients.

In a 3-year multicenter trial of 622 postmenopausal women with established osteoporosis, women receiving 0.25 µg of calcitriol twice daily had a significant reduction in the rate of new vertebral fractures, compared with women receiving a 1 gm per day supplement of elemental calcium in the easily digestible form of calcium gluconate (Tilyard et al. 1992). Women with greater than 5 fractures at baseline failed to benefit from treatment. In these women, the architectural integrity of bone may have been so severely disrupted that bone strength could not be significantly influenced by treatment.

Chapuy et al. (1992) found that daily administration of 800 IU of vitamin D with 1.2 g elemental calcium for 18 months, to elderly nursing home and seniors apartment residents, significantly reduced the incidence of hip and other non-vertebral fractures when compared with placebo controls. France does not fortify dairy products with vitamin D.

This fact, in combination with limited sunlight exposure due to institutionalization, may put this particular group at higher than average risk for vitamin D insufficiency.

In women without severe kidney or liver dysfunction, treatment with the parent vitamin may be effective in reducing secondary hyperparathyroidism and reducing aging bone loss. In a 1-year trial of vitamin D supplementation in 142 elderly women and men in nursing and senior's homes, Lips et al. (1988) found that either 400 or 800 IU vitamin D daily improved serum 25-(OH)D levels and reduced serum levels of intact PTH-(1-84) by 15% (intra-assay CV = 8%). The improvement in PTH concentrations occurred in spite of baseline levels which were within the normal range (mean 3.46; normal range 1.0 - 10.6 pmol/l). Serum 1,25-$(OH)_2D$ levels were improved in subjects whose initial serum 25-(OH)D concentrations were below 30 nmol/l suggesting that at these levels, substrate availability was a limiting factor. Serum alkaline phosphatase and urinary hydroxyproline levels were not altered by vitamin D supplementation. Serum osteocalcin concentrations were improved in the nursing home residents only, mainly attributable to a substantial decline (greater than 50%) transiently at the 3-month interval for the group receiving 400 IU per day. The reason for this unusual event is uncertain. It appears not to be laboratory error since all samples were frozen and analyzed in a single batch at the end of the study.

Dawson-Hughes et al. (1991) studied the seasonal response of bone to vitamin D supplementation (400 IU daily) in a placebo-controlled trial of 249 older postmenopausal women (mean age 62; mean YSM 13) in Boston. Bone mineral density of the spine and whole body increased in the six month period from June or July to December or January, and decreased in the following six months. During the period of bone loss, loss at the spine was significantly greater in the placebo group than in the vitamin D supplemented group (-1.22% versus -0.54%, $p = 0.032$). For the whole body, similar seasonal variations in BMD were observed, but differences between placebo and control groups did not reach

statistical significance. Plasma 25-(OH)D levels were lower during the second measurement period in both groups, but more so in the placebo group. Plasma 1,25-$(OH)_2D$ levels were not influenced by season or by treatment. A wintertime increase in serum PTH was attenuated in the vitamin D-treated group. Seasonal changes in body composition were observed in both groups. During the period of bone loss, women tended also to lose lean tissue and gain fat mass. Changes in soft tissue composition were similar in the treatment and placebo groups, therefore the differences in rates of change of BMD between groups are unlikely to be attributable to measurement artifact.

In a subsequent 2-year trial many of the same women (n = 261) were randomly assigned to one of two vitamin D treatment groups (100 IU or 700 IU per day) plus 500 mg elemental calcium as calcium citrate malate (Dawson-Hughes et al. 1995). BMD of the femoral neck declined in both groups, but less so in the 700 IU group (-1.06 +/- 0.34% versus -2.54 +/- 0.37% in the 100 IU group) with most of the effect due to a partial attenuation of the wintertime loss in BMD during the first year. There was no significant bone loss at the spine or whole body in either group over the 2 year period. Seasonal variation in plasma 25-(OH)D and serum osteocalcin was evident in the 100 IU group but not the 700 IU group, yet both groups exhibited a seasonal variation in femoral neck BMD.

Freaney et al. (1993), examined the influence of season and vitamin D supplementation on the parathyroid axis and one marker of bone turnover, serum alkaline phosphatase, in a small group of community elderly men and women (mean age 75). Blood samples were drawn at baseline (wintertime; January-March), after 4 weeks of treatment, and again at the end of the summer. At baseline, hypovitaminosis D was prevalent among the group in that 86% of subjects had 25-(OH)D levels below 25 nmol/l. Mean intact PTH was also above the reference range at baseline (mean 6.3 pmol/l;

reference range 0.85 - 5.40 pmol/l). After supplementation with 800 IU vitamin D_3, parathyroid hormone levels were reduced significantly to 5.8 pmol/l, and 25-(OH)D levels improved. By the end of the summer, 25-(OH)D levels had continued to rise (only 32 % had levels below 25 nmol/l) and PTH concentrations to fall (mean 5.0 pmol/l). There were no significant changes in alkaline phosphatase. Although the decline in PTH was coincident with vitamin D therapy and seasonal improvement in calcidiol levels, the best predictor of PTH concentration was serum creatinine. This suggests that although substrate availability was a significant factor contributing to relative hyperparathyroidism, the ability of the kidneys to convert 25-(OH)D to the more active metabolite, 1,25-$(OH)_2D$, is also important.

The relationship between 25-(OH)D levels and those of the more active metabolite 1,25-$(OH)_2D$ is complex. Bouillon et al. (1987) found that 1,25-$(OH)_2D$ levels were influenced by brief (2 week) treatment with oral 25-(OH)D only in 10 elderly patients who were frankly vitamin deficient (25-(OH)D < 5 ng/ml or 12.5 nmol/l) but not in 8 elderly controls. However, in a larger group of 240 elderly patients (mean age 78 years) sampled on hospital admission and having a mean serum 25-(OH)D concentration of 72 nmol/l, a significant seasonal variation in 1,25-$(OH)_2D$ was observed. This suggests that concentrations of 1,25-$(OH)_2D$ could be influenced by substrate availability at levels above those normally considered deficient. Others, however, have found no relationship between circulating concentrations of 1,25-$(OH)_2D$ and 25-(OH)D among institutionalized and community elderly men and women (n = 44) who were not frankly deficient in spite of significant correlations between 25-(OH)D and PTH in the same population ($r = -0.40$, $p < 0.05$) (Webb et al. 1990). Unfortunately, not all 25(OH)- and 1,25-$(OH)_2D$ samples were drawn in the same month. In this population, most (73%; n =

6) of those with elevated PTH levels had 25-(OH)D levels below 37.5 nmol/l, and serum creatinine levels within the normal range.

Generally the findings suggest that there is increased risk of hypovitaminosis D in older adults when exposure to sunlight is reduced and dietary sources are inadequate. Declining kidney function may increase the risk of adverse effects resulting from decreased conversion of 25(OH)-D to 1,25-$(OH)_2$D. It appears, however, that increases in serum PTH, and therefore the risk of increased bone loss, may also occur in response to seasonal declines in 25(OH)-D, in spite of kidney function and 25(OH)-D levels which appear by common standards to be within population norms. Because of these risks and because of the intimate relationship between vitamin D metabolism and calcium absorption, it seems prudent to monitor vitamin D status in women approaching their elderly years.

We considered that in Southern Ontario, seasonal cutaneous production of vitamin D may be reduced in postmenopausal women who have minimal sun exposure; and dietary intake may be low in women who avoid milk products. We chose therefore to include a measure of vitamin D status as a candidate variable for predicting age-related bone loss in older postmenopausal women.

CHAPTER 7

METHODS

7.1 The Proposed Study

A prospective 2-year study was designed to test the hypothesis that there are at least 3 core factors which contribute significantly to the preservation, or conversely the loss, of bone mineral in normal postmenopausal women. These factors are: the adequacy of the supply of calcium to the skeleton, the influence of the endogenous production of estrogens by peripheral fat and other soft tissues, and the adequacy of habitual levels of physical activity as mechanical stressors. In the proposed study, these core factors and their subcomponents, as described below, were investigated for their independent ability to predict the rate of decline in bone mineral. Thus the null hypothesis was that the rate of change in bone mineral was not dependent upon any of these core factors. Failure to accept the null hypothesis would support the supposition that the rate of change in BMD is a function of the separate or combined influences of calcium intake, calcium absorption, serum estrogen, body mass, and physical activity.

Calcium intake and calcium absorption were considered representative of the calcium made available to the skeleton. Dietary intake of phosphorus and protein were included as possible confounding variables. Seasonal measurements of vitamin D were considered important to assess the prevalence of vitamin D insufficiency in the subject population, its impact on absorption and ultimately its influence on the rate of bone loss. Because of the possible impact of kidney function on the formation of the active metabolite of vitamin D, creatinine clearance was included as an estimate of glomerular filtration rate.

Evaluation of body mass, including its fat and lean components, was included as a surrogate of the metabolic potential of the body for the peripheral conversion of androgens to estrogens. Serum estradiol was measured to determine its direct contribution to change in BMD, as well as confirm its relationship to body mass.

Three components related to physical activity were included as possible predictor variables for change in BMD. Grip strength was included as representative of genetically determined muscle size as well as muscular development related to upper extremity activities. Aerobic fitness was measured to represent habits of regular repeated and sustained large muscle activity (most likely of the lower extremities). Cumulative daily movement of any intensity, and its influence on bone, was also evaluated using a portable accelerometer.

The rate of change in bone mineral density (BMD) of the whole body, proximal femur and the lumbar spine was evaluated using the technology of dual energy X-ray absorptiometry (Hologic QDR 1000W; Hologic Inc., Waltham, Mass.). This method was chosen due to the high reproducibility of the technique, the availability of the technology at reasonable cost, and the safety of the technique with respect to radiation exposure. Urinary hydroxyproline was included as a marker of bone resorption, and serum alkaline phosphatase as a marker of bone formation. Extreme values of these markers may suggest undetected metabolic bone disease. Relatively high values on one or both markers should reflect high rates of change in BMD.

Because of the exponential decline in BMD after the menopause, and the uncertainty of the consistency of the rate of change in BMD with age, the variables age and the number of years since menopause were considered as possible confounders in the determination of the relationship between rate of change in BMD and the core factors.

Finally, the data collected for the prospective study was also examined in a cross-sectional manner to determine if similar core factors also appeared to have had a long-standing influence on BMD.

7.2 Study Design

Rate of change in BMD was determined from repeated measurements over a 2-year time span. BMD of the lumbar spine (L1-4) and proximal femur (total hip, femoral neck, Ward's triangle, and trochanter) were measured semi-annually (analysis software version 4.57P). Whole body BMD was measured annually (whole body analysis software version 5.63). Body composition (fat mass, lean body mass, and percent fat mass) was assessed using the whole body scan mode of the densitometer. This schedule of visits was arranged to accommodate the 2-year limit of the study grant, to avoid overburdening study subjects or creating unnecessary radiation exposure, and to maximize the possibility of detecting a change in BMD. Power of the study design to detect change in BMD, and confidence limits around the anticipated rate of change in BMD (slope of the regression of BMD on time), were calculated based on the following estimates of machine precision.

Short term measurement error, expressed as a coefficient of variation, for lumbar spine measurements, *in vivo* with repositioning, is approximately 1% (Johnson and Dawson-Hughes 1991). This reflects the precision or reproducibility of the technique. In house measurement error for the femoral neck, Ward's triangle, and trochanter have been determined as 2.2%, 2.5% and 1.1% respectively, from repeated measurements on 21 young women (Chilibeck et al. 1994). Measurement errors of whole body BMD, fat mass and lean body mass were 1.1%, 1.8% and 1.4% respectively. Others have reported similar values for short-term precision of BMD measurements at the lumbar spine, femoral neck

and whole body, in postmenopausal women (Johnson and Dawson-Hughes 1991; Mazess et al. 1989).

The confidence interval for the rate of change in bone mineral over a limited 2-year time span is based upon the frequency of measurement and the precision of the technique at each site. At the femoral neck, assuming a precision of 2.2% or 0.021 gm/cm^2 (Chilibeck et al. 1994), the following argument shows that the 95% confidence interval for the slope is ± 4.3% of baseline BMD, if measurements are made at 0, 6, 12, 18, and 24 months. The confidence interval for the slope (β_1) of femoral neck BMD is determined from the equation (Payne 1988):

$$\hat{\beta}_1 \pm t_{n-2,1-\alpha/2} \times \sigma_{\beta_1}$$

where $\hat{\beta}_1$ is the sample estimate of the true slope, β_1, and $\sigma_{\beta 1}$ is the standard deviation of the slope of the fitted regression line. $\sigma_{\beta 1}$ may be estimated from

$$\frac{S_{Y/X}}{S_X \sqrt{n-1}}$$

where $S_{Y/X}$ is the measurement error or precision of the measurement, S_X is the standard deviation of the average of the length of time from baseline to each measurement of BMD (in years), and n is the total number of measurements of BMD. At the femoral neck, the confidence interval is calculated as follows:

$$\hat{\beta}_1 \pm 3.182 \frac{0.021}{0.79\sqrt{5-1}}$$

or

$$\hat{\beta}_1 \pm 0.042 \text{ gm/cm}^2 / \text{y}$$

In young women, in whom average BMD at the femoral neck is approximately 0.97 gm/cm^2, the confidence interval is equivalent to the rate of change ± 4.2% of baseline BMD. In older women, however, in whom BMD at the femoral neck may be approximately 0.7 gm/cm^2, the confidence interval is equivalent to the rate of change ± 6.0% of baseline BMD.

At the lumbar spine, assuming a precision of 1.0%, the 95% confidence interval for the rate of change is ± 2.0%. Extension of the period of follow-up by an additional 2 observation periods (1 year) would narrow the confidence interval for the slope of the rate of change in BMD of the lumbar spine to ± 1.1%.

The power (1-β) of the study to detect a specified change in BMD over the duration of the study is determined by the following calculation, where n is the total number of observations. Here σ represents the standard deviation of a single measurement of BMD within an individual at a single point in time. This is based on the normal distribution of the measured BMD (Y) at each time point (X), which again may be estimated by $S_{Y/X}$, the precision of the measurement. Δ is the expected total change in BMD. $Z_{1-\alpha}$ is the z-score, or number of standard deviations from the population mean, within which we are not willing to reject the null hypothesis (that there has been no change in BMD), and is determined by our chosen α level (usually $p < 0.05$). $Z_{1-\beta}$ refers to the z-score defining the power of the study to detect a true change from the null hypothesis. This is the number of standard deviations from the alternative population mean which would include the percent of this population likely to be identified by rejection of the null hypothesis.

$$n \geq \frac{2(z_{1-\alpha} + z_{1-\beta})^2 \times \sigma^2}{\Delta^2}$$

Substituting $S_{Y/X}$ for σ, this becomes

$$n \geq \frac{2(z_{1-\alpha} + z_{1-\beta})^2 \times S_{Y/X}{}^2}{\Delta^2}$$

If 2 measurements of lumbar BMD, with a precision of 1%, are performed at baseline and again at 2 years, the power of the model to detect a change in BMD of 2% is given by

$$4 = \frac{2(1.96 + z_{1-\beta})^2 \times .01^2}{.02^2}$$

$$8 = (1.96 + z_{1-\beta})^2$$

$$\sqrt{8} - 1.96 = z_{1-\beta}$$

$$z_{1-\beta} = 0.87$$

Looking this value up in a table of z-values, we find that the percent of the population defined by this value is 61%. Therefore the power of the study to detect a change in BMD of 2% at the lumbar spine is

$$1 - \beta = 61\%$$

If the number of measurements of BMD is reduced, and only one measurement is taken at baseline and again at 2 years, the power to detect a change in BMD is severely reduced as follows:

$$2 = \frac{2(1.96 + z_{1-\beta})^2 \times .01^2}{.02^2}$$

$$4 = (1.96 + z_{1-\beta})^2$$

$$\sqrt{4} - 1.96 = z_{1-\beta}$$

$$z_{1-\beta} = 0.04$$

$$1 - \beta = 3\%$$

However, if measurements are equally spaced throughout the 2 years, rather than clustered at baseline and the 2-year mark, power is improved. Calculation of power based on the uncertainty of the slope again is given by

$$n \geq \frac{2(z_{1-\alpha} + z_{1-\beta})^2 \times \sigma^2}{\Delta^2}$$

where σ is now the standard deviation of the slope, $\sigma_{\beta 1}$,and Δ is the expected rate of change in BMD (the slope, β_1). The power to detect a 1% change in lumbar spine BMD per year is calculated as

$$n \geq \frac{2(z_{1-\alpha} + z_{1-\beta})^2 \times \sigma_{\beta_1}{}^2}{\beta_1{}^2}$$

$$5 \geq \frac{2(1.96 + z_{1-\beta})^2 \times .0063^2}{.01^2}$$

$$\sqrt{6.31} - 1.96 = z_{1-\beta}$$

$$z_{1-\beta} = 0.55$$

$$1 - \beta = 42\%$$

Although this power is less than that achieved by clustering measurements at the beginning and termination of the study, evenly spaced measurements may reduce the influence of cyclical fluctuations in BMD, such as might occur due to seasonal influences, and isolated perturbations or shifts in measurement (Heaney 1986). Regular monitoring may also maintain subject interest and reduce attrition.

7.3 Sample Size Calculations

The main independent predictors under investigation for their relationship to the rate of change in bone loss were calcium intake, calcium absorption, serum estrogen concentration, body mass, and the level of physical activity. Assuming a minimum of 5 subjects (observations) per predictor variable, the minimum enrollment was set at 25 subjects. Because of the potential for interactions and collinearity between predictor variables, as well as the subdivision of some of the major predictors into subcomponents and related variables (i.e. physical activity becomes grip strength, aerobic fitness or habitual daily activity; body mass is subdivided into its components; inclusion of vitamin D as a separate variable), this minimum was increased to 15 subjects per initial core variable for a total of 75. Assuming a drop-out rate of 20%, target enrollment was set at 94.

7.4 Subjects

Women, 2 years or more post-menopause and not on estrogen replacement therapy, were recruited in response to advertisements distributed by posters, newspaper, and cable TV in the general community. Separate advertisements invited responses from interested women who considered themselves to be "somewhat underweight" or "somewhat overweight" or women "of all shapes and sizes". This approach was used to maximize the range of body composition values and increase the probability of sampling a wide range of dietary calcium intakes. By telephone interview, prospective subjects were screened for eligibility on the basis of reported menopausal status, absence of any complicating medical conditions or medications as described below, and BMI above 25 or less than 22. Approximately 300 subjects were interviewed by telephone, 80 were invited to the medical centre to further discuss the study and confirm eligibility. At the medical centre, women were interviewed by a physician to confirm general health and menopausal status. If suitable to continue in the study, written consent was obtained, height and weight were measured, and an initial walk test (to familiarize subjects with the procedure for testing aerobic fitness) was performed. Postmenopausal status was confirmed on the basis of FSH levels of 30 IU/l or greater. Target enrollment was 94 subjects. Due to time constraints, enrollment was closed when 62 subjects had been recruited.

Women with known metabolic bone disease, endocrine disorders (including hyperparathyroidism, insulin-dependent diabetes mellitus, and hyperthyroidism), chronic gastrointestinal disorders, malignancy (excluding squamous cell and basal cell skin cancers), or use of medications known to influence bone or calcium metabolism were excluded from the study. More specifically, exclusion criteria included current use of the following medications: estrogens, other steroid hormones, anticonvulsants, diuretics, and aluminum-containing antacids.

Sixty two women were admitted to the study. One dropped out early in the study, due to illness. The remaining 61 women completed the study. The mean age of the women was 62.9 years, and the average number of years since menopause was 12.9.

7.5 BMD and Body Composition

Spine and hip scans were performed according to the manufacturer's specifications using a knee block to reduce lumbar lordosis for spine scans, and a foot immobilizer to stabilize the leg in internal rotation for the hip scans. All scans were performed by a single experienced technician. Scan analysis was performed for each site using the automated analysis sequence built into the analysis software. Included in this sequence were the following manual steps. For the lumbar spine scans, the region of interest, defined as that portion of the scan including vertebrae L1 to L4, was identified by the technician as an initial step in analysis. For proximal femur scans, the position of the region of interest for the femoral neck was located manually according to the manufacturer's recommendations. The femoral neck was defined as a region 1.5 cm high and 6.0 cm or less wide extending across the narrow portion of the femoral neck perpendicular to the long axis of the femoral neck, and bordered laterally by the medial margin of the greater trochanter. The regions of the total proximal femur, trochanter, and Ward's triangle were chosen by the automated analysis sequence. Ward's triangle is a square approximately 1 cm by 1 cm (11 X 11 pixels) defined by the densitometer software as an area of minimum density in the region of the femoral neck. The greater trochanter region is defined as a triangular region bounded medially by the lateral edge of the femoral neck, and inferiorly by a line connecting the midpoint of the femoral midline to the site of the change in curvature at the inferior edge of the greater trochanter. The intertrochanteric region lies inferior and medial to this same line, is bordered superiorly by the femoral neck and includes the lesser

trochanter. It extends to approximately 1 cm below the lesser trochanter. The TOTAL is the sum of the femoral neck, greater trochanter and intertrochanteric regions.

All scans were initially analyzed on the appointment date of the scan. Lumbar spine scans were reanalyzed retrospectively using the Compare mode of the software which ensures that identical regions of interest are chosen on subsequent scans. This was done to maximize long term precision.

Review of daily phantom spine measurements revealed no evidence of machine drift. The regression of phantom spine BMD over time showed that the slope was not significantly different from zero. There was similarly no evidence of any abrupt change in mean phantom density.

7.6 Anthropometric and Fitness Measurements

Weight was assessed on a sliding beam balance scale to the nearest 0.5 kg. Height was assessed using the sliding vertical scale of the same instrument, instructing subjects to stand comfortably erect without shoes, the back against the measurement instrument and the head kept straight. Height was measured to the nearest 1 cm. All measurements were taken by the same technician. Body mass index (in units of kg/m^2) was calculated as $weight/(height)^2$.

Cardiovascular fitness (estimated $\dot{V}O_{2max}$; l/min) was estimated mathematically from age, weight, speed of walking, and heart rate response in a submaximal 1-mile walk test according to the method developed and validated by Kline et al. (1987). Because the test was developed and tested on an outdoor track, yet seasonal variations in our region make testing on an outdoor track throughout the year impractical, we also piloted the use of this test in an indoor setting with a small group of older adult volunteers (n=17) enrolled in a seniors exercise group (Appendix A).

Walk tests were conducted on a 6-month basis, on a measured and marked indoor track in back corridors of the medical centre. Subjects were instructed to wear comfortable clothing and shoes, and to walk as quickly as possible. Standardized instructions are presented in Appendix A. $\dot{V}O_{2max}$ (ml/min) was calculated using the equation:

$$\dot{V}O_{2max} = 6.9652 + (0.0091 * \text{weight}) - (0.0257 * \text{age}) + (0.5955 * \text{sex}) - (0.224 * \text{time}) - (0.0115 * \text{final heart rate})$$

Because the code for female sex is zero, this term is dropped in the final equation. Final heart rate (beats per minute, bpm) was determined immediately on completion of the walk by auscultation for 15 seconds by stethoscope (Francis 1991). Weight was determined in kilograms, age in years, and time to complete the walk in minutes, by stopwatch. All subjects participated with the exception of one woman who had a history of suspected myocardial infarction.

Grip strength was determined at each 6-month visit using the Jamar hand dynamometer and was calculated as the mean of 3 maximal squeezes with the non-dominant hand according to a standardized published procedure (Mathiowetz et al. 1984). The positioning for this technique is with the shoulder in neutral, elbow flexed 90 degrees, forearm in neutral pronation/supination, and wrist in neutral radial/ulnar deviation. The weight of the instrument is lightly supported by the examiner while the subject squeezes the dynamometer with maximal effort. Encouragement is given by the examiner throughout the effort in a loud voice. One minute rest is provided between efforts. Grip strength was determined by the same examiner for all tests.

Daily habitual activity was assessed by monitoring body motion using a portable accelerometer. Six Tritrac accelerometers were purchased from Hemokinetics Inc. (Madison, WI). The machines were first bench tested in a laboratory setting to assess the between-machine variability and short-term reproducibility. Long term reproducibility was also assessed by repeat bench-testing following field use (Appendix B).

For the assessment of habitual physical activity, accelerometer monitoring of 60 subjects was performed twice, approximately 6 months apart, to reduce the influence of season on physical activity level. Incomplete records were obtained from the remaining two subjects, therefore their data did not contribute to the final analysis. For 3 days (2 weekdays and 1 weekend day), accelerometers were worn around the waist in an adapted belt pouch which, when properly adjusted, positioned the accelerometer just medial to the anterior superior iliac spine, and reduced extraneous motion of the device relative to the body. Subjects were instructed to wear the accelerometer for all waking hours, continue usual activities, and remove the monitor only for activities such as swimming or bathing.

The accelerometer was programmed via a computer interface port to record activity values for intervals of 5 minutes. The activity value for each time interval was assigned to one of 5 distinct categories representing a range of activity intensity. This system was developed within our laboratory by having individual subjects perform standardized activities while wearing the activity monitor. Sedentary, standing, mixed ambulatory, steady ambulatory and high activity categories could be identified during standardized testing with >80% confidence (Appendix B). For the study, the percentage of time each day spent in activities assigned to these 5 categories was calculated from the 3-day data record. Total activity counts for the entire 72-h testing period were also recorded as representative of the daily total (cumulative) body motion for each individual.

7.7 Diet: Calcium, Phosphorus, and Protein Intakes

Calcium intake was measured by food frequency questionnaire, developed according to a method described by Block et al. (1986; 1985) and Cummings et al. (1987). The questionnaire was designed to include the major dietary contributors of calcium, phosphorus, and protein. Nutrient values were obtained from the Nutrient Analysis Program (Elizabeth Warwick, 1991) based on the 1991 Canadian Nutrient and Condensed Files. Details of the questionnaire design and validation are presented in Appendix C.

Subjects were instructed to estimate the frequency of intake and usual portion size of selected foods according to their dietary habits during the preceding 6 months. The questionnaire was administered semi-annually. During the initial session, the instrument was completed at the hospital and verbal instruction and explanation was given as required. Representative examples of portion sizes were available for inspection. During subsequent administrations, subjects were given the option of completing the questionnaire during a regularly scheduled study visit at the hospital, or of returning the questionnaire by mail after a brief review of the proper procedure for completion, and inspection of the sample portion sizes.

The results of the questionnaires were entered into a database computer program designed to yield average estimates of the daily intake of each nutrient. The results of all questionnaires completed by each subject were averaged to give an overall estimate of the dietary habits of the subject during the course of the study.

7.8 Calcium Absorption

Calcium absorption was measured at baseline in 58 women by a single-isotope method according to the procedure of Heaney and Recker (1985; 1988). Calcium-45 as an aqueous solution of calcium chloride was purchased from Diagnostic and

Biotechnology Systems (Markham, ON) and prepared at a concentration of 0.4 MBq/ml oral solution. On the morning of the test, 0.5 ml of oral solution containing 0.2 MBq ^{45}Ca was added to 60 ml of milk from a standardized meal. The standardized test meal contained approximately 200 mg of calcium and consisted of 2 slices of whole wheat toast served with 1 commercially prepared packet of strawberry jam, coffee or tea, and 125 ml of 2% milk (which was divided into labeled and unlabeled portions).

Subjects were instructed to drink the labeled milk through a straw. The cup was then rinsed with the remaining milk from the test breakfast (excluding any milk which had been added to the coffee or tea). Subjects ingested the remaining milk through the same straw, and completed the meal in a leisurely fashion. Three hours later, subjects were given a standardized lunch consisting of a bagel with cream cheese, commercially packaged fruit cocktail, and water.

Five hours after administration of the oral calcium dose, a single blood sample was drawn. Blood was allowed to clot overnight. A 1 ml aliquot of serum was transferred in duplicate into scintillation vials. 10 ml of scintillation fluid was added, the vials capped, and the contents mixed by agitation. Vials were counted in a liquid scintillation-counter for 100 minutes per sample, along with prepared standards. At least 10,000 counts were accumulated per sample. All samples were counted within 48 hours. Fractional concentration of the ^{45}Ca dose per sample (percentage of the administered oral dose per litre of serum) was calculated from comparison of sample counts with the standard curve derived for each batch. Preparation of the standards is described in Appendix D.

Fractional absorption (FxAbs) was calculated from the specific activity of serum calcium and the size of the miscible calcium pool estimated from height and weight, according to the equation

$$FxAbs = 0.3924 \times SA5 \times (0.39398 \times weight) \times (0.46316 \times height)$$

where SA5 is the serum specific activity expressed as a fraction of the oral ^{45}Ca dose per gram of calcium; calculated as the ratio of the fractional concentration of the oral dose per sample to the serum calcium concentration. Serum calcium (grams per litre) was measured by standard hospital automated methodology.

7.9 Laboratory Tests

All laboratory tests described below were performed in the hospital laboratory as routine out-patient blood work and urinalysis. At baseline, a blood sample was collected from all subjects after an overnight fast, and a 24-h urine sample collected. Serum calcium, alkaline phosphatase, and creatinine as well as urinary calcium and creatinine were quantitatively measured using automated test methodology on a Vitros 700 XR analyzer. In this system, the danger of sample contamination is reduced by the use of separate pipetting tips for each sample.

Measurement of calcium involves dissociation from binding proteins, and formation of a complex with Arsenazo III dye, which is measured spectrophotometrically at a wavelength of 680 nm. The lower limit of sensitivity for both urine and serum calcium is 0.25 mmol/l (1.00 mg/dl). The reported interassay coefficient of variation (CV) for serum calcium at a concentration of 2.4 mmol/l is 1.4%. The interassay CV for urinary calcium at a concentration of 2.5 mmol/l is 3.1%. The correlation coefficient between this method and calcium measurement by atomic absorption spectrometry is 0.997.

The colourimetric measurement of creatinine involves initial hydrolysis of creatinine to creatine then to sarcosine, followed by several oxidation steps to the end product hydrogen peroxide. Reaction of hydrogen peroxide with leuco dye produces a

coloured product which is measured at a wavelength of 670 nm. The lower limit of sensitivity is 4 μmol/l for serum and urine. The interassay CV for serum creatinine is 1.0% at serum concentrations of 548 μmol/l. The interassay CV for urinary creatinine is 3.1% at a mean concentration of 98.6 μmol/l. The correlation between this method and HPLC methods is 0.999 for serum and 0.996 for urine.

The glomerular filtration rate was estimated from creatinine clearance by the following formula:

$$\frac{Creatinine\ Excreted\ in\ 24\ hours}{Plasma\ Creatinine\ Concentration} = GFR$$

$$\frac{mg/s}{mg/mL} = ml/s$$

Alkaline phosphatase was measured colourimetrically in a system which uses the ability of alkaline phosphatase to catalyze the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol. P-nitrophenol absorbs light at wavelengths of 400 nm. The rate of change in reflection density monitored by spectrophotometry, is related to enzyme activity. The lower limit of sensitivity of this method is 20 U/l. The interassay CV at a concentration of 78 U/l is 3.5% and at 135 U/l is 5.7%.

Hydroxyproline (total in urine) was measured using a Hyprognosticon kit (Organon Teknika, Boxtel, Holland) in a method involving initial hydrolysis of the peptide-bound fraction. Following elution from an exchange resin, the amino acid is oxidized with chloramine T to form a pyruvole derivative which is then coloured with a reagent and determined photometrically.

Serum 25-(OH)D was measured twice, at 2 successive 6-month visits. Laboratory analysis involved an alcohol extraction technique followed by competitive-binding assay using diluted human serum. The lower limit of sensitivity was 5 nmol/l.

7.10 Measurement of Estradiol

Plasma 17β-estradiols were determined for the initial 52 subjects in a single batch by direct ^{125}I radioimmunoassay (Pantex, Santa Monica, CA) in the laboratory of Dr. BR Bhavnani of St. Michael's Hospital, Toronto. The antigen used in the assay is 17β-estradiol-6(O-carboxymethyl) oxime-bovine serum albumin. The lower limit of sensitivity of this assay is 18-37 pmol/l and the intra-assay CV is 8%. There is a relative cross reactivity of 6% with estrone and 1% with estriol. All samples were drawn in heparinized tubes between 13:00 and 14:00 h, centrifuged immediately, and the plasma stored at -20 degrees Celsius prior to assay.

7.11 Statistical Analysis of Results

Examination of the data: transformations, and missing data

The data was first examined for transcription errors and omissions, as well as to determine if the data for each variable was normal in distribution. The data for the following variables was transformed logarithmically to more closely approximate a normal distribution: daily intake of calcium, intake of calcium plus supplements, intakes of alcohol and tea, body mass index, total daily activity counts, and the percentage of daily activity spent in steady walking or high energy activity. Constants were added to the variables prior to transformation to eliminate zeros or negative numbers. The value of the constant was chosen by its ability to reduce the skew of the transformed data (Norman and Streiner 1994). Because a logarithmic transformation failed to improve the

distribution of the daily intake of alcohol, this variable was transformed using a square root function. Transformed data was used in all statistical analyses. The means and standard deviations for these variables are presented untransformed in the tables and text, for purposes of clarity and simplified presentation alongside variables not requiring transformation.

Some variables contained missing data. In order to avoid casewise deletion and therefore reduced sample size during multiple regression analysis, missing data was handled in the following manner. For variables which contained only 1 or 2 missing values (accelerometer estimates of daily activity, $\dot{V}O_{2max}$, and average 25-(OH)vitamin D), these were estimated using a regression technique. The variable with the missing data was treated as a dependent variable. A second variable, most highly correlated with the first, was treated as a predictor variable. After determination of the regression equation, the predictor value for the missing case was used to calculate the dependent value. In most instances, this procedure resulted in no change in the strength of the simple correlation between BMD and the variable. In only 2 instances r changed: the correlation of whole body BMD with percentage of the day standing decreased from -0.34 to -0.33, and the correlation of lumbar BMD with total activity count decreased from 0.14 to 0.13.

For independent variables containing a greater number of missing values, no estimation was attempted. Serum estradiol was missing 9 values. 4 values were missing from each of calcium absorption (FxAbs) and its product with intake, FxAbs*Ca. Inclusion of estradiol, FxAbs and FxAbs*Ca concurrently in final multiple regression analyses to predict BMD, would reduce the available sample size by 13 cases, from 62 to 49. The predictive equations for BMD were, therefore, initially derived using the full available sample, excluding estradiol, FxAbs and FxAbs*Ca as potential predictors. Estradiol, FxAbs and FxAbs*Ca were then introduced into the final model thus

determined, and the significance of their contribution assessed by the proportion of additional variance explained in the model. As previously stated, any alterations in the final predictive equations determined in this manner were influenced by a change in the size, and possibly the characteristics, of the final sample. In analyses not including calcium absorption, both calcium intake and calcium intake plus supplements were included as possible independent predictors.

Examination of the Independent Variables

The individual variables were examined for logical associations using Pearson correlations. Urinary calcium excretion (24-h collection), corrected for urinary creatinine as an adjustment for completeness of the urine collection, was examined for its strength of association (Pearson correlations) with dietary calcium, with calcium absorption, and with the product of these variables (FxAbs*Ca) which is an estimate of total absorbed calcium. The association of excreted calcium with serum estradiol and 25-(OH)vitamin D was also explored because of the suspected influence of these later variables on calcium excretion and absorption respectively. Glomerular filtration rate was examined for its influence on calcium absorption.

Residual estrogen levels (estradiol) were also examined separately for their strength of association with the number of years since menopause (lnYSM), and with body weight and its components.

An effect of season (winter versus summer; November-April and May-October respectively) on 25-(OH)vitamin D levels was tested using a paired *t*-test. Winter and summer vitamin D levels as well as the average vitamin D level, were examined for their associations with BMD and the rate of change in BMD. The average value was entered as an independent variable in regression analysis.

Cross-sectional Analysis of Intake Data:

The data was examined cross-sectionally to explore the possibility of longstanding relationships between predictor variables and bone density. Such relationships would have contributed to the sample variance in BMD and be dependent on established patterns of diet or physical activity. For this reason, average dietary intakes sampled over the 2-year study were thought to best reflect long-term habit. Similarly, the accelerometer estimates of daily activity are the averages of two sets of measurements performed 6 months apart, and the estimates of cardiovascular fitness and grip strength are the averages of all semi-annual assessments, in order to reduce the influence of day to day variation. Average values of 25-(OH)vitamin D measurements were used to reduce the influence of season and give an estimate of total yearly exposure.

Statistical analyses of the relationships of lumbar, femoral and whole body BMD with variables representing the availability of calcium, estrogen, physical activity, and body mass or composition were performed in 2 phases because of the large number of independent variables. The first phase served as a mechanism of item reduction, as described in the paragraphs following, by exploring the associations of BMD with the various measurements of the following factors: time (chronological age and time since menopause), body size and mass, strength, fitness and daily activity. The second phase then explored the relationships of BMD with dietary and hormonal factors. Finally, the technique of stepwise multiple regression analysis was used to determine the strongest combination of predictors of BMD at each site.

In the first phase of analysis, the possible influences on BMD of age (Age, yrs) and the length of time since the onset of menopause (YSM, yrs) were examined using Pearson correlation coefficients. The possibility of an exponential decline in BMD following menopause was examined by including the natural logarithm of the number of years since

menopause (lnYSM) as a separate variable. lnYSM was noted to demonstrate stronger simple associations with BMD and therefore substituted for YSM in subsequent analyses. Because of the possible independent effects of age and menopause on bone loss, both Age and lnYSM were included as potential predictors in final regression analysis.

The relationships between BMD and the various markers of bone turnover were also examined, although these variables were not included in final regression analysis as possible predictor variables.

Because of the partial dependency of BMD on skeletal size, and therefore an anticipated positive relationship between BMD and body mass, the strengths of association of BMD with the various measurements of body mass were also examined using simple then partial correlation coefficients, controlling for Age and lnYSM. The strongest correlations thus identified were included in the second phase of analysis. The associations of BMD with the percentage of time per day spent in each of the 5 Activity Categories, were examined using partial correlations controlling for Age and lnYSM. The Activity Category demonstrating the strongest association with BMD was included in subsequent regression analyses. The association of BMD with total daily activity counts (Total Activity), strength (Grip) and cardiovascular fitness ($\dot{V}O_{2max}$, l/min and ml/kg/min) were similarly examined, although no item reduction was necessary.

In the second phase of analysis, the strengths of association of BMD with all dietary variables, calcium absorption (FxAbs), 25-(OH)vitamin D, and serum estradiol were also examined using simple (Pearson) and partial correlations, controlling for Age and lnYSM. The product of dietary calcium and fractional absorption of calcium (FxAbs*Ca) was examined as a distinct variable. The best predictors of BMD at each site were determined by the technique of stepwise forward regression analysis, controlling for age or years since menopause, and including the following independent variables:

FxAbs*Ca, 25-(OH)vitamin D, estradiol, a body mass variable (determined from phase one), Grip, VO_{2max} (cardiovascular fitness), an Activity Category, and total daily activity. Because of their potential confounding influences, the following additional variables were also included as independent variables: intakes of protein, phosphorus, tea, coffee, and alcohol.

All *p* values refer to two-tailed tests of the null hypothesis. Statistical tests were performed using Statistix statistical package (version 4.0).

Analysis of Prospective Data:

Statistical analyses of the relationships of calcium absorption, intake, vitamin D, body mass and composition, physical activity, and residual estrogen (independent variables) to the rates of change of BMD (ΔBMD) at the lumbar spine, all femoral sites and whole body (dependent variables), were performed in a similar manner to the analyses of cross-sectional data.

In the initial phase of analysis, the strengths of association of each of the time variables with ΔBMD were explored using Pearson correlation coefficients. The associations of ΔBMD with anthropometric variables were explored as simple correlations and as partial correlations controlling for lnYSM. The strongest of the anthropometric variables was entered as a possible predictor variable in the final regression analyses. Similarly, the associations of the rate of change of BMD with each estimate of daily activity were first examined using partial correlations (controlling for the influence of lnYSM), then the variable demonstrating the strongest association included in the final regression.

In the second stage of analysis, the strengths of association of ΔBMD with all the remaining variables; dietary intakes, FxAbs*Ca, serum estradiol, 25-(OH)vitamin D, grip

strength, and $\dot{V}O_{2max}$ (l/min and ml/kg/min), were examined using partial correlations. Multiple regression analysis was then used to determine which of the body mass and physical activity variables, along with age, lnYSM, FxAbs, Ca*FxAbs, and the intakes of calcium, protein, phosphorus, tea, coffee, and alcohol, best predicted rate of change in BMD. Markers of bone turnover, alkaline phosphatase and urinary hydroxyproline were examined separately for their strength of association with BMD and the measured rate of change in BMD, using Pearson correlation coefficients. They were not included in the regression analyses because they are measurements reflective of bone turnover, not factors under consideration for a causative role.

CHAPTER 8

RESULTS

Part I. Cross-Sectional Data

Baseline characteristics of the 62 postmenopausal women enrolled in the study are presented in tables 8.1, 8.2, and 8.3. The average age of the women was 63 years (48 to 77 years). The average number of years since menopause was 12.9 with a range from 2 to 26 years. Over 90% of the women were more than 5 years since menopause at baseline.

Calculated from the cross-sectional data, the apparent rate of decline in BMD was 0.8% per year at the lumbar spine, and 1%, 0.8% and 0.6% per year at the femoral neck, total femur and whole body respectively.

8.1 Examination of the Independent Variables

Body mass and Composition

There was no definite trend toward either an increase or decrease in body weight, or change in body composition with age. Height was significantly associated with lean body mass ($r = 0.53$, $p < 0.001$) but failed to demonstrate a significant association with any of the other body mass variables. Conversely body weight demonstrated the strongest associations with body mass index, and with total absolute fat mass ($r = 0.93$ and 0.92 respectively).

Table 8.1. Intake characteristics of 62 postmenopausal women not on estrogen replacement therapy.

	Mean	SD
Age (yrs)	62.9	6.0
Years since menopause	12.9	6.7
Height (m)	1.62	0.06
Weight (kg)	68.3	13.8
BMI (kg/m^2)	26.1	5.2
Lean mass (kg)	41.9	5.2
Fat mass (kg)	23.9	10.5
% Fat	33.9	8.0
Lumbar BMD (g/cm^2)	0.910	0.152
Femoral Neck BMD (g/cm^2)	0.704	0.121
Total Femur BMD (g/cm^2)	0.806	0.131
Whole Body BMD (g/cm^2)	1.022	0.097
Estradiol (pmol/l)[a]	61.8	23.0
25-(OH)vitamin D (nmol/l)	65.9	21.1
Alkaline Phosphatase (IU/l)	77.5	16.2
Serum Creatinine (μmol/l)	78.6	8.9
Urinary Creatinine (mmol/24h)[b]	9.6	1.9
GFR (creatinine clearance) (ml/s)[b]	1.43	0.29
Hydroxyproline (μmol/mmol creatinine)	22.0	6.7
Urinary Calcium (mmol/mmol creatinine)[b]	0.44	0.21

[a] n = 53, [b] n = 45

Table 8.2. Characteristics of dietary intake based on averages of all semi-annual assessments by food frequency questionnaire.

	Mean	SD	Minimum	Maximum
Dietary Calcium(mg/day)	872	465	147	3252
Total Calcium(mg/day) *(including supplements)*	1114	594	147	3852
FxAbs (%)	24.4	6.8	11.3	41.8
Ca*FxAbs (mg)	205	108	18	613
Phosphorus (mg/day)	1272	473	252	3285
Protein (g/day)	65	20	15	129
Alcohol (drinks/day)	0.4	0.5	0.0	2.1
Coffee (8oz cups/day)	1.7	1.3	0.0	5.3
Tea (6oz cups/day)	1.2	1.7	0.0	11.0

Table 8.3. Physical activity characteristics; averages of semi-annual measurements.

	Mean	SD	Minimum	Maximum
Grip Strength (kg)	27	4	17	38
Estimated VO_2 (l/min)	1.7	0.5	0.1	2.6
Estimated VO_{2max} (ml/kg/min)	24.9	8.4	4.6	41.4
Activity (% of 24 h)				
Sedentary	58	8	42	77
Standing	13	4	4	25
Mixed Ambulatory	25	6	12	37
Steady Ambulatory	2	1	0	7
High Energy	1	2	0	7
Total Activity Counts/Day	4214	1933	1278	13470

Serum Estradiol

Serum estradiol failed to demonstrate a significant association with either age or years since menopause (r = 0.04 and 0.04, respectively). There was a trend toward a positive association of estradiol with serum alkaline phosphatase (r = 0.24) and a negative association with urinary hydroxyproline (r = -0.23) but these trends failed to reach statistical significance (p = 0.08 and 0.09 respectively).

Estradiol was positively associated with weight (r = 0.32, p = 0.02), fat mass (r = 0.34, p = 0.02), lean mass (r = 0.30, p = 0.03), and percent body fat (r = 0.28, p = 0.04).

Serum Alkaline Phosphatase and Urinary Hydroxyproline

All of the women had serum alkaline phosphatase levels within the normal range. Serum alkaline phosphatase was positively associated with both body weight (r = 0.30, p = 0.02) and fat mass (r = 0.38, p = 0.002) and urinary hydroxyproline was negatively associated with body weight (r = -0.27, p = 0.04). Neither of the markers of bone turnover demonstrated significant correlations with either age or the number of years since menopause.

Creatinine Clearance

None of the women had serum creatinine levels above the normal range reported by our laboratory (normal range 50 - 110 µmol/l). Values for urinary creatinine were reported for only 45 of the 62 women, therefore calculation of GFR (creatinine clearance) was limited to this subgroup. Of this group, 2 had 24-h urinary creatinine excretions below the normal for adult women (< 7 mmol/day). When corrected for body weight, 11 of the values fell below the normal range.

Eleven of the 45 women had GFRs below the normal range of 1.24 to 2.08 ml/s. Those women with low GFRs were not significantly older than women with GFRs within the normal range (*t*-test, p = 0.39). The average serum creatinine level was slightly higher for women with low GFRs when compared to women with GFRs within the normal range (83.2 and 77.9, respectively) but the difference did not reach statistical significance (p = 0.09).

Serum 25-(OH)vitamin D

The average winter value for serum 25-(OH)vitamin D was 60.0 nmol/l (range 5.0 to 122.3 nmol/l). During the summer, the average value was higher, 70.8 nmol/l (range 10.0 to 132.3 nmol/l). This difference was statistically significant ($p < 0.0001$).

The normal range for 25-(OH)vitamin D, reported by our lab, is 45 to 90 nmol/l. Eight women (13%) exhibited values for average 25-(OH)vitamin D below 45 nmol/l, and 3 had values below 30 nmol/l. When winter values alone were considered, a greater number of women exhibited low levels of 25-(OH)vitamin D; 24 women (39%) had values below 45 nmol/l, and 6 (10%) below 30 nmol/l.

Calcium Intake, Calcium Excretion, and Calcium Absorption

Urinary calcium excretion (24-h collections) although failing to demonstrate a significant association with calcium intake alone, demonstrated a positive association with calcium absorption (FxAbs) and with the product of calcium intake and absorption (r = 0.35 and 0.37, respectively; p = 0.02). Calcium excretion was not associated with kidney function (creatinine clearance) either before or after correction for calcium intake or the product of calcium intake and fractional absorption.

Calcium intake tended to increase with age, but not significantly so (r = 0.14, p = 0.29). Calcium intake was not significantly associated with body weight or composition. FxAbs, however, was positively associated with body weight and fat mass (r = 0.29 and 0.32 respectively).

FxAbs was not significantly different in those women with low serum 25-(OH)vitamin D levels from those women with vitamin D levels within the normal range (mean 23.9% versus 24.4% respectively, p = 0.29). In addition, there was no obvious effect of kidney function on calcium absorption. FxAbs did not differ significantly in women with creatinine clearance values below the normal range, versus women with creatinine clearance values within the normal range (mean FxAbs 25.2% versus 24.8% respectively, p = 0.18).

Estimates of Physical Activity

The different measurements of physical activity demonstrated the expected interrelationships. VO_{2max} (ml/kg/min) was negatively associated with the percentage of time spent in sedentary activity (r = -0.48, p = 0.0001), and positively associated with the percentage of the day spent in steady walking or high energy activity (r = 0.26 and 0.47, p = 0.04 and 0.0002, respectively) and with total activity counts (r = 0.54, p < 0.0001). Similarly, grip strength was negatively associated with the percentage of time spent in sedentary activity (r = -0.44, p = 0.0005) and positively associated with the total activity count in 24 hours (r = 0.33, p = 0.01).

8.2 Correlates and Predictors of Bone Density

The associations of BMD with the time variables, and with biochemical markers of bone turnover are presented in table 8.4. Both age at baseline (Age) and the number of

years since menopause (YSM and lnYSM) demonstrated significant negative associations with BMD at all sites, with the exception of the lumbar spine, where only the negative associations with YSM and lnYSM were significant. For the femur and whole body, the differences in magnitude between these associations was minimal. Expressing the years since menopause as its natural logarithm improved the association with BMD at all sites.

Table 8.4. Pearson correlation coefficients of the association of BMD with time and with markers of bone turnover.

	Lumbar Spine	Femoral Neck	Total Femur	Whole Body
Age	-0.25	-0.37 †	-0.31*	-0.37 †
YSM	-0.26 *	-0.29 *	-0.24	-0.29 *
lnYSM	-0.35 †	-0.34 †	-0.30*	-0.36 †
Laboratory Results				
AlkalinePhosphatase	-0.01	0.08	-0.01	-0.03
Hydroxyproline	-0.18	-0.16	-0.20	-0.14
Urinary Calcium[a]	-0.19	0.12	-0.07	-0.23

[a]n = 45; * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$

In stepwise regression analysis, lumbar BMD was best predicted by lnYSM, while femoral and whole body BMD were best predicted by age. Inclusion of additional time variables did not significantly contribute to the predictive power of the equations.

All anthropometric variables except height demonstrated significant correlations with BMD (table 8.5). Body weight (Weight; kg) correlated most strongly with lumbar BMD, explaining an additional 22% of the variability in measurements after adjustment for Age and lnYSM. Similarly, lean body mass (Lean; kg) explained approximately 25% of

Table 8.5. Partial correlations of the association of BMD with anthropometric variables, controlling for Age and lnYSM.

	Lumbar Spine	Femoral Neck	Total Femur	Whole Body
Height	0.14	0.24	0.11	0.09
Weight	0.51 ‡	0.45 ‡	0.48 ‡	0.48 ‡
BMI	0.46 ‡	0.36 †	0.45 ‡	0.45 ‡
Lean	0.50 ‡	0.55 ‡	0.52 ‡	0.53 ‡
Fat	0.41 †	0.32 *	0.39 †	0.36 †
%Fat	0.30 *	0.21	0.31 *	0.30 *

* $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$

Table 8.6. Partial correlation coefficients for the relationship of BMD with dietary data, absorption data, serum estradiol and markers of bone turnover, controlled for Age and lnYSM .

	Lumbar BMD	Femoral Neck	Total Femur	Whole Body
Dietary Variables:[a]				
Calcium, no supplements	-0.04	0.19	0.21	0.16
Calcium, with supplements	-0.07	0.11	0.13	0.13
Phosphorus	0.04	0.30*	0.29*	0.23
Protein	0.10	0.33*	0.32*	0.26*
Alcohol	0.06	0.18	0.10	0.09
Tea	0.30*	0.40†	0.34†	0.34†
Coffee	0.03	0.07	-0.01	0.02
Absorption Data:[b]				
FxAbs	0.11	0.13	0.10	0.05
FxAbs*Ca	0.11	0.17	0.15	0.08
FxAbs*Ca + supplements	0.09	0.14	0.12	0.06
Laboratory Results				
Serum Estradiol [c]	0.37†	0.29*	0.33*	0.40†
25-(OH)vitamin D	-0.15	-0.10	-0.13	-0.05

[a]n = 62, [b]n = 58, [c]n = 53; * $p < 0.05$, † $p < 0.01$

additional variation in BMD at the femoral neck after adjustment for Age and lnYSM, and 23% of additional variability in BMD of the total femur and whole body BMD.

Table 8.6 presents the partial correlations for the relationships of BMD with the dietary and absorption variables. Both calcium intake and calcium absorption failed to demonstrate a statistically significant relationship with BMD, either as simple correlations (data not shown) or when controlled for Age or lnYSM. Correcting intake for absorption (FxAbs*Ca) did not improve the ability to detect a relationship between dietary calcium and BMD. A strong trend toward a positive association of BMD with protein intake reached statistical significance at the femoral sites both before and after adjustment for Age and lnYSM.

Of the other dietary factors, the daily intake of tea (Tea; number of 6-oz cups per day) demonstrated the strongest relationship with BMD at all sites, significantly improving the predictive power of Age and lnYSM by an additional 7% at the lumbar spine, 9% at the total hip and whole body, and 13% at the femoral neck.

Serum estradiol levels demonstrated significant positive correlations with BMD at all sites. These associations were not reduced by adjustment for Age and lnYSM (table 8.6). Estradiol was also positively associated with body mass measurements ($r = 0.32$, 0.30 and 0.34 for the association of estradiol with weight, lean body mass and fat mass respectively).

Serum 25-(OH)vitamin D levels demonstrated a trend toward negative associations with BMD at all sites prior to adjustment for Age and lnYSM ($r = -0.17$ to -0.23, ns). After adjustment, these trends were reduced. Serum 25-(OH)vitamin D levels increased with age in this population of women. There was an average 42% increase in serum 25-(OH)vitamin D levels between the ages of 50 and 70 years.

In women with 25-(OH)vitamin D levels below 45 nmol/l (n = 9) or below 30 nmol/l (n = 3), there was no significant difference in BMD at any site compared with women with average serum levels above 45 nmol/l (n = 53).

Maximum oxygen consumption ($\dot{V}O_{2max}$; l/min), demonstrated positive associations with BMD at all sites prior to adjustment for Age and lnYSM (r = 0.28 to 0.37). The magnitude of this association was reduced after adjusting for Age and lnYSM. After further adjustment for body mass ($\dot{V}O_{2max}$; ml/kg/min), this positive association with BMD was eliminated and there was a trend toward an inverse assocation between the two variables. This trend was not statistically significant (table 8.7). Grip strength, although independently related to lean body mass (r = 0.32, p = 0.01), failed to demonstrate a significant association with BMD at any site. Of the daily activity variables, only the percentage of the day spent in standing was significantly associated with BMD. Unexpectedly, this association between a weight-bearing activity and BMD was negative in direction, and persisted after adjustment for the time variables.

Table 8.7. Activity characteristics: strength, fitness, and habitual daily activity. Partial correlations controlled for Age and lnYSM.

	Lumbar BMD	Femoral Neck	Total Femur	Whole Body
Grip Strength (kg)	-0.04	0.07	-0.02	0.06
$\dot{V}O_{2max}$ (l/min)	0.26	0.22	0.14	0.18
$\dot{V}O_{2max}$ (ml/kg/min)	-0.11	-0.11	-0.20	-0.19
Activity (% of 24 h)				
sedentary	0.07	0.13	0.19	0.22
standing	-0.32*	-0.30*	-0.33*	-0.33*
mixed ambulatory	0.10	0.07	0.01	-0.05
steady ambulatory	0.02	0.04	-0.02	-0.01
high energy	0.04	-0.06	-0.07	-0.01
Total Activity Counts	0.12	-0.01	-0.03	0.05

* = $p < 0.05$, † = $p < 0.01$

The final regression equations for the prediction of BMD are presented in tables 8.8 through 8.12. All of the predictive equations included the variable lnYSM. The influence of the menopause was greatest at the lumbar spine (table 8.8). The calculated decline in BMD between years 5 and 6 postmenopause at this site was 0.015 g/cm^2, or about 1.7% of baseline BMD (group average). At 10 years postmenopause, the calculated decline in BMD per year was reduced to 0.008 g/cm^2, about 0.9% of baseline average BMD.

Table 8.8. Regression of lumbar BMD on diet, physical activity, body mass, and vitamin D

Variable	Coefficient *b*	Standard Error *se(b)*	*t*	*P*
Constant	0.7512	0.1003		
lnYSM	-0.0826	0.0241	-3.42	0.0012
Weight	0.0052	0.0011	4.57	0.0001

Variables not included in the final equation: age, intakes of calcium, protein, phosphorus, coffee, tea and alcohol, total activity count, percentage of the day standing, grip strength, $\dot{V}O_{2max(ml/kg/min)}$, and 25-(OH)vitamin D. Adjusted $R^2 = 0.35$

The only additional variable contributing significantly to the predictive power of lnYSM at the lumbar spine was that of body weight. There was 6% (0.052 g/cm^2) advantage in BMD for every 10 kg increment of body weight.

The variability in the data explained by this model was 35% (adjusted R^2). Neither FxAbs, FxAbs*Ca, nor Estradiol contributed significantly to the equation (p = 0.78, 0.76, and 0.14, respectively).

The final regression equation for the prediction of femoral neck BMD, included 3 predictor variables (table 8.9). As for lumbar BMD, the predictors included lnYSM rather

than Age. The decline in BMD between years 5 and 6 postmenopause was 0.0115 g/cm^2, or 1.6% of the average femoral neck BMD for this population. The decline in BMD between years 10 and 11 postmenopause, at this site, was reduced to 0.006 g/cm^2, or about 0.8% of the group average BMD.

Table 8.9. Regression of femoral neck BMD on diet, physical activity, body mass, and vitamin D.

Variable	Coefficient *b*	Standard Error *se(b)*	*t*	*P*
Constant	0.4075	0.1079		
lnYSM	-0.0635	0.0180	-3.52	0.0008
Lean	0.0111	0.0023	4.84	0.0001
lnTea	0.0211	0.0079	2.66	0.0100

Variables not included in the final equation: age, intakes of calcium, protein, phosphorus, coffee, and alcohol, total activity count, percentage of the day standing, grip strength, $\dot{V}O_{2max(ml/kg/min)}$, and 25-(OH)vitamin D. Adjusted $R^2 = 0.43$

The increase in BMD per kg of body weight was greater at the femoral neck than at the lumbar spine. The change in BMD per 10 kg of <u>lean</u> mass was 0.111 g/cm^2, approximately 16% of average BMD. For the group, the average body composition was 62% lean tissue mass. Therefore, the average change in femoral neck BMD per 10 kg of body weight could be estimated at 0.069 g/cm^2, approximately 10%. However, this approximation is not accurate at the extremes of body mass, because lean tissue occupies a larger proportion of body mass for women with a lower body weight, and a smaller proportion of total body mass for women with higher body weight. At the 25th percentile of body weight, lean tissue occupied 66% of the total mass. At the 75th percentile of body weight, lean tissue occupied only 49% of total body mass. Therefore, the influence

on BMD of a 10 kg weight differential, if attributable to differences in lean mass, will appear less in heavier individuals.

lnTea, which had demonstrated significant simple correlations with femoral neck BMD, also entered into the final equation as an independent predictor. Determined from the logarithmically transformed data, there was a 4% difference in BMD, between subjects with a tea intake of ½ cup per day (the geometric mean of the transformed data) versus those with intakes of 2 cups per day (approximately 1 standard deviation of tea intake). The calculations are described as follows. An increase of 4% in BMD or 0.028 g/cm^2, would require a change in the value of lnTea of 1.3270. For a daily intake of a half cup of tea, the value of lnTea is -0.5978 (a constant of 0.05 was added to the original data prior to logarithmic transformation). Addition of the increase in lnTea required for the desired effect on BMD, results in the following value, 0.7292. To the base *e*, the actual tea intake becomes 2.07 cups. Subtracting 0.05 cups, the tea intake becomes 2.02 cups, a reasonable amount. Further increases in BMD, however, become more difficult to attain, as the additional intake of tea required to achieve a 10% increase in BMD is 15 cups per day!

The predictors lnYSM, lnTea, and Lean, explained 43% of the variance of BMD at the femoral neck (adjusted R squared). Estradiol, when entered into the model, demonstrated a partial correlation coefficient of 0.19 with the residual variance. The additional variance explained by the addition of estradiol was <1 %, which was not significant ($p = 0.28$). No additional variance was explained by the addition of FxAbs or FxAbs*Ca.

The final equation for BMD of the total femur is presented in table 8.10. The decline in total femur BMD between 5 and 6 years postmenopause was 0.011 g/cm^2 or

1.4% per year. Between years 10 and 11 postmenopause, the decline was 0.006 g/cm^2 or 0.7%.

The relative contribution of lean mass to BMD at the total femur was similar to that at the femoral neck. There was a 14% increase in BMD for each 10 kg of lean mass.

Table 8.10. Regression of total femur BMD on diet, physical activity, body mass, and vitamin D.

Variable	Coefficient *b*	Standard Error *se(b)*	*t*	*P*
Constant	0.4808	0.1235		
lnYSM	-0.0608	0.0206	-2.95	0.0046
Lean	0.0115	0.0026	4.41	0.0000
lnTea	0.0190	0.0091	2.09	0.0407

Variables not included in the final equation: age, intakes of calcium, protein, phosphorus, coffee, and alcohol, total activity count, percentage of the day standing, grip strength, $\dot{V}O_{2max(ml/kg/min)}$, and 25-(OH)vitamin D. Adjusted $R^2 = 0.36$

The influence of the intake of tea was slightly smaller at the total femur than at the femoral neck. The change in BMD in response to a change in tea intake from ½ to 2 cups per day was 0.025 g/cm^2, or about 3% of baseline BMD.

The adjusted R squared for this model was 36%. Estradiol explained an additional 2% (adjusted R squared) of the residual variance when the model was fitted to the smaller sample. The partial correlation for estradiol was 0.21, but again, its contribution to the model was not statistically significant ($p = 0.13$).

The final regression model for whole body BMD is presented in table 8.11. The coefficient for lnYSM was similar to that in the predictive equation for total femur BMD, suggesting similar absolute losses and decline over time. The percentage changes were, however, smaller for the whole body because of the larger baseline BMD for this

measurement. The approximate bone loss from the whole body between 5 and 6 years postmenopause was 1.0%, whereas the loss between years 10 and 11 postmenopause was 0.5%.

The influence of lean body mass was 0.086 g/cm^2 per 10 kg of lean mass, or approximately 0.053 g/cm^2 per 10 kg of body weight. The corresponding percentage changes in BMD were 8% and 5% respectively.

Table 8.11. Regression of whole body BMD on diet, physical activity, body mass, and vitamin D.

Variable	Coefficient *b*	Standard Error *se(b)*	*t*	*P*
Constant	0.7995	0.0891		
lnYSM	-0.0539	0.0149	-3.62	0.0006
Lean	0.0086	0.0019	4.56	0.0000
lnTea	0.0139	0.0066	2.12	0.0384

Variables not included in the final equation: age, intakes of calcium, protein, phosphorus, coffee, and alcohol, total activity count, percentage of the day standing, grip strength, $\dot{V}O_{2max(ml/kg/min)}$, and 25-(OH)vitamin D. Adjusted $R^2 = 0.40$

The increment in BMD in response to a change in tea intake from ½ cup per day to 2 cups per day was 0.018 g/cm^2, which is about 1.8% of the average whole body BMD for this group.

This model explained 40% of the variability in whole body BMD. When the model was fitted to the reduced sample, estradiol contributed significantly to the model (p = 0.04), and the contribution of tea intake was slightly reduced such that this variable no longer reached statistical significance (p = 0.06) and was dropped from the equation (table 8.12).

Table 8.12. Linear regression of whole body BMD in a subset of 53 women.

Variable	Coefficient b	Standard Error $se(b)$	t	P
Constant	0.7453	0.0893		
lnYSM	-0.0507	0.0175	-2.90	0.0056
Lean	0.0078	0.0021	3.76	0.0004
Estradiol	0.0038	0.0018	2.11	0.0402

Not included in the final equation: lnTea.

This model explained 38% of the variance of whole body BMD. The standard deviation of serum estradiol for the group was 23.0 pmol/l. The change in whole body BMD corresponding to a 1 standard deviation difference in serum estradiol was 0.024 g/cm^2, or 2.3%.

Part II. Prospective Data

8.3 Correlates and Predictors of the Rate of Change in Bone Density

Table 8.13 presents the rate of change data (positive indicating an increase, negative a loss) for BMD at all sites. For the group as a whole, the average rate of change in BMD (ΔBMD) at each site did not differ significantly from zero, with both gains and losses being observed (figure 8.1).

Table 8.13. Rates of change in BMD, body mass and body composition for 61 post-menopausal women; calculated from repeated measurements over 2 years .

	Baseline	Rate of Change (g/cm^2/year)	SE of the Rate of Change	Maximum Rate of Loss	Maximum Rate of Gain
Lumbar BMD	0.910	2.15E-03	1.65E-03	-0.0257	0.0278
Fem. Neck BMD	0.704	-2.68E-03	2.08E-03	-0.0443	0.0355
Total Femur BMD	0.806	4.87E-04	1.87E-03	-0.0518	0.0342
Whole Body BMD	1.022	-5.76E-04	1.39E-03	-0.0269	0.0261

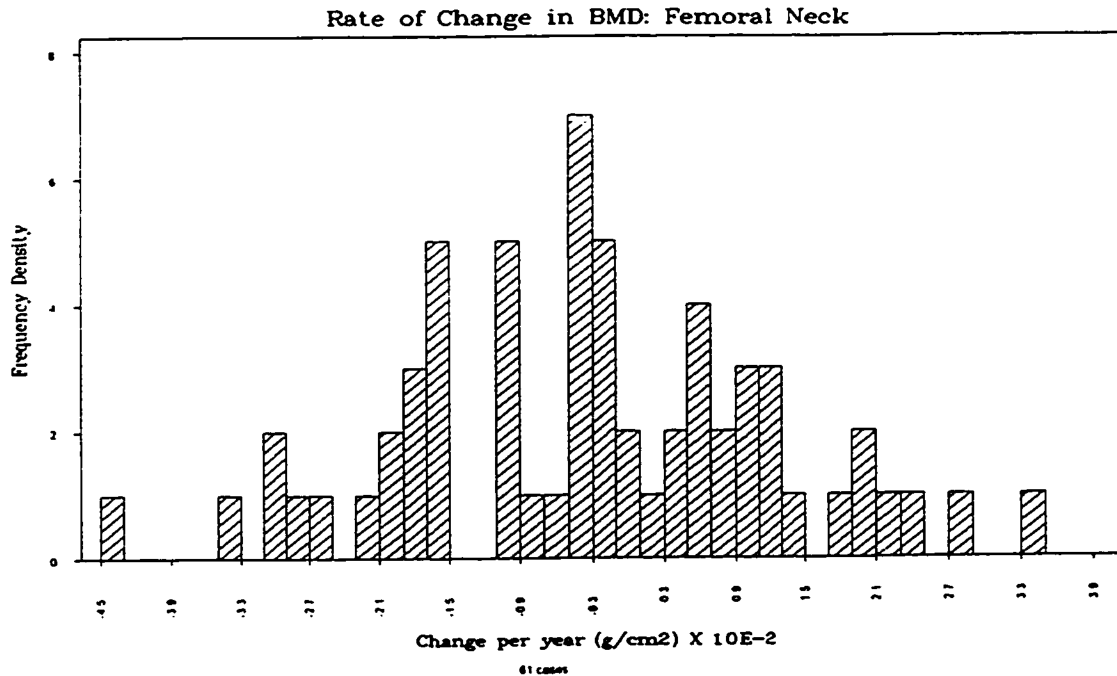

Figure 8.1 Frequency distribution for the rates of change in femoral neck BMD.

Expressed as a percentage of baseline BMD, the confidence interval for ΔBMD at the lumbar spine was -0.1% to 0.6% per year. The confidence interval for ΔBMD at femoral neck BMD was -0.9% to 0.2% per year. At the total hip, the confidence interval was -0.4% to 0.5% per year, and at the whole body, -0.2% to 0.4% per year. Negative values indicate bone loss, whereas positive values indicate bone accretion.

Calculated from the individual coefficients (β) for the regression of BMD on time, and the standard errors of those coefficients (*se*β), 15% of the women demonstrated a significant gain or loss of BMD at the lumbar spine ($p < 0.05$). For the femoral neck, 11% of the women demonstrated a statistically significant gain or loss, and for the total femur 18%. However, 34% of subjects significantly gained or lost BMD of the whole body.

The estimated measurement error at the lumbar spine (the average standard error of the Y estimate for individual regressions) was 0.014 g/cm^2, which expressed as a coefficient of variation relative to the group mean at baseline was 1.7%. Re-analysis of all lumbar spine scans by a single operator using the compare mode of the software reduced the scatter about the regression line to an average of 1.5%. Measurement error averaged 0.017 g/cm^2 at the femoral neck, or 2.4% of baseline, and 0.018 g/cm^2 for the total femur, or 2.2%. The average measurement error for whole body BMD was 0.011 g/cm^2, or 1.0%.

The rates of change in BMD at the different anatomical sites tended to be weakly correlated. Only the associations of ΔBMD at the lumbar spine with ΔBMD of the total femur, and the association of ΔBMD of the femoral neck with that of the total femur were significant (figure 8.2).

Serial measurements using the whole body scan mode of the densitometer were used to calculate changes in body mass and composition over the 2 year study period. These are presented in table 8.14. There was considerable weight gain and loss in the group, with over half the women gaining or losing 2 kg or more during the study period. 18.6% of women lost weight at a rate of greater than 1 kg per year, whereas 32.2% gained weight at a rate greater than 1 kg per year.

	Δ Lumbar Spine	**Δ Femoral Neck**	**Δ Total Femur**
Δ Femoral Neck	0.21		
Δ Total Femur	0.30*	0.28*	
Δ Whole Body	0.09	0.20	-0.10

* $p < 0.05$

Figure 8.2. Correlational matrix of the associations of rates of change in bone mineral density at different anatomical sites.

The extent of weight gain or loss was not related to initial body weight, absolute fat mass, percent body fat, or any physical activity or dietary variable. However, 89% of the change in body weight was attributable to change in fat mass (figure 8.3).

Table 8.14. Changes in body weight, lean mass, and fat mass over the 2 year study period.

	Baseline	Rate of Change (kg/year)	SD of the Rate of Change	Maximum Rate of Loss	Maximum Rate of Gain
Weight (kg)	68.3	0.01	1.74	-6.23	3.09
Lean mass (kg)	41.9	0.09	0.58	-1.15	1.49
Fat mass (kg)	23.9	-0.10	1.56	-5.89	3.06

Regression of Change in Body Weight on Change in Absolute Fat Mass

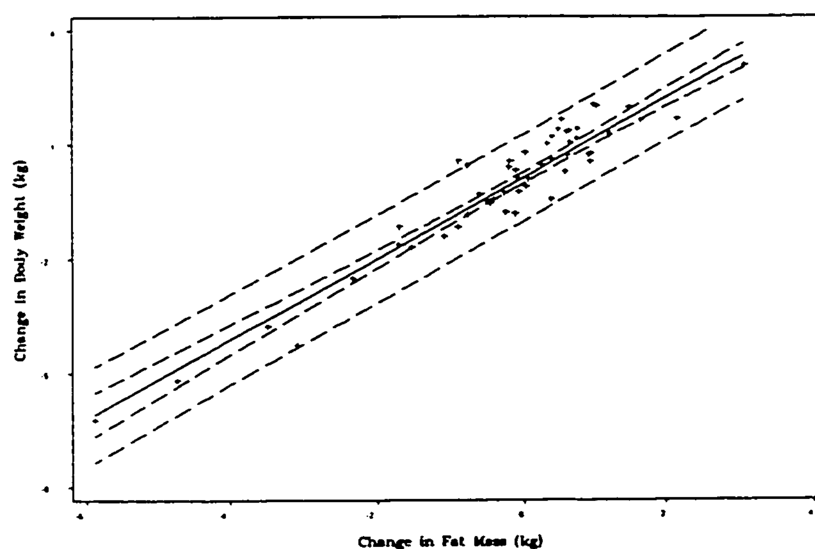

Figure 8.3 Regression plot illustrating the contributions of changes in body fat to changes in total body mass. ΔBodyWeight = 0.116 + 1.058 * Δ Fat Mass

Simple correlations of ΔBMD with markers of bone turnover, and with age and years since menopause (at intake) are presented in table 8.15. The associations of ΔBMD with the number of years since the menopause were weaker than the associations of BMD with the number of years since menopause (observed for the cross-sectional data), but remained significant at the most trabecular site, the lumbar spine, as well as at the total femur. For the most cortical regions of interest (the total femur and whole body), positive associations of ΔBMD with age were also suggested, reaching significance for the whole body. Positive associations suggest slower rates of bone loss (or greater annual gains in BMD) with advancing years.

Table 8.15. The strengths of association of the rate of change in BMD with age, the number of years since menopause, and markers of bone turnover (Pearson correlations).

	Δ Lumbar BMD	Δ Femoral Neck BMD	Δ Total Femur BMD	Δ Whole Body BMD
Age	0.18	0.07	0.21	0.29*
YSM	0.24	0.15	0.27*	0.15
lnYSM	0.26 *	0.14	0.24	0.21
Alkaline Phosphatase	0.10	0.00	0.06	0.09
Hydroxyproline	-0.15	-0.01	0.02	-0.18
UrinaryCalcium	-0.18	-0.50 ‡	-0.20	-0.21

* $p < 0.05$, ‡ $p < 0.001$

Table 8.16 shows the associations of ΔBMD with variables representing body size and composition. The rate of change in lumbar spine BMD was positively associated with body mass, particularly, but not exclusively, lean mass. None of the other sites of BMD measurement demonstrated this relationship.

Table 8.16. Partial correlations of the rate of change in BMD with body size, mass, and composition, controlled for lnYSM.

	Δ Lumbar BMD	Δ Femoral Neck BMD	Δ Total Femur BMD	Δ Whole Body BMD
Height	0.15	-0.02	0.06	-0.11
Weight	0.39 †	0.04	-0.08	0.12
BMI	0.33 †	0.03	-0.11	0.18
Lean	0.45 ‡	0.02	0.04	0.06
Fat	0.27 *	0.05	-0.11	0.16
%Fat	0.19	0.08	-0.06	0.14
ΔWeight	0.45 ‡	0.30 *	0.38 †	-0.05

* $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$

Both lumbar and femoral ΔBMD were significantly and positively associated with changes in body weight, such that weight loss was associated with more rapid bone loss, and weight gain with a slower rate of loss or an increase in BMD.

There were few consistent associations of any of the dietary variables with ΔBMD at all sites (table 8.17). Protein intake appeared to be positively associated with changes in lumbar BMD, but there was no such trend at the other sites. The intake of tea demonstrated significant associations with ΔBMD at the whole body. However, at the total femur, the intake of coffee demonstrated a positive association with ΔBMD, of similar magnitude.

The average dietary intake of calcium and annual changes in calcium intake failed to demonstrate an association with ΔBMD at any site. Fractional absorption and its product with calcium intake, however, demonstrated significant inverse associations with ΔBMD at the femoral neck, suggesting more rapid bone loss among women with high fractional absorption.

Serum 25-(OH)vitamin D demonstrated a trend toward positive associations with the ΔBMD at all sites. However, these trends failed to reach statistical significance.

Measurements of physical activity for the most part failed to demonstrate the expected associations with ΔBMD (table 8.18). Aerobic fitness (VO_{2max}, ml/kg/min) tended to demonstrate inverse associations with ΔBMD, reaching statistical significance at the lumbar spine prior to adjustment for lnYSM (r = -0.26, p = 0.04). This indicates more rapid bone loss in fitter women. These associations were, however, reduced after correction for lnYSM. Similarly, the percentage of the 24 hour day spent in mixed ambulatory activity was inversely associated with ΔBMD, suggesting more rapid bone loss in women who make frequent position changes. This inverse association was significant at the whole body site.

Table 8.17. Partial correlations of the rate of change in BMD with dietary variables, controlled for lnYSM.

	Δ Lumbar BMD	Δ Femoral Neck BMD	Δ Total Femur BMD	Δ Whole Body BMD
DietaryVariables:[a]				
Calcium, no supplements	0.08	0.01	0.06	0.16
Calcium, with supplements	0.14	0.10	-0.03	0.22
ΔCalcium intake	0.16	-0.05	-0.03	0.01
Phosphorus	0.17	-0.01	0.04	0.19
Protein	0.33*	-0.01	0.04	0.18
Alcohol	0.07	0.08	0.16	0.00
Tea	0.11	-0.03	-0.24	0.41†
Coffee	0.18	0.13	0.39*	-0.15
Absorption Data:[b]				
FxAbs	0.01	-0.29*	-0.12	-0.20
FxAbs*Ca	0.03	-0.30*	-0.12	-0.16
FxAbs*Ca+Suppl.	0.04	-0.27*	-0.13	-0.16
Laboratory Results				
Serum Estradiol[c]	0.23	-0.03	-0.11	0.10
25-(OH) vitaminD	0.12	0.20	0.15	0.20

[a] n = 61; [b] n = 58; [c] n = 53 ; * $p < 0.05$, † $p < 0.01$

Table 8.18. Partial correlations of the rates of change in BMD with activity characteristics: strength, fitness, and habitual daily activity, controlled for lnYSM.

	ΔLumbar BMD	ΔFemoral Neck BMD	ΔTotal Femur BMD	ΔWhole Body BMD
Grip Strength (kg)	-0.01	-0.22	-0.08	-0.10
$\dot{V}O_{2max}$ (l/min)	0.06	-0.13	-0.08	-0.20
$\dot{V}O_{2max}$ (ml/kg/min)	-0.21	-0.16	-0.04	-0.22
Activity (% of 24 h)				
sedentary	0.08	0.06	0.00	0.23
standing	-0.05	-0.01	0.06	-0.14
mixed ambulatory	-0.18	-0.11	-0.12	-0.28*
steady ambulatory	0.09	0.07	0.18	-0.01
high energy	0.22	0.03	0.25	0.10
Total Activity Counts	0.07	0.05	0.13	0.06

* = $p < 0.05$

Despite the apparent associations between ΔBMD and physical activity, none of the physical activity variables were independent predictors of the rate of change of spine, hip or whole body BMD in multiple regression analysis. The final predictive equations for the regression of ΔBMD on age, lnYSM, body mass, physical activity and diet, are presented in tables 8.19 through 8.23.

Change in body weight appeared as a positive independent predictor of ΔBMD at the lumbar spine and femoral sites. Determined from the logarithmically transformed data, the influence of a 1 kg per year increase in body weight (from previously stable body weight) was an improvement of 0.0041 g/cm^2 per year in the rate of change in lumbar BMD, or a percentage change of approximately 0.5% (of the mean baseline BMD) per year. The influence of a 1 kg per year weight loss (from previously stable body weight) was a 0.0033 g/cm^2 per year deterioration in the rate of change in BMD (about 0.4% of

baseline BMD). The proportional influence of the change in body weight on ΔBMD was similar at the femoral neck and total femur.

Age also appeared as an independent predictor of ΔBMD at all sites except the femoral neck. The influence of age was much less than that of weight gain or loss. The advance of age was predictive of a slowing of bone loss (or greater likelihood of improvement in bone mass). With advancing age, the rate of change in BMD was positively influenced by approximately 0.06% (of baseline BMD) per year at the lumbar spine, 0.09% of baseline BMD per year at the total femur, and 0.05% of baseline BMD per year for the whole body. This could effectively change a BMD loss of 1% per year at baseline, to 0% per year over the course of 10 to 20 years.

ΔBMD at the lumbar spine was also positively influenced by lean body mass (table 8.19). A 1.0 kg difference in body weight resulted in a 0.1% (of baseline) difference in the yearly rate of change in BMD. A 5 kg difference in lean body mass could therefore potentially decrease or increase by half, the expected 1% per year rate of decline of BMD at the lumbar spine.

Table 8.19. Regression of the rate of change in lumbar BMD on diet, physical activity, body mass, and vitamin D.

Variable	Coefficient *b*	Standard Error *se(b)*	*t*	*p*
Constant	-0.0733	0.0173		
Age	5.845E-04	2.134E-04	2.74	0.0084
Lean	9.132E-04	2.414E-04	3.78	0.0004
ln Δ Weight	0.0169	0.0034	5.00	0.0000
Protein Intake	1.270E-04	6.218E-05	2.04	0.0460
ΔCalcium Intake	1.604E-05	7.008E-06	2.29	0.0261

Variables not included in the final equation: lnYSM, intakes of calcium, phosphorus, coffee, and alcohol, total activity count, percentage of the day in high energy activity, grip strength, $\dot{V}O_{2max(ml/kg/min)}$, and 25-(OH)vitamin D. Adjusted $R^2 = 0.47$

Due to the reduction in sample size when fractional absorption and estradiol were considered as possible predictor variables, the predictive power of the dietary variables was altered. In the reduced sample, protein intake and the change in calcium intake did not explain a significant amount of the variance in ΔBMD and were dropped from the model. Neither FxAbs nor FxAbs*Ca improved the power of age, lean body mass, and change in body mass to predict the ΔBMD at the lumbar spine (p = 0.55 and 0.51 respectively). The influence of estradiol was borderline, explaining an additional 2.4% of the variance in ΔBMD (p = 0.06).

In the full sample, ΔBMD of the femoral neck was predicted only by change in body weight, which explained only 8% of the variance in the data (table 8.20). According to this model, stable body weight was permissive of an annual decline in BMD at the femoral neck of 0.0042 g/cm^2 or 0.6% of baseline BMD per year. A weight gain of 1 kg per year reduced the rate of decline in BMD to 0.1% per year.

Table 8.20. Regression of the rate of change in femoral neck BMD on diet, physical activity, body mass, and vitamin D.

Variable	Coefficient *b*	Standard Error *se(b)*	*t*	*p*
Constant	0.0027	0.0031	0.87	0.3860
ln Δ Weight	0.0137	0.0056	2.44	0.0179

Variables not included in the final equation: age, lnYSM, % fat mass, intakes of calcium, protein, phosphorus, coffee, and alcohol, change in calcium intake, total activity count, percentage of the day in mixed ambulatory activity, grip strength, $\dot{V}O_{2max(ml/kg/min)}$, and 25-(OH)vitamin D. $R^2 = 0.08$

The introduction of fractional absorption, explained an additional 6.5% of the variance in the smaller sample (p = 0.03). The resulting model explained 15% of the

variance in the data (table 8.21). Estradiol did not contribute to the original model either alone or in conjunction with fractional absorption (p = 0.85 and 0.80 respectively).

Table 8.21. Linear regression of the rate of change in femoral neck BMD in a subset of 55 women.

Variable	Coefficient *b*	Standard Error *se(b)*	*t*	*p*
Constant	0.0166	0.0073	2.26	0.0278
ln Δ Weight	0.0116	0.0056	2.07	0.0431
FxAbs	-6.302E-04	2.951E-04	-2.14	0.0374

$R^2 = 0.15$

The influence of fractional absorption at the femoral neck was negative in direction. The influence of a one standard deviation change in fractional absorption (6%), was a shift in the annual rate of change of BMD of 0.0038 g/cm^2 per year (0.5% of baseline BMD at the femoral neck).

At the total femur, ΔBMD was predicted by change in body weight, age and also in a positive manner by the daily intake of coffee (table 8.22). The influence of age on the rate of change in BMD was positive. There was about a 0.9% improvement in ΔBMD per decade. At this site, a weight gain of 1 kg per year improved ΔBMD by 0.0036 g/cm^2 or 0.4% of baseline BMD per year.

The difference in the rate of change of BMD, per cup of coffee was 0.004 g/cm^2 per year or about 0.5% of baseline BMD. This is substantial in that it suggests that a difference in intake of 2 cups of coffee per day could completely attenuate an annual rate of decline of 1% per year.

Table 8.22. Regression of the rate of change in total femur BMD on diet, physical activity, body mass, and vitamin D.

Variable	Coefficient *b*	Standard Error *se(b)*	*t*	*p*
Constant	-0.0481	0.0177	-2.73	0.0086
ln Δ Weight	0.0144	0.0045	3.17	0.0025
Age	7.555E-04	2.681E-04	2.82	0.0067
Coffee Intake	0.0044	0.0012	3.55	0.0008

Variables not included in the final equation: lnYSM, BMI, intakes of calcium, protein, phosphorus, tea, and alcohol, total activity count, percentage of the day in high energy activity, grip strength, $\dot{V}O_{2max(ml/kg/min)}$, and 25-(OH)vitamin D. Adjusted $R^2 = 0.31$

Neither FxAbs, FxAbs*Ca, nor estradiol added significantly to the predictive equation (p = 0.70, 0.81, and 0.61 respectively).

ΔBMD of the whole body was dependent on age and also the daily intake of tea (table 8.23). Advancing age had a positive influence on the rate of loss of 0.45% (of baseline BMD) per decade.

Table 8.23. Regression of the rate of change in whole body BMD on diet, physical activity, body mass, and vitamin D.

Variable	Coefficient *b*	Standard Error *se(b)*	*t*	*p*
Constant	-0.0278	0.0134	-2.08	0.0422
Age	4.621E-04	2.113E-04	2.19	0.0329
lnTea	0.0026	8.152E-04	3.24	0.0020

Variables not included in the final equation: lnYSM, BMI, change in body weight, intakes of calcium, protein, phosphorus, coffee, and alcohol, total activity count, percentage of the day in mixed ambulatory activity, grip strength, $\dot{V}O_{2max(ml/kg/min)}$, and 25-(OH)vitamin D. $R^2 = 0.20$

The influence of tea was such that the difference in the annual change in BMD in women consuming 2 cups of tea per day compared with those consuming ½ cup of tea per day, was 0.003 gm/cm^2 per year, or approximately 0.3% (of baseline). Thus a small difference in tea consumption could half the expected rate of decline in BMD for the whole body. Neither FxAbs, FxAbs*Ca nor estradiol added significantly to the final regression equation ($p = 0.23$, 0.28 and 0.44 respectively).

CHAPTER 9

DISCUSSION

9.1 Age-Related Decline in BMD

It is generally accepted that bone mass declines with age (Aloia 1994). Cross-sectional examination of baseline data from our study also revealed an age-related decline in bone density which, when described by a linear function, was similar in magnitude to that reported in other cross-sectional studies (Gotfredsen et al. 1987; Mazess et al. 1987; Riggs et al. 1981). The age-related decline determined by this method was greater at more trabecular bone sites. Similar to others, however, we also found that this decline could be adequately described by a non-linear function based upon the number of years since the cessation of menses (Gotfredsen et al. 1987). Although we intentionally excluded women who were within 3 years of the menopause (average 12.9 years since menopause), the fact that an exponential decline in BMD was an independent predictor of bone mineral density in multiple regression analysis suggests that the influence of the menopause was still significant in our group. The fact that the natural logarithm of the number of years since menopause, rather than age, emerged as an independent predictor of baseline BMD at all sites suggests that the early rapid phase of bone loss was still operative in some women. We found that exclusion of women less than 6 years since menopause eliminated the choice of lnYSM as a predictor variable. Although the influence of the menopause may extend beyond 5 years, with advancing years it would be increasingly difficult to distinguish a curvilinear relationship from a linear one with such a small sample.

Controlling for an exponential decline due to the menopause largely eliminated any association between age and BMD at the lumbar site. At the other sites, however, a trend for an independent negative association between age and BMD persisted, although our sample size was insufficient for the partial correlations to reach statistical significance, or for age to appear as an independent predictor in regression analysis. Higher bone densities observed in women nearer to the menopause may be the consequence of higher peak bone densities or other factors. It is interesting that in the models predicting ΔBMD rather than baseline BMD, the emergence of Age rather than lnYSM as a positive predictor of rates of change in bone density suggests a gradual attenuation of bone loss with advancing years, rather than an exponential change early in an early postmenopausal period followed by a continuous rate of loss into old age.

With advancing age, an apparent attenuation of bone loss at the lumbar spine has been described previously, in cross-sectional studies using dual photon technology (Mazess et al. 1987; Riggs et al. 1986a). Ongoing linear bone loss from other anatomical sites has been described in both cross-sectional and prospective studies which have focused on the pattern of bone loss in older adults (Hannan et al. 1992; Jones et al. 1994). The apparent attenuation of bone loss at the lumbar site may be the result of age-related increases in spinal osteophytes or soft tissue calcifications within the scanning area. This may affect the accuracy of the assessment of BMD and may obscure age-related losses in bone mineral from the vertebral bodies (Masud et al. 1993).

9.2 Lack of Agreement Between Cross-Sectional and Prospective Data

Our prospective data failed to confirm a definitive ongoing age-related decline (linear or non-linear) in BMD at any site, for the group as a whole. Although it may be argued that 2 years is insufficient time to detect low rates of change, some of our women

demonstrated significant gains in BMD. The greatest increases occured at the lumbar spine, where 25% of the subjects demonstrated increases in BMD of 1.5% per year or greater. In the absence of true changes in bone mass, statistically some women are likely to demonstrate what appear to be relatively large positive rates of change due to occurrence of a Type I error. This does not explain the inability to detect a significant rate of loss in this population, however, nor the discrepancy between the apparent rates of bone loss described by cross-sectional versus prospective analysis of the data. The confidence intervals surrounding the mean rates of change in BMD determined prospectively, did not include the apparent rates of loss suggested by cross-sectional examination of the data. For example, the estimated rates of loss from the lumbar spine determined from cross-sectional data was -0.8% per year, whereas the 95% confidence interval for the rate of change in BMD at this site, from the prospective data, was -0.1% to 0.6% per year.

The apparent discrepancy between our cross-sectional and prospective data may be the result of skewing of the cross-sectional data due to fortuitous recruitment of younger individuals who possessed relatively high bone mass (large skeletons) prior to the onset of bone loss, and older individuals whose peak bone mass was lower (smaller skeletons). This would give the appearance of age-related decline. However, neither height nor weight (both of which contribute to total body mass and bone mass) were related to age or the number of years since menopause.

A portion of the discrepancy between rates of bone loss determined from cross-sectional and prospective data, could be explained by the intrinsic variability of rates of bone loss, or by global changes in health-related behaviours of the study group during the 2-year period of observation. Pouilles et al. (1996) have reported that the rate of bone loss identified in early postmenopausal women is not stable over time, and when stratified

into tertiles according to individual rates of bone loss, less than 1/3 of women retain their initial classification over a subsequent 2 years. Causes of individual variations in rates of bone loss may include any short or long term influence on the rate of bone turnover, including temporary or permanent lifestyle changes. Changes in lifestyle may be prompted by study involvement or vice versa, or in response to increasing public attention to osteoporosis and women's health issues. None of our women initiated the use of hormone replacement therapy during the 2-year study. Changes in diet or physical activity therefore may be the most likely candidates for a positive short term influence on bone turnover and mass.

9.3 Change in Health-Related Behaviour as a Reason for Attenuation of Bone Loss

There was a mean annual 1% improvement in aerobic fitness in the group throughout the study. This change was not significantly different from zero. However, when compared with an anticipated annual decline in maximal oxygen uptake of approximately 1% per annum after the age of 25 (McArdle et al. 1996b), the annual 1% increase in our study population was statistically significant. It may be suggested, therefore that the maintenance or improvement in fitness in our group attenuated normal age-related bone loss. We cannot, however, make such a statement with confidence due to the absence of a control group. This arguement is also not supported by the prospective data, which suggests negative associations of fitness with ΔBMD at an individual level.

It is also possible that the apparent improvement in fitness demonstrated by the study population as a whole were artifactual due to learned familiarity with the testing procedure. Although not easily quantifiable, and in spite of initial instructions to dress

appropriately, we noted that some women appeared to wear more appropriate footwear and clothing after a number of testing sessions.

Calcium supplementation has been demonstrated to cause a decrease in biochemical markers indicating a reduction in bone turnover (Fardellone et al. 1998). Sudden reductions in remodeling space increase bone density. It is possible that sudden increases in dietary calcium may have a similar effect. Such a change in dietary habit does not, however, emerge as a strong candidate for explaining the attenuation of bone loss in our women. Rather than an improvement in dietary habit over the course of the study, the group demonstrated a significant decline of 62 mg per year (approximately 7%) in calcium intake. Questionable results were obtained from one subject who reported a calcium intake in excess of 3,000 mg per day on her initial questionnaire, and an average calcium intake of approximately 1000 mg per day on subsequent questionnaires. When this woman's data was excluded, the group continued to demonstrate a decline in calcium intake of 45 mg per year (5% of baseline).

The reason for the decline in calcium intake is uncertain. The tendency to over-report calcium intakes (observer bias) may have diminished during the study as subjects became more familiar with and less concerned with meeting the expectations of the investigators. Similarly, subjects entering a study on bone health may have made recent changes to their diet prior to enrollment, because of public attention and personal interest in osteoporosis. Over the two year follow-up, their dietary patterns may have drifted back toward previous habits. Both of these explanations would suggest that other dietary habits remained relatively consistent, while the nutrient of focus, calcium, demonstrated the greatest fluctuations. In fact, dietary protein also demonstrated a significant decline of approximately 8% over the course of the study. This change was positively associated with the change in calcium intake ($r = 0.50$) but not with change in body weight ($r = 0.10$).

This may indicate that subjects over-reported their dietary intakes in the early part of the study, or under-reported dietary intakes toward the end of the study. Regardless of the reasons for an apparent change in calcium intake, however, it appears that positive dietary changes were not primarily responsible for the maintenance of bone mass in the group as a whole, either directly or indirectly through effects on body mass.

9.4 Measurement Error and Artifact as a Reason for Apparent Attenuation of Bone Loss

Other reasons for absence of a decline in BMD during the study may have been technical in nature. Increasing arthritic osteophytosis in the posterior spinal elements or aortic calcification may obscure losses detectable by PA densitometry scans. Absence of an age-related decline at the lumbar spine has been reported in other prospective studies using dual energy absorptiometry (Jones et al. 1994). Yet, continued decline in trabecular bone density, to age 80, has been suggested using CT scanning (Cann et al. 1985). Examination of the purely trabecular component using this technique would eliminate the artifactual contribution of extra-articular osseous outcroppings at the vertebral end plates or posterior spinal elements, or calcifications in adjacent soft tissues such as the aorta. The prevalence of aortic calcifications in women over the age of 65 has been determined from population based studies as 60% or greater (Vogt et al. 1997), so these features may have been present in a significant proportion of our study population. The presence of osteophytes has also been shown to increase the ratio of lumbar to femoral neck BMD in women over age 60, and reduce the correlation of age with lumbar BMD (Liu et al. 1996). Reid, however, in a study of postmenopausal women, reported that the magnitude of the relationship of BMD with the extent of osteophytosis was weak, and that the contribution could be considered insignificant (Reid et al. 1991). Close examination of the regression equations from Reid's study still suggest a potentially clinically important effect.

Coefficients for the regression of lumbar BMD on age, weight, and osteophyte score, demonstrated that a change in osteophyte score by one category influenced BMD by 4 to 5%, i.e. about half of one standard deviation of the age-stratified distribution of lumbar BMD in adult women. For our women, however, we did not have radiographs of the lumbar spine from which to assess the presence, age-related distribution, and subsequent contribution of such calcifications to BMD and ΔBMD.

Absence of statistically significant bone loss at the femur and whole body, in the group as a whole, is more difficult to explain, although our confidence limits (CI) do not exclude a slow rate of bone loss at the femur (95% CI -0.9 to 0.2% BMD per year) and minimal loss at the whole body (95% CI -0.2 to 0.4% BMD per year). Exclusion of early postmenopausal women, and less severe bone loss with advancing age may make bone loss difficult to detect. Our power to detect a 1% per year decline in BMD at the femoral neck in the group as a whole was 33%. The power to detect a similar magnitude of bone loss at the total femur was 72%, and at the lumbar spine and whole body power was greater than 95%. However, the power to detect a rate of change of 0.5% per year in the group was only 52% at the whole body, and less at the other sites.

At the lumbar spine, total femur and whole body sites, age had a positive independent influence on the rate of change in bone density, suggesting that bone loss is not constant across the decades and that bone mass may stabilize with age. Our observations are in general agreement with those of Davis et al. (1989), who reported waning rates of bone loss at the radius with age, in a large cohort of Japanese-American women. At odds with our results are those of Jones et al. (1994) and Ensrud et al. (1995), who reported accelerated bone loss with age, at the femur. Although our confidence limits for the rate of change at the femoral neck do not entirely exclude the possibility of a 1% per year decline in BMD, our results did not support an acceleration in this rate with

age. Disagreement between our results and those of others may be due to differences in age and health of the study populations. Results from the Study of Osteoporotic Fractures (Ensrud et al. 1995) suggested increased rates of decline at the hip and calcaneus in women over the age of 80. In this large cohort, between the ages of 67 and 69, there was an average decline in bone mineral of 0.3% per year from the total femur. By ages 75 to 79, the annual rate of loss had accelerated slightly to 0.7% per year, whereas those women age 85+ exhibited doubling of that rate at an annual loss of 1.4% (of the group mean). The study by Jones et al. (1994) included only adults over the age of 60, both ambulatory and institutionalized (n = 769). Subjects were not excluded on the basis of fracture or diagnosis of osteoporosis. Because co-morbidities increase with age, this increases the likelihood of a negative bias on rates of bone loss in the older individuals. Our study included relatively younger postmenopausal women; most of whom were in their early to mid 60's (interquartile range 59 to 66 years), healthy, ambulatory, and living in the community. Our population may not have included sufficiently elderly individuals to allow detection of this accelerated phase of bone loss. Also, the maintenance of bone mass in our group may reflect the positive effect of health and mobility status on the preservation of bone mass, although we were unable to clearly identify a positive relationship between current level of fitness and bone mass, within this ambulatory population.

9.5 Predictors of BMD and ΔBMD

Paucity of Evidence for a Role for Habitual Activity in the Maintenance of Bone Mass

Maximal oxygen uptake ($\dot{V}O_{2max}$; l/min), as estimated by the 1-mile walk test, was positively associated with femoral BMD. However, oxygen uptake (l/min) for a given physical task increases with body mass. Adjusted for body mass, differences in oxygen consumption (ml/kg/min) are influenced by training and therefore reflect aerobic fitness.

Training influences the efficiency of oxygen transfer, as well as uptake and utilization of oxygen by exercising musculature. The association between $\dot{V}O_{2max}$ (l/min) and BMD was reduced after controlling for age, and eliminated following adjustment for body mass ($\dot{V}O_{2max}$; ml/kg/min). This suggests that at a given age, little of the variability in bone mass can be explained by differences in aerobic fitness.

It is possible that a large range of body mass and a small range of aerobic fitness in our group of normal women may have minimized a true influence of fitness on BMD. However, the range of oxygen consumption ($\dot{V}O_{2max}$; l/min) in our population, after controlling for age (95% predictive limits from the regression of oxygen consumption on age), was substantial (± 0.75 l/min or 45% of the mean), as was the range of age and weight-adjusted oxygen consumption (predictive limits ± 14 ml/kg/min or 56% of the mean value). This range of aerobic fitness ($\dot{V}O_{2max}$; ml/kg/min) includes values from the 5th to 95th percentiles of published norms based on data from the Canada Fitness Survey, 1981 (Pollock and Wilmore 1990).

The positive association of oxygen consumption ($\dot{V}O_{2max}$; l/min) with BMD is likely the result of the co-dependency of both variables on body size. Body mass has been reported to explain up to 70% of the variability in oxygen consumption measured during brisk walking (McArdle et al. 1996a). We also found that approximately 20% of the variability in BMD could be explained by body mass alone. At least a portion of this dependency is due to the fact that BMD (g/cm^2) is not a true volumetric measure. Since BMD is the mass of bone mineral contained within a projected area of bone, bones of the same mineral concentration will have increasing BMD as bone size increases. For individuals with larger skeletons and larger body mass, BMD will be overestimated. The association of oxygen consumption with BMD may therefore be partly attributed to this measurement error.

By eliminating the co-dependency of BMD and oxygen consumption on body size by expressing oxygen consumption in units of ml/kg/min, the positive association of oxygen consumption with BMD was abolished. Similarly, the prospective data failed to support a positive influence of fitness on ΔBMD at any site. On the contrary, at the lumbar spine, there was a negative association of fitness with ΔBMD, and a similar trend was evident at the whole body.

The inability to detect an association of fitness with BMD in the presence of a wide range of fitness levels may be explained by absence of a significant influence of fitness in this group, or possibly by presence of an underlying non-linear association between the two variables. It is possible that physical activity in postmenopausal women is beneficial at moderate levels, but that negative effects occur in response to overexercise. Although numbers were few at the extremes of fitness, examination of the residuals revealed no clear evidence for non-linearity of the relationship of fitness with ΔBMD at the lumbar spine or whole body.

Trends toward negative associations of BMD and fitness at some sites may be due to the indirect influence of physical activity upon bone mass through changes in body weight and fat mass. Negative effects of excessive physical activity on bone mass have been associated with weight loss in young women (Arena et al. 1995; Warren et al. 1991), although interference with the neuroendocrine regulation of cyclic hormone production in young women is also involved. Among our women, fitness was not associated with the extent of weight loss during the 2-year study, but was negatively associated with both baseline body weight and fat mass. Low soft tissue mass may reduce the conversion of androgens to estrogens in postmenopausal women (Longcope et al. 1978; Longcope et al. 1986) and reduce the gravitational forces imposed upon the skeleton by total soft tissue mass. Although we failed to identify a clear association between fitness and serum

estradiol levels ($r = -0.15$, $p = 0.29$), the associations of estradiol with body weight and fat mass were significant. Fitness may therefore have indirectly contributed to low serum estradiol levels through the maintenance of low body mass and fat mass.

At an individual level, the data did not support a positive effect of a change in fitness on the preservation of bone mass. Change in fitness, calculated as the slopes of the individual linear regressions of estimated $\dot{V}O_{2max}$ on time (interquartile range -4% to 5% change), failed to demonstrate an association with the rate of change in BMD, and was not an independent predictor of ΔBMD in regression analysis, at any anatomical site. This does not suggest a strong direct or immediate effect of fitness on BMD within the range of fitness tested, although it does not exclude the possibility of latent or indirect effects of fitness, or an influence of more profound modifications in activity levels.

Although we failed to establish a positive link between fitness and BMD or ΔBMD in this population, we had anticipated a positive association between BMD and other mechanical forces acting upon the skeleton. We chose grip strength as a simple surrogate for muscular development, and activity monitoring to quantify the pattern and extent of cumulative daily movement requiring muscular contraction.

Neither grip strength nor any of the measures of daily activity or activity intensity were independent predictors of BMD or ΔBMD. The absence of an association of grip strength with bone mass was disappointing. Several investigators have reported good correlations between grip strength and decreased risk of fracture (Cooper et al. 1988; Gardsell et al. 1989; Wickham et al. 1989). Grip strength may, however, have acted as a surrogate for general health and resistance to falling. Conversely, grip strength may be a poor surrogate for the local muscular forces acting at the spine and hip, although positive correlations of grip strength with upper body strength (Sinaki et al. 1974) and with axial muscle strength (Petrie et al. 1993) have been demonstrated. In men, grip strength tends

to be higher in those classified as highly active (Myburgh et al. 1993) and is more strongly associated with ulnar bone mass than is activity level. Tests of muscular strength have also been shown to demonstrate positive associations with BMD and BMC at distant anatomical sites in young adults (Sinaki et al. 1996; Snow-Harter et al. 1992). In older adult women, a simple squeezing exercise was shown to elicit a positive change in radial BMC (Beverly et al. 1989). This response was, however, both locally and temporally linked to a change in usual activity level. The resulting increase in BMC may have been a transient remodeling response (i.e. a closing of remodeling space due to a decrease in activation frequency) or an osteogenic response (increased osteoblastic activity with resulting bone formation) in response to unusual strain levels and novel strain distributions (Kannus et al. 1996; Smith and Gilligan 1996). We did not impose a change in activity level in our study, but instead evaluated existing strength and activity.

The range of grip strength and daily activity measured in our study population may have been neither low enough to induce disuse osteoporosis, nor intense or novel enough to induce significant osteogenesis. We have confidence that our estimates of activity measured true differences within the population in that good internal consistency was demonstrated by the associations of the measures of physical activity with each other, and with body weight and composition.

Neither percentage of the 24-h day spent in steady ambulation nor total activity level, assessed by 3-day monitoring with a Tritrac accelerometer, correlated significantly with either BMD or ΔBMD. Failure to comply with full day use of the monitor may have interfered with accurate assessment of daily activity. Both of these variables were, however, positively correlated with aerobic fitness and grip strength, and negatively correlated with body fatness, again demonstrating good internal consistency.

It is well known that prolonged bed rest induces bone loss. We did not detect a significant trend of bone loss due to low levels of activity. The greatest proportion of time (77%) was spent in sedentary behaviour (lying or quiet sitting) within this group. This would suggest that as little as 5.5 hours of the day in greater than minimal activity is sufficient to maintain bone mass. This agrees with the results of animal studies which have demonstrated that the minimal movement requirements for the maintenance of bone mass are low. Ulnar bone mass in the rooster wing is maintained by only 4 loading cycles of normal physiological magnitude per day (Rubin and Lanyon 1987). The minimum movement requirement in humans is more difficult to quantify. In 1966, Issekutz et al., found that 2 to 3 hours of quiet standing per day was sufficient to reduce the excess urinary calcium excretion induced by 18 days of bedrest. Estimates such as these based on relatively few healthy young male volunteers may not be appropriate for postmenopausal women. (Issekutz et al. 1966). It has been suggested that estrogen deficiency may reduce the osteogenic influence of physical loading (Lanyon 1996), in some manner raising the minimum exercise stimulus required to prevent disuse osteopenia. Our group apparently did not breach this minimum requirement.

The percentage of the 24-h day spent in standing was negatively associated with baseline BMD at all sites. It seems counter-intuitive that a variable representing weight bearing activity would be negatively associated with BMD. Standing did not persist as an independent predictor in multiple regression analysis. This variable was negatively correlated with body weight ($r = -0.29$) and demonstrated a trend toward a positive association with increasing years since menopause ($r = 0.19$). It would appear therefore that its association with bone mass was largely attributable to its relationship to these two independent predictors.

Similarly, correlation coefficients showed that increasing proportions of the day spent in mixed activity were associated with more rapid bone loss in the whole body. In our study participation in mixed activity is not indicative of a measured level of resistive activity, but instead suggests frequent position changes. It is unlikely to be acting as a surrogate for sedentary activity, nor is participation in mixed intensity activity likely to be replacing participation in perhaps more beneficial high energy activity, because it was negatively correlated with the former and not significantly associated with the latter. In this population of older women, the percentage of the 24-h day spent in mixed activity may be representative of what appears to be a relatively busy lifestyle. An average of 25% of the day was spent in mixed activity (interquartile range 20 to 30%), whereas less than 4% of the day (interquartile range 0 to 2%) was spent in more vigorous activity. The reason for a negative association of mixed activity with ΔBMD is uncertain in that it implies that repeated short bursts of movement decrease bone formation or increase resorption. However, in both organ culture and animal experiments intermittently applied strains have been demonstrated to increase osteoblastic activity and increase or maintain bone mass (Lanyon and Rubin 1984; Rubin and Lanyon 1984). A possible explanation for our contradictory results is that the mixed activity variable was negatively correlated with fat mass and with age, both of which were predictive of positive changes in whole body BMD. Adjusting for age reduced the magnitude of the association of ΔBMD with mixed activity below the level of significance for this sample size ($r = -0.21$). Mixed activity did not persist as an independent predictor in multiple regression. Therefore, although the natural decline in physical activity with age partially explains the negative association of mixed activity with ΔBMD in this group, a negative trend persisted. Again, within the range of activity studied, we were unable to support a positive effect of increased physical activity or fitness on BMD or ΔBMD in this group of healthy postmenopausal women.

We chose to use activity counts as an assessment of cumulative movement. Thus, we monitored movement in terms of distance and time, but not in terms of the work required to produce this movement. When compared with the non-obese, obese adults expend more energy during performance of a given exercise i.e. to move the torso and limbs against gravity (McArdle et al. 1996a). Stronger muscular contraction will also be associated with movement of a heavier body or a body part through a distance per unit time. The cumulative performance of similar activities may not correlate well with physiological outcomes which are more strongly related to muscular forces and energy expenditure. It may be for this reason that although heavier weight was associated with lower activity counts that this apparent inactivity seemed not to be detrimental to bone mass within the physiological range of activity tested.

In all instances in which a physical activity variable demonstrated a positive simple association with BMD, that variable was replaced by a body mass variable in multiple regression analysis. Grip strength was positively associated with lean body mass. Maximal oxygen consumption ($\dot{V}O_{2max}$; l/min) was also positively associated with lean tissue mass, although aerobic fitness ($\dot{V}O_{2max}$; ml/kg/min) was not. Lean body mass had a positive influence on BMD at a number of sites, as well as on ΔBMD at the lumbar spine. Lean body mass may be the best surrogate for local osteogenic effects of muscular forces, including the muscular forces necessary to stabilize the body against gravity in a static system and also move the body against gravity in a dynamic system.

Participation of Endogenous Estrogens in Bone Mass Maintenance

It is also possible that lean body mass acts as a surrogate for an osteogenic hormonal climate. Muscular tissue may act as a site for conversion of androgens to estrogens. Also, muscular development may reflect the anabolic effects of serum

androgens and their influence at the tissue level. Muscle cross-sectional area and force production have been reported to correlate positively with serum testosterone levels in elderly females (Hakkinen and Pakarinen 1993). We did not assess serum total or free testosterone in this group. Serum estradiol, however, was positively associated with lean body mass as well as the other anthropometric characteristics. In spite of this, body weight and lean body mass were better predictors of baseline BMD in multiple regression analysis than was estradiol. The strength of the association between lean mass or body mass and BMD may to some extent be explained by a partial dependence of measurement of areal BMD on skeletal and hence bone size. A larger skeleton may also support a greater soft tissue mass. This co-dependence of BMD and body mass on skeletal size could exaggerate the strength of association between body mass and BMD, and favour the selection of weight, lean or fat mass in multiple regression analysis rather than a variable such as estradiol, for which soft tissue mass might act as a surrogate.

Total serum estradiol had a borderline independent impact on ΔBMD at the lumbar spine, a highly trabecular site. At the menopause, estrogen withdrawal causes a disproportionate loss in trabecular bone (Geusens et al. 1986). Our results suggest that the influence of residual estrogen levels may continue to influence primarily trabecular sites regardless of age. Slemenda et al., in a study of pre-, peri, and postmenopausal women, found that postmenopausal changes in bone mass were correlated with serum estrone and estradiol levels at the lumbar spine (highly trabecular) and mid-radius (cortical) but not at the femoral neck (Slemenda et al. 1996).

The role of residual estrogen levels in age-related bone loss and in the maintenance of bone mass has recently been given renewed attention. A recent unifying theorem proposed by Riggs, Khosla, and Melton (1998a) suggests that estrogen influences both the early rapid and later slow phases of bone loss in women. We found that serum estradiol

did not demonstrate a significant association with either YSM or lnYSM. All women had estrogen levels within a range expected for postmenopausal women (range 18 to 128 pmol/l). In these postmenopausal women, then, the controlling influence on the levels of circulating estrogens appears to be soft tissue mass.

It is also possible that other physiological effects of body size modify the influence of serum estradiol or other sex steroids in a manner not detected by using total serum levels. Estradiol and testosterone circulate bound to sex hormone-binding globulin (SHBG). This transport protein is decreased in obesity (Haffner and Bauer 1992) which may in turn increase the free levels of the hormones, and their availability to tissue receptors. Body weight may therefore not only be associated with production of osteogenic hormones, but also with their biological availability. Body size and weight may therefore act as surrogates for a number of osteogenic factors, including mechanical loading, hormone production and activity, and perhaps also some aspects of diet.

Nutrition, Bone Mass and Bone Maintenance

We found no association between body weight and dietary calcium intake. There is no evidence then to suggest that heavier individuals benefit by a higher absolute intake of daily calcium. Lean body mass and dietary protein, however, were positively associated with each other and with BMD at both femoral sites, as well as with ΔBMD of the lumbar spine. These findings support the work of Cooper et al. (1995) who reported a positive association of dietary protein with BMD in premenopausal women, suggesting an effect on peak bone mass. Our findings also agree with those of Michaelsson et al. (1995) who reported positive associations of dietary protein with BMD across a wide age range of pre- and postmenopausal women in a population-based study in Sweden, although this association was not examined separately for postmenopausal women. We did not find a

negative influence of protein intake on BMD of the proximal femur, as might be suggested by cross-cultural associations of high protein intake with high hip fracture rates (Abelow et al. 1992). Within populations, there is evidence to suggest that the energy and protein intakes of elderly patients sustaining hip fractures are inadequate, and that clinical outcomes may be improved by provision of a protein-rich dietary supplement daily during hospitalization (Delmi et al. 1990). Our results suggest that protein intake may have some positive independent influence on the rate of bone loss across a wide age range of postmenopausal women.

High protein intakes are thought to be detrimental due to the promotion of a negative calcium balance through increased urinary losses (Kerstetter and Allen 1994). In the current study, protein intake was not correlated significantly with 24-h urinary calcium losses (either simple association or controlled for calcium intake). This lack of association is not a benefit of uniformly low protein intakes since the average protein intake (65 g/day) was 11% higher than that recommended by Canadian guidelines. On an individual basis, 58% of subjects reported protein intakes greater than the Canadian recommended nutrient intake (RNI) of 0.86 g/kg/day (Health and Welfare Canada 1990). However the intakes of protein and calcium were positively correlated, ($r = 0.68$) and fractional absorption of calcium was not influenced by protein intake. Linear regression of FxAbs*Ca on protein intake showed an average increase of 3.6 mg of absorbed calcium for each additional gram of dietary protein. These values suggest more than adequate compensation for an estimated calciuric response of approximately 60 mg calcium per day for each 50 g increment in dietary protein (Kerstetter and Allen 1994). In this group of women, protein intake and positive changes in calcium intake, as discussed in the following paragraphs, may together represent good nutrition.

Calcium intake, calcium absorption (FxAbs), and the product of these two variables failed to demonstrate significant simple correlations with baseline BMD at any site. Both FxAbs and FxAbs*Ca did, however, demonstrate significant negative associations with ΔBMD at the femoral neck, which suggests that the higher the percentage of dietary calcium absorbed, the more rapidly bone is lost. FxAbs emerged as an independent predictor of ΔBMD at the femoral neck, in final regression analysis. FxAbs may act as a surrogate measure for PTH. Calcium absorption is controlled by a negative feedback mechanism involving the parathyroid response to serum calcium. In both animals and man, absorption is depressed in the presence of chronically high calcium intakes, and elevated in the presence of chronic calcium insufficiency (Heaney et al. 1975; Ireland and Fordtran 1973; Kemm 1972). We found an expected positive association of both FxAbs and FxAbs*Ca with urinary calcium ($r = 0.35$ and 0.37 respectively, $p < 0.05$) but only a low negative association between fractional absorption and dietary intake, which failed to reach statistical significance ($r = -0.19$, $p = 0.16$). This may indicate that our food frequency questionnaire performed poorly in the separation of individuals in terms of usual dietary habit. Individuals with slightly low calcium intakes may unwittingly overestimate their usual intakes, further perpetuating their poor diets. Fractional absorption may act as a more accurate surrogate for usual calcium intake than dietary questionnaires. Alternatively, calcium may act as a threshold nutrient, and absorption may fail to correlate with intake above a certain threshold. However, we found no evidence of a negative association between intake and fractional absorption even at intakes less than 400 mg per day (range of intake 147 to 376 mg; $n = 5$). Others have similarly reported a lack of association between fractional absorption and intake in adults over the age of 65 (Gallagher et al. 1979). Fractional absorption did not appear to be limited by vitamin D availability among our women as there was no detectable association between the two

variables. Fractional absorption in this population may be driven by urinary losses which, by lowering serum calcium levels, prompt PTH secretion and ultimately increase fractional absorption. If a portion of the study population is unable to compensate for calcium losses, fractional absorption will remain high even as dietary intake increases from low to moderate or high levels. This will reduce the simple association between dietary intake and fractional absorption.

On initial examination of the data, the lack of association between dietary calcium and FxAbs appeared largely attributable to the influence of two women who demonstrated both low to average calcium intakes and very low values for FxAbs (11%). The reason for low FxAbs for these women is uncertain. It seems unlikely that balance was achieved at such low absorption. For one woman, urinary calcium excretion exceeded reported calcium intake. For the other woman, excretion exceeded the product of intake and absorption. Both women were greater than 10 years since menopause, had 25-(OH)vitamin D levels within the normal range, and normal values for creatinine clearance. Poor absorption may have been mediated by inadequate conversion of 25-(OH)vitamin D to the active di-hydroxylated form (Tsai et al. 1984), or through some refractoriness of the gut to the actions of 1,25-$(OH)_2$ vitamin D (Francis et al. 1984). However, both women exhibited BMD values within 1 standard deviation of the age-adjusted norm, and neither demonstrated rates of loss significantly different from zero. This may indicate that calcium intake was much higher than recorded or that some error had occured in the determination of fractional absorption. Exclusion of these women from the analysis, however, failed to influence the relative associations of the independent variables with each other and with ΔBMD at the femoral neck, indicating that the dissociation between calcium intake and absorption was not entirely attributable to these two outliers.

The reason for the association of FxAbs with ΔBMD at the femoral neck and not the other sites is unclear, although it may reflect the preferential influence of PTH on cortical bone and the increasing contribution of cortical bone loss to the clinical presentation of senile osteoporosis. Other cortical sites did not, however, demonstrate a similar relationship with FxAbs, nor could we evaluate the proportion of cortical versus trabecular bone loss at the femoral neck.

Vitamin D sufficiency did not appear to exert a significant influence on BMD or ΔBMD within this group of healthy women. Average 25-(OH)vitamin D levels were positively correlated with the percentage of the 24-h day spent in vigorous activity which may act as a surrogate for vigorous outdoor recreation and therefore sunlight exposure. When vitamin D levels were divided by season, the association with vigorous activity was significant for the summer months but not for the winter months. This suggests that sun exposure does contribute significantly to the circulating vitamin D levels in southern Ontario and that more active women are more likely to benefit from this exposure.

Although our results support dermal sun exposure as a significant source of this hormone even at this latitude, we could not establish the contribution of diet. Kinyamu et al. (1997) noted a positive association of vitamin D intake with both serum 25-(OH)vitamin D levels and with dietary calcium in 176 adult women in Omaha, Nebraska, a third of whom were confined to nursing homes. We did not attempt to directly evaluate vitamin D in the diet. However, a major dietary source of vitamin D in North America is fortified milk. It might therefore be anticipated that serum 25-(OH)vitamin D levels would demonstrate a positive correlation with dietary calcium, particularly among individuals for whom the solar contribution was minimal. Kinyamu et al. (1998) noted that serum 25-(OH)vitamin D was positively associated with milk calcium intake. We were unable to detect such an association. Our assessment of dietary calcium intake included both dairy

and non-dairy products, and would therefore be a poorer surrogate for dietary vitamin D than would milk intake alone. The difficulty in detecting a dietary contribution to 25-(OH)vitamin D levels may also be the result of the greater solar contribution in our ambulatory cohort, even at this latitude.

Serum 25-(OH)vitamin D was not correlated with ΔBMD at any site, although it approached statistical significance at the whole body ($p = 0.07$). Although some of our women had relatively low levels, deficiency may not have reached the severity to impede bone calcification. We assessed serum levels only twice and cannot be certain of the duration of relatively low serum levels. As previously noted, vitamin D levels were not correlated with fractional absorption, suggesting that 1,25-(OH)vitamin D levels were not limited by the availability of the mono-hydroxylated precursor, 25-(OH)vitamin D. Visual inspection of the data revealed that for only one woman, whose winter vitamin D level fell below 5 nmol, did fractional absorption demonstrate a low value (17%) in spite of a low calcium intake (147 mg per day). Vitamin D deficiency did not therefore appear to be a limiting factor to fractional absorption and calcium availability for the majority of this group of healthy ambulatory postmenopausal women.

When women were divided according to calcium intake (the median calcium intake for the group was 807 mg per day), associations of ΔBMD with vitamin D status were strengthened in the low calcium group (figure 9.1). Positive correlations of ΔBMD with total calcium intake (supplements included) were strengthened at the hip and spine ($r = 0.27$ to 0.32) but did not reach statistical significance due to the reduced sample size (figure 9.2). Also the presence of a significant negative association of urinary calcium with ΔBMD at the femoral neck and total femur ($r = -0.43$ and -0.41 respectively, $p < 0.05$) in the low calcium group suggests that a threshold effect may be operative.

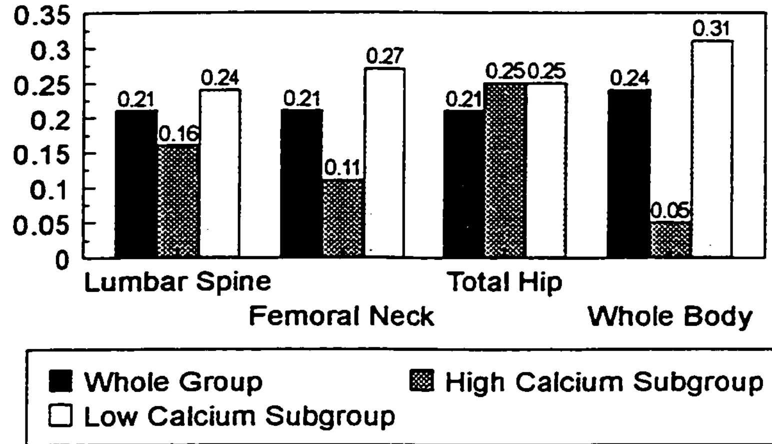

Figure 9.1. The effect of calcium intake on the Pearson associations of vitamin D status and ΔBMD. Low calcium group = calcium intake less than 807 mg per day. High calcium = intakes greater than 807 mg per day.

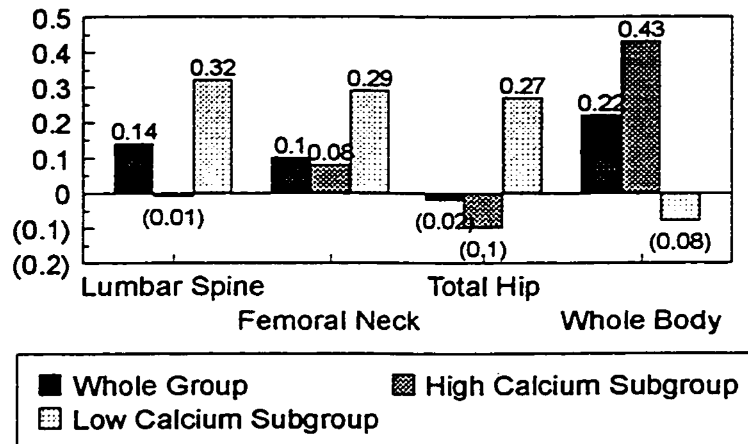

Figure 9.2. Pearson associations of total calcium intake and ΔBMD, by subgroup. Low calcium group = calcium intake less than 807 mg per day. High calcium = intakes greater than 807 mg per day.

Together these suggest that inadequate compensation for urinary calcium losses (i.e. negative calcium balance) may contribute to bone loss in otherwise healthy women. However, the post-hoc division into low and high calcium groups, and the lack of statistical power demand confirmation of these observations with an appropriate study design.

Although factors representing calcium intake or availability at baseline were poor predictors of ΔBMD, dietary fluctuations in this nutrient appeared to exert at least some acute influence on trabecular bone loss. At the lumbar spine, individual changes in calcium intake over the study period (mg per year) significantly contributed to the prediction of ΔBMD in conjunction with changes in body mass and baseline lean mass. This suggests that women who did not reduce their calcium intakes in conjunction with weight loss, or who increased calcium intake during weight gain were able to maintain or improve bone mass, whereas those women who reduced calcium intakes tended to make smaller gains or lose bone. Ramsdale and Bassey (1994) also reported a correlation between bone loss at the lumbar spine and changes in calcium intake during planned weight loss in young women, but did not indicate if this association remained independent beyond the association of bone loss with weight loss.

We noted that changes in calcium intake appeared to potentiate the effects of weight gain or loss at the lumbar spine. The relative influences of the effects of weight gain or loss and change in calcium intake on ΔBMD are illustrated in figure 9.3. Patients were divided by quartiles according to absolute gain or loss in body weight (kg), and increases or decreases in calcium intake. Because of the post-hoc assignment, the subjects were not initially randomized into these groups and the potential exists for group differences in other important characteristics.

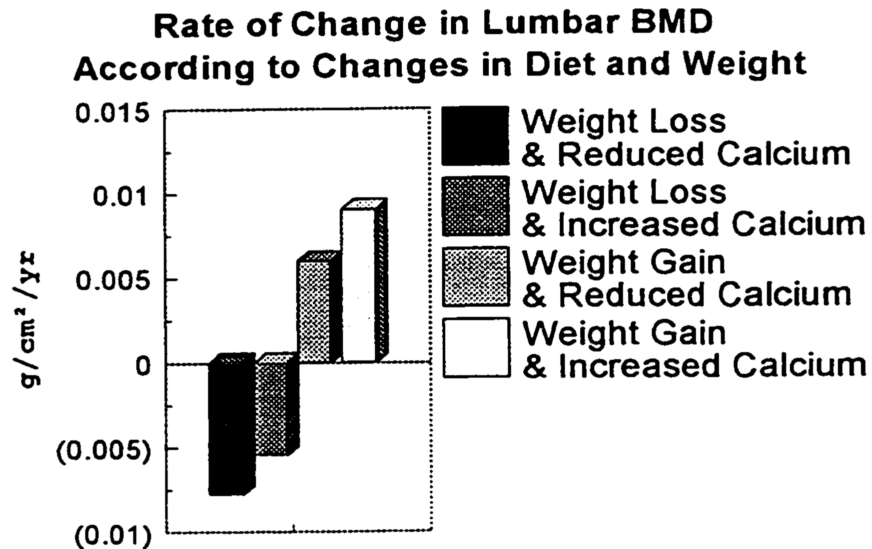

Figure 9.3. The relative influence of changes in body weight and changes in calcium intake on the rate of change of bone mineral density of the lumbar spine. The two groups demonstrating weight gain were significantly different from the two groups demonstrating weight loss.

These groups do not, however differ according to age or the number of years since menopause. It is apparent that the dominant influence on the gain or loss of bone mass is a change in body weight. However, dietary changes appear to potentiate these effects. The group differences are not significant for the effect of calcium intake when analyzed in this manner, but the trend is attractive and supports the findings from the regression equation. This relationship did not emerge at the other sites. The lumbar spine is highly trabecular, and may be more responsive to short term perturbations than more cortical sites.

The Substantial Influence of Weight Gain or Loss on Short Term Changes in Bone Mass

Change in body weight emerged as a strong predictor of the rates of change in bone mass at the lumbar spine and both femoral sites. Change in body weight was also highly correlated with the rate of change in total body bone mineral content (ΔBMC; $r = 0.76$, $p < 0.001$), but was not correlated with ΔBMD for the whole body. Our findings are similar in magnitude to those of Ryan et al. (1998) and in partial agreement with those of Slemenda et al. (1996). Ryan et al. (1998) reported a 1 to 4% decline in BMD at the lumbar spine and hip, in response to a modest 7 kg weight loss over a 6 month period in a small group of postmenopausal women ($n = 18$). Interestingly, the decline in bone mass appeared to be attenuated in a second group of women who supplemented weight loss with a modest exercise program consisting of 35 minutes of treadmill walking 3 times per week. This suggests that changes in mechanical loading characteristics may be responsible for the observed relationship between body weight changes and changes in bone mass. The intensity of the exercise component of this study may be questioned, however, on the basis of an apparent lack of influence of exercise on body composition or the extent of total weight loss, as well as the modest increment in $\dot{V}O_{2max}$ of 8% over the 6-month period of training. Slemenda et al. (1996), in a longitudinal study of the relationship of sex steroids with bone mass and bone loss in postmenopausal women, noted positive correlations of changes in body weight with changes in bone mass at the femoral neck but not the lumbar spine. Unfortunately the authors did not report any concurrent changes in sex hormone levels. The difference in the rate of bone loss was 0.4% per year for each 1 kg change in body weight. We noted a similar effect size at the spine as well as the hip.

The rapid and sizable influence of changes in body weight on bone mass at a number of anatomical sites may be physiologically attributed to changes in both local patterns of bone loading, and to changes in the endocrine environment. Although low

body weight may be associated with increased fitness, and physical activity may contribute to the maintenance of low body weight, caloric restriction rather than increased energy expenditure is generally the dominant mechanism driving acute weight loss (Segal and Pi-Sunyer 1989). Increases in activity during this period of body mass reduction may be inadequate to prevent simultaneous downward adjustment of bone mass to match the new loading environment. Dietary changes and weight loss may also be accompanied by alterations in the endocrine environment through changes in the circulating levels of insulin, insulin sensitivity, lipoprotein fractions, sex hormone levels and sex hormone binding globulin levels (Bray 1992; O'dea et al. 1979), some of which may influence bone turnover (Slemenda et al. 1996). Although we collected baseline measurements of estradiol, serial measurements of this and other hormones would be necessary in order to examine these interactions in detail.

Physiological influences of weight loss on bone metabolism should be confirmed with other measurement methods, including biological markers of bone turnover. Hyldstrup et al. (1993) reported an increase in serum osteocalcin levels at month 2 of an 8 month weight loss program for morbidly obese patents, and an increase in urinary hydroxyproline at months 2 and 8, suggesting an increase in bone turnover. Osteocalcin levels were depressed in obese patients relative to controls prior to weight loss. Sartorio et al. (1990), had, however, reported elevated serum osteocalcin levels and urinary hydroxyproline/creatinine ratios in obese patients relative to controls at baseline, and further increases in osteocalcin in response to weight loss but a normalization of hydroxyproline values. Svendsen et al. (1994) reported no detrimental changes in markers of bone turnover 6 months following a 12-week diet and exercise intervention in 118 overweight women, in spite of maintenance of significant weight loss and beneficial changes in blood lipids.

Although it is interesting to speculate upon the potential physiological impact of weight gain or loss, concern has also been raised in the scientific community, both past and present, over the possible technical problems introduced by changes in tissue composition and thickness to the accurate and precise measurement of bone mass. There have been various estimates of the contribution of tissue thickness and tissue composition to error in the estimation of total bone mass and BMD. Mazess et al. (1989) found that including lard in plastic bags containing hydroxyapatite decreased the measured BMC, but found no effect of varying thicknesses of water in the assessment of spinal or femoral BMD. Johnson and Dawson-Hughes (1991) reported that the measured density of an aluminum phantom decreased when scanned in a water or oil/water mixture of less than 18 cm depth (corresponding to approximately 56 kg body weight), but increased when scanned in oil/water mixture at depths simulating average truncal thicknesses. They did not test the effect of increasing the ratio of oil to water as thickness was increased. Morita et al. (1993) varied both the oil/water ratio and depth overlying cylindrical phantoms, and reported errors of less than 3% when phantoms were scanned beneath uniform layers of these substances. Although small, such a variation could be of clinical significance. This group did, however, report that BMD fell linearly when the oil/water ratio in the section of the water bath immediately above and below the phantom (but not either side) was increased. Recently, in a series in simulation studies, Bolotin (1998) demonstrated that DXA-measured BMD at the spine and hip increased in response to increasing ratios of fat to lean areal density in the soft tissue regions surrounding bone, and increases in the ratio of yellow to red marrow within the scanned bone. The combination of low bone density, with a high yellow/red marrow mix typical of advancing age and a low body fat mass contributed to measurement errors of 20% or greater.

Although Morita et al. (1993) suggested that manipulation of the oil/water ratio in a central fluid chamber overlying bone might mimic physiological differences in the yellow/red composition of marrow within bone, this system may also simulate inhomogeneity of the extraosseous soft tissue. Previous studies of dual photon technology have demonstrated that such variations in the distribution of estraosseous fat may contribute significantly to measurement error. Using ^{153}Gd-sourced DPA technology which calculates bone density based on similar assumptions to current DXA technology, Farrell and Webber (1989) examined the influence on measured lumbar BMD, of inhomogeneous distributions of fat in the regions adjacent-to and above-and-below the spine. Their results demonstrated that if the ratio of fat to lean tissue was greater in the region adjacent to the scanned bone than in the region above and below the scanned bone, the result would be an artifactual increase in the measured BMD. Although their estimate of error elicited some controversy, the potential for error was not disputed so much as the magnitude of that error (Mazess and Sorenson 1989; Sorenson 1990; Pye 1990; Farrell and Webber 1990). A similar effect of fat inhomogeneity (determined by QCT) on BMD (measured by DXA) was more recently reported by Formica et al. (1995). Although these findings are potent reminders of the potential for measurement error, the extent that machine error is responsible for the changes in BMD observed during serial measurements taken during a period of weight fluctuation remains to be established, and yet has serious clinical implications. Although Bolotin's work suggests that weight gain (a generalized increase in the proportion of extraosseous fat to lean tissue) may systematically increase regional BMD, the total amount of local fat accumulation and the local distribution of subcutaneous and visceral fat deposits adjacent to bone may be subject to individual variability (Farrell and Webber 1989) making it difficult to estimate the magnitude of the error at an individual or site specific level. We noted strong positive correlations between

body weight changes and whole body bone mass (BMC), but not BMD. Svendsen et al. (1993), using a Lunar corporation DXA system, noted that simulated weight gain of 8.8 kg, produced a significant 7% increase in whole body BMC (similar in magnitude to our *in vivo* observations) and a non-significant 1.4% increase in BMD. The sample size for the simulation study was small (N=6), however, and included only young women within the ideal range of body mass index. They were therefore unable to confirm the phenomenon of systematic error at lower bone densities typical of postmenopausal women, and at the extremes of body fatness and thinness.

It is possible that the greatest risk of systematic error in the clinical setting occurs during assessment of whole body rather than regional BMC and BMD. Determination of whole body bone mass and density require the system to perform calculations based on predetermined assumptions about the depth and composition of soft tissue in areas where bone-free soft tissue is difficult to isolate, such as throughout the thorax and head. Hologic has introduced software upgrades for densitometer models in the 4500 series to improve determination of bone mass and density in the head region, in recognition of problems involved with accurate assessment of this area (Hologic, Inc. 1996). Errors may therefore be specific to individual densitometers and the software version in use at any given time. In an attempt the establish the contribution of systematic error to the observed relationships between change in body weight and bone mass in our study, we performed a series of similar weight gain simulations using the Hologic QDR 1000W densitometer used for all our subject examinations. *In vivo* weight-gain simulations such as this may be useful as they introduce the normal variability in tissue composition, thickness, and bone mass encountered in the clinical environment. *In vivo* simulations demand operator decision and software recognition of bony edges and contours through a soft tissue mass

of altered composition and non-uniform tissue depth within a single region of interest. These simulation studies are described in Appendix E.

The findings of our simulation experiments support those of Svendsen et al. (1993) by demonstrating a systematic bias of whole body BMC in the presence of simulated weight gain. This suggests that a similar association of change in body weight with change in whole body BMC detected in our prospective study was artifactual. In the simulation studies we also found a small positive influence of simulated weight gain on whole body BMD. We did not identify this association with whole body BMD among our postmenopausal women who gained weight. This may suggest that some women actually lost BMD as they gained weight, attenuating the systematic measurement error, or more likely, that differences in the distribution of simulated weight gain and true weight gain cause minor differences in the magnitude of the systematic error of the measurement of BMD, and that clinically the influence is negligible.

At the lumbar spine, simulated weight gain, as a uniformly distributed fat layer, failed to significantly influence measured BMD. Asymmetrical placement of fat layers, however, increased the measurement error, and increased measured BMD. Although we are unable to determine the pattern of fat distribution inherent in true weight gain among our women, these simulations suggest that systematic error may be partially or wholly responsible for the detected associations at this site. Although it would seem prudent to develop a correction factor for density results obtained during weight gain, this would require careful study of the redistribution of fat during actual weight gain or loss, perhaps using CT, establishment of the relationship of fat distribution to body mass, BMI, hip-to-waist ratio and other anthropometric parameters. Confirmation of the magnitude of the scanning error due to fat mass changes may require cadaver studies in which scans are followed by ashing of bone specimens.

Additional Unexpected Dietary Influences on Bone Mass and Maintenance

In this two year study we detected two unexpected predictors of bone density and the rate of change in bone density. Daily intakes of coffee and tea were included as potential confounders due to their caffeine contribution to the diet, and therefore the potential for a negative effect on bone mineral. Both, however, demonstrated positive contributions to the maintenance of bone mass at various anatomical sites. The intake of tea was associated with both baseline bone mass at both femoral sites and the whole body, and with the rate of change in BMD of the whole body. Coffee intake was associated only with the rate of change of the total proximal femur. Neither variable was an independent predictor of BMD or ΔBMD at the more trabecular spinal region.

The physiological mechanism responsible for these associations are uncertain. It is possible that these associations are spurious due to the large number of analyses run, or as a result of the confounding effects of other variables. For instance, coffee intake tended to be positively associated with lean body mass ($r = 0.26$), grip strength ($r = 0.26$) and percentage of the 24 hour day spent in steady ambulation ($r = 0.38$). However, none of these physical activity measures when entered as possible predictor variables, appeared as independent predictors in multiple regression analysis, regardless of the inclusion or exclusion of coffee intake as a potential predictor in the stepwise analysis.

The negative effects of caffeine are widely accepted. Caffeine consumption has been associated with increased urinary and intestinal calcium excretion and negative calcium balance (Heaney and Recker 1982). Caffeine intake acutely increases calcium excretion in young women (Massey and Whiting 1993).

Our results support those of others who have failed to demonstrate a negative influence of caffeine intake on bone mass or fracture (Cooper et al. 1992; Lloyd et al.

1997), but disagrees with others (Hernandez-Avila et al. 1991; Kiel et al. 1990). This discrepancy may be partly attributable to differences in both caffeine and calcium intake. Results from the Framingham study suggest increased risk of hip fracture for coffee intakes of greater than 2.5 cups per day, but not for intakes of 2 or fewer cups per day (Kiel et al. 1990). Our women consumed on average 1.7 cups per day. The Framingham study did not consider the possible confounding effect of calcium intake. In the study by Lloyd et al. (1997) average calcium intake approached 800 mg per day, similar to that in our study. It has been suggested that the negative effects of coffee are realized only in the presence of low-calcium diets (Massey 1991). Barger-Lux et al. (1990) found that among adult women with calcium intakes of 600 mg per day, administration of a daily caffeine dose of 400 mg (the equivalent of approximately 5 cups of coffee) had a minimal influence (-4% NS) on calcium balance. In a population based cohort of postmenopausal women, Barret-Conner et al. (1994) also noted that consumption of at least one glass of milk per day during adulthood eliminated any negative association of lifetime caffeine intake with BMD. Perhaps due to both adequate calcium intake and low to moderate caffeine consumption, we failed to detect negative associations with ΔBMD even at calcium intakes below 800 mg per day.

The positive associations of coffee intake with ΔBMD in our study remain unexplained. Cooper et al. (1992) note trends toward positive associations of caffeine intake with BMD at the spine and whole body in a sample of adult women in Rochester Minnesota, but a negative association with BMD of the proximal femur. Controlling for age, cigarette smoking and alcohol consumption eliminated these associations. We also noted a trend toward a negative association of coffee intake with age ($r = -0.22$) of borderline significance ($p = 0.09$). The effects of age and coffee intake were independent in our study, however, as both were included in the predictive equation for ΔBMD of the

total hip. Also, the association of coffee with BMD and ΔBMD appears not to have been influenced by confounding effects of smoking, alcohol or calcium consumption. Only two of our subjects were current smokers, and coffee intake was not significantly associated with either alcohol or calcium intake. It therefore appears that the negative influence of caffeine may be minimized in healthy populations with sufficient calcium intakes and the positive association with ΔBMD of the total hip remains to be explained.

The intake of tea demonstrated more consistent positive associations with BMD and ΔBMD than that of coffee. Tea also tends to be a weaker source of caffeine, therefore considering the absence of a negative effect of coffee, a negative effect of tea would be surprising. Our findings of a positive association of tea intake with BMD and ΔBMD support the findings of Johnell et al. (1995) who have reported decreased risk of hip fracture among tea drinkers in five European countries. Explanations for the positive effects of tea in this study may ultimately be found to have physiological or cultural bases. Tea leaves have been reported to contain significant amounts of natural fluoride (Linder 1991; Rao 1984), and in some cultures may increase the environmental exposure to fluoride by 1.5 to 3.2 mg per litre of ingested beverage (Gulati et al. 1993). The Food and Nutrition Board, USA has recommended that daily total intakes for adults not exceed 4 mg (Lentner 1981a) and intakes above 8 mg per day may result in toxicity. Therapeutically, sodium fluoride has been used to stimulate bone formation with a preferential influence on trabecular bone, and has been used as an alternative treatment to estrogen and other therapies (Murray and Ste-Marie 1996). Although the dosages used in fluoride therapy are a magnitude greater than that likely to be ingested in beverage form given the estimates cited above, therapeutic exposure is controlled in duration, whereas the cumulative environmental and lifestyle exposure is uncontrolled and may be substantial. It may therefore be worthwhile to establish the fluoride content of locally

brewed teas and evaluate the influence of lifelong patterns of tea intake. We, however, found no evidence to suggest a preferential effect of tea at trabecular as opposed to cortical sites.

To date a strong role for environmental sources of fluoride in the establishment of peak bone mass or rates of loss has not been unequivocally demonstrated. Generally these studies have investigated the impact of regional variations in the fluoride content of drinking water. It is worthwhile to note that the investigation of fluoride intake should take into account various fluoride sources including toothpaste and dental mouthwashes (Wiktorsson et al. 1991).

Recent evidence suggests the polyphenols and flavonoids in tea may have positive antioxidant properties. Tea, in particular green tea, has been investigated for its positive effects on cardiovascular risk factors and incidence of cancer in humans, and tumour growth in animal models. The mechanism of action, particularly at estrogen sensitive tissues, however, remains uncertain. Recently, oral administration of green tea to rats has been shown to stimulate glucuronidation of estrone and estradiol, whereas *in vitro* mixtures of green or black tea polyphenols inhibited glucuronidation of estrone and estradiol by liver microsomes (Zhu et al. 1998). The consumption of green tea has also been associated with higher levels of sex hormone binding globulin, which in turn could reduce binding of endogenous estrogens to tissue receptors (Nagata et al. 1998). This would presumably be detrimental to the preservation of bone mass. Tea also contains oxalic acid, which although modest in quantity, could impair the absorption of calcium in the gut (Lentner 1981b). Clearly the direction of the skeletal impact as well as the total health impact of this common beverage deserves ongoing investigation.

It is possible that the associations of tea with bone mass observed in our study were the result of confounding influences of cultural and lifestyle factors. Our group

contained a considerable number of women with both British and non-British heritage. A number of behavioral patterns and genetic differences may therefore be linked to specific dietary patterns. The association of tea intake with different cultural heritages of our women, however, remains to be determined.

9.6 Summary

We hypothesized that the rate of change in bone density in postmenopausal women might be determined by 3 core factors; available calcium, physical activity, and endogenous estrogen levels. We report positive associations of residual estradiol levels with BMD at all sites. The predictive ability of estradiol in regression analysis was reduced or eliminated by the strong predictive power of measurements of body or soft tissue mass. However, the importance of residual estrogen levels postmenopausally was suggested by a continued independent influence on whole body BMD and an influence of borderline significance on ΔBMD at the lumbar spine. The long term importance of this association may be clarified by the use of serial measurements to establish the consistency of ranking of serum estrogen levels among postmenopausal women, and long term associations with rates of loss.

Endogenous estrogen levels were consistently related to the soft tissue mass and body weight. The strength of association of residual estrogen levels with body weight should also be further documented by following both factors, as well as sex hormone binding globulin and other potentially osteogenic hormones, longitudinally during weight gain and weight loss.

Body mass may influence bone density through hormonal or mechanical mechanisms. The strength of the association between body mass and bone density may also be exaggerated by the co-dependency of both measurements on body size. The

dependency of bone density on body size is a limitation imposed by the areal rather than volumetric nature of the measurement. Therefore body mass may be acting as a surrogate for estradiol even though the associations of BMD with estradiol were weaker. Our findings more strongly support a hormonal basis for the association of body mass with BMD, rather than mechanical basis.

We failed to find support for mechanical influences on bone mass in this group of healthy women. We measured 3 aspects of physical activity; fitness, strength and daily movement, but failed to detect any unifying support for a positive influence on BMD or ΔBMD. Although not all measurements were site specific, with the variety of measurements employed and the internal consistency demonstrated in the results of the different measures, we are confident this represents a true phenomena. The most likely explanation for a lack of association is that the physical lifestyles of these healthy postmenopausal women were neither intense nor sedentary enough, nor demonstrated sufficiently novel perturbations during the period of study to significantly influence bone turnover. It is unlikely that physical activity plays a major role in the regulation of postmenopausal bone loss in healthy, ambulatory postmenopausal women.

It is interesting that in spite of including estimates of calcium absorption, vitamin D sufficiency, and including other possible dietary confounders, we were unable to establish a firm association of calcium intake and rates of change in BMD. Only at the lumbar spine was a role for calcium suggested by the positive influence of increases in dietary intake of this mineral over the two year period of observation. Because only the change in dietary calcium intake rather than absolute calcium intake was associated with ΔBMD, this may represent a temporary reduction in bone turnover and may not be sustained.

A number of limitations of the study may have contributed to the difficulty in establishing firm associations between dietary calcium intake and rates of change in BMD.

Our group of women were all healthy and ambulatory, and demonstrated an average calcium intake similar to that previously recommended by the World Health Organization. Associations of calcium intake with BMD have most frequently been reported in populations with relatively low intakes (Dawson-Hughes et al. 1990; Lau et al. 1988). Although recently reviewed Dietary Reference Intakes for North America suggest that higher calcium intakes are appropriate for older women, it may be more difficult to identify significant differences in bone loss in women with only marginally inadequate diets.

Our study was also grossly limited by sample size, the healthy status of the women, and the large number of variables. This both reduces the possibility of detecting a true association and increases the possibility of committing a type I statistical error. Heaney et. al. (1991) suggested that the contribution of calcium intake to bone mass was in the order of about 5% and the contribution of calcium absorption to bone mass was of a similar magnitude. If the combined magnitude of intake and absorption is approximately 10% (and would exhibit an r value of 0.32), this would be detectable with a sample size of less than 100 if the measurement of these variables is without error. However, intake is notoriously difficult to measure, and we did not find fractional absorption to improve the strength of association of calcium intake with bone mass or change in bone mass.

Fractional absorption of calcium was unrelated to intake in this population and tended to be negatively associated with ΔBMD at the femoral sites. This may represent the actions of PTH at cortical sites, but remains to be substantiated with concurrent measurements of this hormone, fractional absorption and rates of bone loss in further studies.

Changes in body weight were consistently the best predictors of changes in bone mass. Through additional simulation experiments, we have found that weight gain may

introduce additional variability into the measurement of bone mass, and that inhomogeneity in changes in soft tissue composition in the baseline sampling regions adjacent to bone, has the potential of producing systematic error in the direction favouring apparent bone mass accumulation during weight gain. At this point we cannot determine the extent to which systematic error may have contributed to the magnitude of the associations between changes in body weight and changes in bone mass. Physiologically, body weight may also influence rates of bone loss through both hormonal and mechanical mechanisms, of which the hormonal mechanism is favoured by the results of this study. Serial measurements of biological markers of bone turnover and other hormones, along with bone mass, during controlled weight gain or loss may assist in the differentiation of systematic error from true biological effects of body weight changes on bone mass. This may help us determine if middle-age weight gain after menopause is a biological protective adaptation for the skeleton, or may seriously mask rapid bone loss in some women. At present, caution is advised when interpreting the results of serial bone measurements for monitoring pathological bone loss or positive effects of intervention therapies, in the presence of concomitant weight gain or loss.

The End.

APPENDIX A

A SUBMAXIMAL WALK TEST TO ESTIMATE MAXIMAL OXYGEN CONSUMPTION

A.1 Introduction

Direct measurement of maximal oxygen consumption using graded exercise testing on bicycle or treadmill, and a metabolic cart to evaluate respiratory gases is considered the most accurate method of determining aerobic fitness (Kline et al. 1987). This method is, however, expensive, cumbersome, and requires maximal subject participation and motivation to achieve a valid test. Walk tests are a simple and inexpensive method of estimating cardiovascular fitness from the heart rate response to submaximal exercise. A number of submaximal exercise tests have been developed that utilize cycling, walking, running, or bench stepping. The practical nature of these tests may however be questioned on the basis of limited applicability to older healthy populations (Francis 1991). Some tests were developed for populations already compromised in their fitness by cardiac or respiratory pathology (Guyatt 1987; Guyatt et al. 1984), while others such as bench stepping may be suitable for young populations (Mazess et al. 1989) but may not be well tolerated by older populations.

Walk tests have the advantage of using a simple and familiar activity with a low injury rate and have been shown to be well tolerated by aged and overweight populations (Donnelly et al. 1992; Laukkanen et al. 1992). Longer walk tests may discriminate better between subjects and are more sensitive to change over time (Guyatt et al. 1984), although tests which discriminate only on the basis of distance walked, may be subject to

variability resulting from individual motivation as well as the extent of encouragement given by the administrator (Guyatt et al. 1984).

An equation was developed by Kline et al. (1987) for predicting maximal oxygen consumption (VO_{2max}) from sex, age, weight, heart rate response and time to complete a 1-mile walk, in a group of 390 men and women between the ages of 30 and 69 years. The following equation estimating VO_{2max} (as determined *a priori* by a graded treadmill test), in litres per minute, was developed by best-subsets regression:

$$VO_{2max} = 6.9652 + (0.0091 * \text{weight}) - (0.0257 * \text{age}) + (0.5955 * \text{sex}) - (0.224 * \text{time}) - (0.0115 * \text{final heart rate})$$

The correlation between measured and estimated VO_{2max} was 0.93 with a standard error of the estimate (SEE) of 0.325 l/min (an error of 10-15% depending on age and sex). Cross-validation of the method on a second group of 169 subjects produced similar results with correlations between predicted and measured VO_{2max} which ranged from 0.89 (for 60-69 year olds, n=36) to 0.93 (for 30-39 year olds, n=48). This suggests that accuracy is relatively stable across age groups and that the procedure is appropriate for older populations.

Because the test was developed and tested on an outdoor track, but seasonal variations in our region make testing on an outdoor track throughout the year impractical, we piloted the use of this test in an indoor setting with a small group of older adult volunteers enrolled in a seniors exercise group.

A.2 Method

Subjects: 15 subjects (9 women, 6 men) who had recently enrolled in a seniors exercise program following a 2-year exercise study, volunteered to participate in the development of the walk test within the facility. All subjects had undergone a symptom-limited graded exercise test by cycle ergometer within the previous 6 months as a requirement for enrollment in the exercise group. None were known to be on medications affecting either the heart rate or blood pressure response to exercise.

Cycle Ergometry: Peak oxygen consumption was determined using a graded resistance cycle ergometer protocol which consisted of cycling at constant pace with a 100 kpm increase in workload each minute. Expired gases were collected, dried and analyzed each minute, and heart rate was monitored by ECG and recorded during the last 5 seconds of every minute. The test was terminated when the subject could no longer continue despite verbal encouragement or the test was terminated by the physician because of unacceptable ECG or blood pressure changes. Peak $\dot{V}O_2$ (the highest oxygen consumption measured during the test) was compared with estimated $\dot{V}O_{2max}$ calculated from the results of the 1-mile walk test.

Walk Test: Walk tests were performed in quiet indoor corridors of the university during the usual supervised exercise sessions. Subjects were therefore dressed appropriately in comfortable clothing and athletic shoes. Subjects were encouraged to walk as quickly as possible but not run. Positive reinforcement was given at each 1/8th of a mile (one lap). Time (minutes; min) to complete the walk was determined by stopwatch. Heart rate (beats per minute; bpm) was measured immediately on completion of the walk for 15 seconds with a stethoscope. Weight was determined to the nearest 0.5 kg on a sliding beam balance.

Statistical Analysis: Observed peak $\dot{V}O_2$ and estimated $\dot{V}O_{2max}$ were compared using Pearson product moment correlations, SEE, and paired t-tests.

A.3 Results

Characteristics of the 15 subjects are presented in table A1. The average number of days elapsed between the cycle ergometer test and walk test was 71 (SD16). The correlation between observed and estimated $\dot{V}O_2$ was 0.86 ($p = 0.0001$). The SEE was 0.250 l/min. The walk test significantly overestimated observed $\dot{V}O_2$ by an average of 0.52 l/min ($p = 0.001$).

Table A1. Subject Characteristics and Outcomes for Walk Test Validation

	Mean	SD	Min	Max
Age (yrs)	69.5	4.2	62	76
Weight (kg)	73.0	16.0	48	106
Peak $\dot{V}O_2$ (L/min)	1.44	0.47	0.90	2.44
Estimated $\dot{V}O_{2max}$ (L/min)	1.96	0.69	0.96	3.16

A.4 Discussion

Correlations obtained in this study between observed and estimated $\dot{V}O_2$ were similar to the original cross validation study of Kline et al. (1987). This suggests that adaptation of the protocol to an indoor setting does not render the procedure invalid. Although our SEEs were similar to those reported by Kline, the estimated values for $\dot{V}O_2$ significantly exceed the observed values. This may be due to a number of factors. There was a slightly greater than 2 month time lapse between performance of the graded exercise test and participation in the walk test. During this time subjects were ongoing participants in a regular weekly exercise program. They may have improved their level of fitness prior

to the walk test. Use of an indoor track may also have improved times in the walk test because of the level walking surface, absence of wind, etc.

Finally, the graded exercise test determined peak $\dot{V}O_2$ (highest oxygen consumption recorded before termination of the test) rather than $\dot{V}O_{2max}$. For those participants that achieved heart rates within 15 bpm of maximum, and respiratory exchange ratios greater than 1.1, the value should be synonymous with $\dot{V}O_{2max}$. It is possible, however, that a proportion of the subjects may have terminated the test before these conditions were achieved, thereby recording $\dot{V}O_2$ peak values that were less than the anticipated maximum. This information was not available for examination.

It appears that the 1 mile walk test functions adequately in an indoor setting to discriminate between subjects on the basis of estimated oxygen consumption. Our correlations between estimated and observed oxygen consumption compare favourably with those reported by Kline et al. (1987) during the initial testing of the instrument in an outdoor setting. Used indoors, the equation may overestimate true $\dot{V}O_{2max}$ somewhat, although more rigorous testing would be required to determine the true magnitude of this discrepancy.

APPENDIX B

BENCH TESTING AND DEVELOPMENT OF ACTIVITY CATEGORIES FOR THE TRITRAC ACCELEROMETER

B.1 Introduction

It has been suggested that some of the problem involved in establishing the influence of physical activity on disease risk lies in the difficulty of accurately and reliably measuring both the quantity and pattern of daily activity (Washburn and Montoye 1986). Direct observation of subjects by trained observers is considered the gold standard for quantification of unencumbered daily activity. This technique is, however, labour intensive and expensive for large population studies (Williams et al. 1989). The presence of an observer may also have the negative consequence of a change in behavior of the subject being observed. Questionnaires have also been used extensively in population studies of the relationships between physical activity and disease. Due to their subjective nature, however, questionnaires may misrepresent quantity and intensity of usual activity and may overemphasize leisure activity, missing the influence of occupational or domestic work. In particular, questionnaires related to leisure time activity have been questioned as to their validity and ability to distinguish between health related activity patterns among women (Blair 1994).

Because of the practical limitations of direct observation, and the subjective nature of questionnaires, investigators have sought other methods to evaluate physical activity, that combine favourable qualities such as practicality, convenience to the subject, and objectivity. Instruments developed for such use include pedometers, heart rate monitors, and accelerometers. Each of these is subject to limitations, particularly the

inability to recognize certain types or intensities of movements or, in the case of the heart rate monitor, the ability to be influenced by non-exercise factors such as ambient temperature, training status of the subject, or emotion (Freedson 1989). Accelerometers may be superior to movement counters and pedometers because of their ability to measure the intensity of movement as well as the frequency (Montoye et al. 1983; Meijer et al. 1991; Klesges and Klesges 1987).

Small portable accelerometers have demonstrated reasonable reliability and validity in laboratory and field settings. Accelerometers are able to discriminate between speeds of walking on a treadmill (Maliszewski et al. 1991; Montoye et al. 1983) with high reproducibility (r = 0.94) (Montoye et al. 1983), but are less able to discriminate the between-subject variation in energy cost of walking at the same speed (Maliszewski et al. 1991; Pambianco et al. 1990).

In field use, some studies have reported good correlations between movement counts recorded by accelerometers, and average or total estimates of energy expenditure determined from observational techniques (Mukeshi et al. 1990; Klesges and Klesges 1987; Noland et al. 1990). Poorer correlations have been reported between the results of accelerometers and activity data from questionnaires. In a validation study of the Lipid Research Clinics Questionnaire, the questionnaire explained less than 10% of the variability in the accelerometer data (Ainsworth et al. 1993). It was suggested that the two techniques may be measuring different aspects of physical activity. In particular, the accelerometer sums both the intense and sedentary activities into a daily total movement score.

More recent accelerometers have been developed that are able to record the intensity of movement within discrete time intervals. This may allow the differentiation of

individual patterns of activity which, when measured, might differ greatly in their relative range of intensities, but be similar in the daily cumulative total.

The Tritrac accelerometer is a 2.5 X 10.0 X 6.75 cm, 227 gm device which incorporates 3 accelerometers oriented at right angles to one another, to detect movement in 3 dimensions (arbitrarily directions X, Y, and Z). Movement (velocity over time) is sampled and recorded at a frequency of 10Hz by the machines, and summed to record activity counts in 1- or 5-minute intervals. Up to 14 days of data can be collected, and the data downloaded for examination and analysis. The movement data recorded by each internal sensor may be examined separately (X, Y, or Z values) or as a composite of all 3 (the Vector Magnitude; $\sqrt{x^2 + y^2 + z^2}$).

Recently Kochersberger et al. (1996) tested the Tritrac accelerometer and reported good minute by minute agreement between machines during bench testing, citing an intraclass correlation coefficient (ICC) of 0.97 as well as high test-retest stability during sitting and slow treadmill walking (ICC 0.97). Concurrent validity was supported by establishing ability of the monitors to distinguish between activity levels of nursing home residents previously categorized as sedentary, moderately active or active by nursing staff ($p < 0.001$). Activity counts measured among community-dwelling subjects during a period of 3 to 7 days of continuous (daily) use demonstrated good short term reliability (ICC 0.81). Similarly, there was no significant difference between mean activity counts recorded during this and a second testing session, 8 weeks later. This supports the reliability of the instrument for activities of relatively low intensity, and the ability of the instrument to distinguish between grossly different levels of function. It does not however, make use of the ability of the instrument to record motion in discrete intervals, and thereby identify infrequent periods of intense activity.

B.2 Methods

We purchased 6 Tritrac accelerometers (serial numbers RD0029, 31, 32, 34, 35 and 36; hereafter referred to as Machines 29 through 36) in 1992, and undertook bench testing for reliability and validity. Recall of the machines rendered this initial period of testing invalid. Beginning in 1993, machines were again bench-tested for reliability. A set of movement categories were also developed for use in categorizing habitual daily activity by intensity and frequency. These categories were developed by having a group of subjects perform standardized tasks while wearing the accelerometers. Finally, following a period of field use, accelerometers were again bench tested to establish long term stability.

Bench Testing: Mechanical Agitation

Short term reliability of the Tritrac accelerometer was examined using a laboratory test tube agitator to generate regular oscillating forces on all accelerometers simultaneously, on repeat occasions. All 6 accelerometers were secured together by multiple adhesive bands to form a single secure unit. This unit was wrapped in plastic to repel water and excess humidity, and fastened to a test tube rack. The rack fit securely in the agitator, with the accelerometers held above a water depth of 1 to 2 cm. The temperature of the water was consistently 37 degrees Celsius. Room air temperature was not controlled.

Frequencies of agitation were selected by pretesting the agitator with the testing unit in place. Four rates of agitation (90, 80, 70, and 63 cycles per minute) were chosen spanning the range of frequencies available, and the dial of the agitator marked to ensure consistent resetting from one testing session to the next. The lowest speed was attained

by turning the agitator dial to its minimum, and was therefore not marked. Testing sessions were 1 hour in duration.

Within Session Intraunit Reliability

Tritrac machines were programmed to collect and record movement counts (Vector Magnitude values) in either 5-minute or 1-minute intervals. Intraunit reliability was determined by calculation of mean Vector Magnitude values for each session, within-session standard deviations and the coefficients of variation for each machine.

Total activity counts (summed Vector Magnitude values for each testing session) for both the 1 minute and 5 minute recording methods were recorded and used to verify the relative equivalence of the 5- and 1-minute recording periods for data collection.

Testing of Interunit Reliability, Sensitivity, and Short Term Stability and Drift

The initial phase of testing was completed over a 2 month period in 1993. A number of testing sessions were performed at each frequency of agitation as follows: 20 tests at 90 cycles per minute, 16 tests at 80 cycles per minute, 12 tests at 70 cycles per minute, and 20 tests at 63 cycles per minute.

To identify differences between the total activity counts recorded by different accelerometers, the data collected at each frequency of testing for all testing sessions in 1993 was pooled, then compared by analysis of variance (ANOVA) followed by the Bonferroni comparison of individual means. Interunit reliability was calculated as the intraclass correlation coefficient (ICC) for random rater effects (Shrout and Fleiss 1979), from the total activity counts collected for individual 1993 testing sessions at 4 frequencies of agitation. Total activity counts rather than minute by minute counts were chosen as the unit of comparison because the between-subject comparisons in the main study would

similarly be based on total activity counts or on activity categories. Activity categories are comprised of groups of data points falling within preset ranges, the development of which is described below.

The sensitivity of each machine to distinguish between different frequencies of agitation was tested by analysis of variance (ANOVA) using the pooled results of testing sessions conducted at each frequency for each of the six machines.

Results for each machine at each frequency were examined for short term stability and drift by plotting total activity count against order of the testing sessions, and fitting a regression line by the least squares method. Consistency or stability was evaluated by examination of the random scatter about the regression line, and calculated from the standard error of the Y estimate, reported as a coefficient of variation for each machine. Drift was determined by testing the significance of the slope of the regression of total activity count on session number for 20 testing sessions in 1993, and again for 10 testing sessions in 1996.

Between Session (Test-Retest) Reliability, Responsiveness and Long Term Stability

Machines were retested following field use, briefly in 1995, and again more extensively in 1996. Only 3 of the original machines remained operational for both subsequent sessions. An additional machine (serial number RD0030, "Machine 30") from another laboratory was included in the later two testing sessions.

By 1996, the laboratory test tube agitator was demonstrating signs of wear. Excessive heating of the motor occurred during agitation at high frequencies. It was felt advisable to repeat tests only at the slowest possible frequency. Only two sessions at higher frequencies were performed, as well as one session when a comparison was made,

of five frequency settings of short (10 minute) duration. All other tests were performed at the lowest dial setting of the agitator.

In order to correct for some inconsistencies in agitator speed that were noted in the 1996 testing sessions, the frequency of the test tube agitator at the lowest dial setting was measured during each session, using a stopwatch to time 100 cycles of agitation (to the nearest second). Between-session variations in frequency were adjusted to a standard speed of 58.5 cycles per minute mathematically, using predictive equations of the regression of activity count on frequency. These equations were derived from the results of a single-day testing session of 5 frequencies in succession. Means and standard deviations of total activity counts for each machine were used to calculate the between session coefficient of variation for each machine. These represent the short term test-retest reliability or precision of the machines. Also using the mathematically adjusted data, data was examined for drift by regressing activity counts on testing session.

The responsiveness of the machines to changes in the frequency of agitation was re-examined in 1996, using the data collected during agitation for 10 minutes at each of 5 different frequencies in succession, without machine repositioning, during a single day. Total activity counts were regressed on frequency of agitation. Linearity was evaluated by examination of the residuals. X- coefficients (slopes) and Y-intercepts were compared by ANOVA followed by the Bonferroni comparison of means. Similar analyses were performed on pooled 1993 data obtained from many tests performed on different days.

The mean activity counts obtained during the 1996 and 1993 trials, for the 3 original machines, were compared by *t*-tests to investigate the long term stability of performance.

Testing of the Effect of Machine Orientation on Vector Magnitude Counts

Machine data, corrected to a consistent frequency of agitation as described above, was also used to examine the effect on the Vector Magnitude activity values of changes in the orientation of the machines relative to the primary direction of movement.

In order to test sensitivity relative to each orientation, the accelerometers were rotated through six position changes relative to the primary and secondary directions of agitator movement. With the x, y, or z axis of the machines aligned with the primary direction of movement (primary orientation), the machines could also be rotated 90 degrees about this axis to change the vertical or horizontal orientation of the remaining axes. For one position, the secondary orientation resulted in the accelerometers standing in an upright position on a relatively narrow base of support. This secondary orientation was described as vertical. The alternate position, with the accelerometers standing on their sides with a wider base of support, was described as being horizontal. Thus, each x, y, and z axis was tested in both horizontal (relatively stable) and vertical (relatively unstable) positions. Results for each x, y, and z primary orientation for each machine were compared by ANOVA.

Horizontal and vertical positions were also pooled for each orientation, and the pooled results compared for each machine. Finally, the x, y, and z values from all 4 machines were pooled to determine if there was any consistent difference between the orientations that might be attributable to a systematic error innate in the design of the units.

Development of Activity Categories

Activity categories were established in order to estimate the percentage of the 24 hour day that study subjects might spend in sedentary, active or vigorous pursuits. While

wearing Tritrac accelerometers, 7 volunteers were instructed to participate in supervised physical tasks chosen to represent different exercise intensities (METS; multiples of resting metabolic rate)(Ainsworth et al. 1992; Bouchard et al. 1983; McArdle et al. 1996a). The physical tasks were: lying quietly (1 MET), sitting eating a meal (approximately 1.5 - 2 METS), standing writing on a chalk board (2 - 3 METS), slow walking on a treadmill at 3.0 km per hour (3 - 4 METS), moderate walking at 4.5 km per hour (4 - 5 METS), vigorous walking at 6.5 km per hour (4 - 11 METS), and jogging/running at 9 km per hour (7 - 15 METS).

As previously described, the machines were worn in modified belt pouches, positioned just medial to the anterior superior iliac spine. The clock used to mark the timing of each physical task was synchronized with the internal clock of the accelerometers which was set via a computer interface. Machines were rested on a level surface in the belt pouch for a minimum of 1 minute prior to start of the activity. This was done to confirm the accuracy of the timing of the activities when examining the data record following downloading via a computer interface. Belt pouches were strapped on immediately prior to the start of the task, and removed and set on a level surface immediately following. The time marking the start and finish of each task was recorded for each subject, and the first and last minute of each data record eliminated to ensure that only the desired activity was included.

Participation in each task lasted a minimum of 15 minutes, and accelerometers were set to record motion in 1-minute intervals. The minute by minute activity counts (Vector Magnitude values) were converted to equivalent 5-minute counts by summation of each 5 successive data points. Each 15 minute testing session therefore contributed 3 values per subject to the final analysis. Data from all subjects was pooled. The ability of accelerometer counts to distinguish between MET levels was examined by ANOVA.

Following this, the pooled data was examined to identify distinct ranges of activity counts (Activity Categories) which might be capable of predicting weight bearing or non weight-bearing activities.

Eight additional unrelated subjects were recruited to test the predictive power of the Activity Categories. Four subjects wore Tritrac accelerometers while participating in treadmill walking, standing, sitting, and lying activities similar to the original subject group. The remaining four subjects agreed to wear accelerometers while participating in usual unstructured but continuous observable occupational or recreational activities which involved sitting, standing or steady walking, or "start and stop" walking. Examples of these activities included watching TV, eating meals, secretarial desk work, standing at a counter taking orders, walking around a block, and walking in a mall. The observed activity, and the start and stop time of that activity, was recorded on a time sheet. The downloaded data was assigned to an Activity Category according to the pre-designated ranges and the proportion of the correct or incorrect responses recorded. Differences among machines in the ability to correctly assign Tritrac data to Activity Categories was tested using the Chi-square statistic. The Chi-square statistic was also used to test for differences among the Activity Categories in the frequency of misclassification of data.

B.3 Results

Bench Testing: Within-Session Intraunit Reliability

The results of intraunit reliability testing are presented in table B1. Within session coefficients of variation were similar for all machines, therefore the results for all 6 machines have been pooled.

Table B1. Mean activity counts (Vector Magnitude) for 1-minute or 5-minute recording intervals, average within-session standard deviations and coefficients of variation, and total counts per session. Pooled data for 6 accelerometers.

Speed	Recording Interval									
	1 Minute					5 Minute				
	N	Vector Mag.	Totals	SD	CV	N	Vector Mag.	Totals	SD	CV
90 cpm	12	923.8	55428	121.3	15.8	8	4699.4	56393	251.5	5.4
80 cpm	10	811.7	48702	64.8	8.0	6	4177.9	50135	216.2	5.1
70 cpm	8	661.2	39671	58.2	8.8	4	3513.2	42158	162.5	4.7
63 cpm	12	536.2	32172	79.4	14.4	8	2887.8	34653	261.7	8.8

Reliability (precision) tended to be poorest at the lowest and highest frequencies with errors ranging from 8.0 to 15.8% for data collected in 1-minute intervals, and 4.7 to 8.8% for data collected in 5-minute intervals. The total activity counts are presented to allow comparison of the relative equivalence the completeness of data collection using either 5- or 1-minute data recording intervals. No differences between the 1- or 5-minute recording methods were detected at any frequency.

Interunit Reliability, Sensitivity and Short Term Stability

There were significant differences between machines, with relative differences in machine output remaining consistent over time and frequency. Machine 35 demonstrated a tendency to produce the highest values and Machine 32 the lowest (figure B1, page 214). At the slowest agitation frequency, only Machine 32 recorded values significantly different from the other machines. At higher frequencies, however, additional machines demonstrated significant differences, suggesting between-machine variability in their responsiveness to changes in the intensity of movement.

In spite of significant differences between machines tested at the same frequency of agitation, the interunit reliability (agreement) of the machines for activity counts collected across the entire range of tested frequencies was very good (ICC 0.89; confidence interval 0.77 - 0.96).

All accelerometers were able to differentiate between the 4 testing frequencies ($p < 0.0001$). Single day testing data (1996) suggested that the relationship between frequency of agitation and total activity count was adequately described by a straight line within the range tested. There was random scatter of the error component around the fitted data (i.e. residuals were normally distributed), although lack of a zero Y intercept suggested a non linear response outside of the tested range (table B2).

When 1993 activity counts were regressed on testing session, at all frequencies, there was wide scatter about the regression lines. The average error of the Y estimate expressed as a coefficient of variation was 8.3% for all machines at all frequencies (range 7.0 to 10.8). There was a tendency for all machines to follow similar patterns of scatter, suggesting that an extrinsic source of variation contributed to the measured error (figure B2, page 215).

Figure B3 (page 215) shows that in 1993, at the lowest agitation frequency, there was a significant decrease in activity values over time ($p = 0.04$). Over a time period of 2 months (Trials 1 through 20), fitted total Vector Magnitude counts fell from approximately 39,000 to 28,000 per testing session. At the highest frequency of agitation, there was a trend toward an increase in activity values for all machines, but this did not reach statistical significance for any machine. When 1996 activity counts collected at low frequency were plotted against testing session, there was no evidence of a decline over time (10 tests).

Responsiveness, Long Term Stability, and Between Session Reliability (Precision)

Slopes and intercepts for the regression of total activity count on frequency of agitation for each machine in 1996 are presented in table B2, along with the slopes and intercepts of similar analyses performed on the 1993 data.

Table B2. Regression of total activity count on frequency of agitation (cycles per minute)

Accelerometer	X-coefficient	SE	Y-Intercept	SE
Pooled Data From All 1993 Trials (20 testing days, 68 1-hr tests, 4 frequencies)				
Machine 29	799.1	40.8	-15132.0	3140.8
Machine 31	853.6	39.8	-21390.8	3064.1
Machine 32	835.2	42.9	-23077.3	3300.3
Machine 34	877.7	44.9	-21097.8	3452.0
Machine 35	1068.9*	53.3	-32244.8*	4102.7
Machine 36	919.3	43.3	-23249.6	3333.2
Pooled Data	892.3	40.7	-22685.2	3127.9
Single Testing Session, 1996 (1 day, 5 10-min tests, 5 frequencies)				
Machine 31	978.9	52.1	-25950.8	4212.8
Machine 32	695.3**	41.1	-16065.9	3324.2
Machine 36	852.6	69.7	-20611.6	5634.3
Machine 30	921.9	62.0	-26146.9	5010.6
Pooled Data	862.2	36.0	-22193.8	2908.4

* slope is significantly greater than all other machines; Y intercept is different from Machine 29
** significantly less than Machine 30 or 31; no difference between intercepts

The Y intercepts were not significantly different between machines, except for Machine 35 which ceased to operate shortly thereafter. In 1996, one machine differed in its responsiveness to changes in frequency of agitation. Machine 32 demonstrated a significantly blunted response to increases in frequency relative to the other machines, as

demonstrated by a smaller X-coefficient (slope). This was consistent with its tendency to underestimate movement at all frequencies as previously described (figure B1, page 214).

Means, standard deviations, and coefficients of variation (between session) for the 1996 testing sessions are presented in table B3. Columns represent data either uncorrected or mathematically adjusted to a standard frequency of agitation. Adjusting data to a consistent frequency of agitation improved coefficients of variation considerably such that the between-session variability was about 7% (6.6 to 7.3%).

Table B3. Mean total activity count, between-session standard deviations and coefficients of variation for 10 testing sessions at low frequency of agitation (data corrected to 58.5 cycles per minute) in 1996.

	Uncorrected Data			Frequency-Adjusted Data		
Machine	Mean	SD	CV	Mean	SD	CV
31	33642.6	3748.3	11.1	33642.6	2264.4	6.7
32	30754.7	3669.8	11.9	30754.7	2254.0	7.3
36	33551.5	3642.8	10.9	33551.5	2241.9	6.7
30	34677.9	4164.4	12.0	34677.9	2276.1	6.6

There was no significant difference between activity counts collected at 63 cycles per minute, in 1993, and those collected at the same frequency in 1995 or 1996 (ANOVA, $p = 0.77$; regression of activity count on year $p = 0.65$). This suggests that the apparent drift detected in the 1993 data may have been the result of extrinsic factors, rather than a decline in the sensitivity of the accelerometers as a result of time and use.

Effect of Machine Orientation

Positioning of the accelerometers (oriented with either x, y, or z axes as the primary direction of acceleration) had a significant influence on total activity counts.

Table B4. Means (Vector Magnitude) and standard deviations according to machine orientation relative to the principal direction of agitation; pooled data from two trials. Corrected to agitator frequency 62.5 cpm.

		Primary Machine Orientation					
Machine	Secondary Orientation	x	pooled x	y	pooled y	z	pooled z
31	horizontal	591.9(126.9)	620.4(139.7)	564.2 (78.5)	644.3(125.1)	491.0(95.2)*c	537.7(110.1)*c
	vertical	648.9(147.0)*a		724.5(111.0)*a		584.4(104.5)*a	
32	horizontal	447.3(100.3)	497.8(118.5)	475.8(60.5)	549.9(104.1)*d	487.9(123.2)	524.8(126.3)
	vertical	548.3(114.3)		624.0(83.8)*b		561.8(119.4)	
36	horizontal	532.4(127.5)	571.5(131.9)	532.2(56.7)	536.5(63.6)*e	521.5(122.6)	573.3(132.8)
	vertical	610.6(125.5)		540.7(70.1)*b		625.1(122.7)	
30	horizontal	498.0(93.1)	566.7(136.8)	492.2(60.7)	520.3(69.1)*b	536.0(86.8)*c	585.6(139.6)
	vertical	635.5(139.4)		548.4(65.8)*b		635.1(163.7)	
			564.1 (138.6)		562.8(105.4)		555.1(129.8)

a : x is significantly different ($p < 0.01$) from y is significantly different from z.
b: y is significantly different ($p < 0.01$) from both x and z
c: z is significantly different ($p < 0.01$) from both x and y
d: y is significantly different ($p < 0.01$) from x
e: y is significantly different ($p < 0.05$) from z

Differences were not, however, consistent between machines (table B4), with pooled results (all 4 machines) yielding no difference between orientations (tables B4 and B5). Expectedly, when machines were positioned such that the secondary alignment of axes rendered the machine position more vertical, the Vector Magnitude counts were significantly higher than those recorded in the horizontal position (table B5).

Table B5. Effect of machine position on Vector Magnitude output; pooled data for all machines. Average Vector Magnitude movement count per minute (means and standard deviations). Agitator frequency: 62.5 cycles per minute.

Secondary Orientation	Machine Primary Orientation Relative to Principal Direction of Agitation			
	x	y	z	
Vertical	610.8 (136.9)	609.4 (112.1)	601.6 (131.7)	p = 0.834 ns
Horizontal	517.4 (124.1)	516.1 (73.0)	509.1 (109.4)	p = 0.803
Pooled Data	564.1 (138.6)	562.8 (105.4)	555.1 (129.8)	

Establishment of Activity Categories

Means, standard deviations, and the range of activity values for each activity category are presented in table B6. Results are pooled data from all 7 subjects, with the exception of level 7. Only 3 subjects were able to complete 15 minutes at this intensity.

Differences in the average activity counts for activity Levels 1 and 2 (lying and sitting) did not reach statistical significance due to large standard deviations. All other activity levels were significantly different from each other and from these 2 levels ($p < 0.0001$). There was some overlap between the activity counts recorded for Levels 2 and 3 (active sitting and standing), and between Levels 4 and 5 (walking on a treadmill at 3.0 or 4.5 km per hour). There was no overlap between the values recorded for Level 2 versus 3, or between Levels 6 and 7 (moderate versus vigorous walking or jogging).

Table B6. Activity counts during performance of standardized activities, presented as Vector Magnitude values per 5-minute recording interval.

Activity Level	Description of Activity	N	Mean Count	SD	Range
1	Lying	21	23.6	23.5	0 - 82
2	Sitting	21	78.9	37.6	26 - 144
3	Standing	18	285.1	112.0	119 - 496
4	Treadmill (3.0 km/h)	18	4059.9	750.9	2526 - 4976
5	Treadmill (4.5km/h)	18	6130.6	702.4	4470 - 7185
6	Treadmill (6.5 km/h)	15	10117.9	913.5	7878 - 11470
7	Jog/Run (9km/h)	9	21066.7	2880.9	16421 - 26158

Using this data, the ranges of accelerometer values thought to best characterize different types of daily activities were defined in the following manner. Levels 1 and 2 were collapsed into one category defined by the 5-minute Vector Magnitude values of 1 to 100 and referred to as "Sedentary". Levels 6 and 7 were collapsed to form a category including all data at or above 7500 counts, because it was felt that few postmenopausal women would be likely to participate in steady activities at an intensity equivalent to level 7. This category was called "High Energy", and collectively contains intensities of activity elsewhere defined as "Heavy and Very Heavy" for middle-aged and older women (McArdle et al. 1996a). Levels 4 and 5 were collapsed into a single category of values from 2501 to 7500, referred to as "Steady Walking". The division between Sedentary and light standing activity (Standing) was arbitrarily set at 100 counts. This division correctly categorized all of the Standing data (data range 101 to 500), and 90% of the Sedentary data (data range 1 to 100; all of the lying data, and 71% of the sitting data). Data values between the upper limit of Standing and the lower limit of Steady Walking (501 to 2500

activity counts) were set into a new category, "Mixed Ambulatory". The resulting categories and ranges are listed below:

ACTIVITY CATEGORIES	
Sedentary	0 - 100
Standing	101 - 500
Mixed Ambulatory	501 - 2500
Steady Walking	2501 - 7500
High Energy	7500 +

In the second phase of testing, use of the Activity Category data ranges to classify activity resulted in the correct classification of 85% of the 67 observed 5-minute time intervals. The 95% confidence interval for this proportion is 0.70 to 0.93. Table B7 presents the number and proportion of correct classifications, according to machine and Activity Category.

Table B7. Number and percentage of correct classifications of observed activities by application of Activity Category data ranges.

Machine	Number of Data points	Number Correct (Percentage)	Activity Category	Number of Data points	Number Correct (Percentage)
31	11	10 (91)	Sedentary	18	14 (77)
32	30	25 (83)	Standing	10	6 (60)
35	8	7 (88)	Mixed	9	9 (100)
36	18	15 (83)	Walking	27	25 (92)
			High Energy	3	3 (100)

Proportions (of correct responses) were not significantly different between machines ($p = 0.45$). Four of the 18 sampling intervals measured during sitting activities were incorrectly assigned to the Standing category. Similarly, 4 intervals sampled during standing activities

were incorrectly assigned to the Sedentary category. Two intervals sampled during slow walking (on a treadmill) were incorrectly assigned to the Mixed ambulatory category. This tendency for greater error of classification amongst the lower categories just failed to reach statistical significance ($p = 0.06$)

B.4 Discussion

We experienced mechanical failure of half of our accelerometers over a 3 year period of heavy field use. Surviving units, however, demonstrated good stability of performance as demonstrated by bench testing. During the initial 2 months of testing of 6 units in 1993 (20 testing sessions), we noted a decline in the activity counts of all units during low frequency agitation. This apparent drift did not occur, however, at other frequencies. Additional Tritrac testing of 3 of the original units in 1996, failed to reveal any drift across an additional 20 testing sessions, and no significant change in activity counts from the 1993 data. This suggests good stability of the surviving units. Because the agitator used for bench testing demonstrated a change in performance over time, it is plausible the decline in activity counts at low frequency agitation in 1993 resulted from drift in the performance of the testing apparatus.

During bench testing, we noted significant differences between accelerometers which remained consistent over time. This suggests systematic error as might be attributed to differences in machine calibration. Because our machines were recently received from the manufacturer at the time of initial bench testing, these differences cannot be attributed to some aspect of machine fatigue or damage due to misuse by study subjects. Nor can they be attributed to the testing apparatus, because all machines were tested simultaneously. In spite of these differences, however, all machines were able to distinguish between different frequencies of agitation. We do not have a direct

measurement of the acceleration forces generated during this activity, but note that the activity counts recorded by the accelerometers were within the range found to represent slow to moderately brisk walking. Differences between machines were in the order of 10 to 20% (about 400 activity counts per 5-minute measurement interval). However, absolute differences in accelerator-generated activity counts indicative of slow versus brisk walking (3.0 versus 4.5 km per hour) were an order of magnitude greater. Therefore, within this range of activity counts, the differences between machines can be considered minor, and the agreement between units can be considered good, as demonstrated by a high intraclass correlation coefficient (ICC 0.89; confidence interval 0.77 to 0.96).

Of clinical note, between-machine differences were position-dependent, suggesting differences in the sensitivity of the 3 internal accelerometers (sensors) contained by each unit. We had initially considered derivation of a correction factor to improve interunit reliability. Development of such correction factors would be complex, however, and may not give predictable results in less predictable environments. In field use, it is anticipated that accelerometers will be subject to considerable variation in the angle and primary axis of acceleration. Correction factors developed during mechanical testing situations in which acceleration is unidirectional, may not accurately adjust activity data collected during field use. Unless all 3 internal sensors within a machine are relatively less sensitive compared with other machines, any interunit differences should be attenuated in complex environments. It is probably advisable, however, that accelerometers be oriented similarly for all sessions of use within a given experiment, particularly if attempted to detect change over time. For this type of experimental design, repeated use of the same accelerometer should also improve precision. For experiments designed to detect differences between individuals, repeated testing (of each subject) with different accelerometers may afford some additional protection against systematic machine bias.

Kochersberger et al. (1996) reported a higher intraclass correlation coefficient (ICC 0.97) when comparing 9 Tritrac accelerometers on a "shaker table" which rotated the accelerometers in two dimensions. The group of accelerometers tested by Kochersberger may have been well calibrated for the axes of movement tested. Also, because the accelerometers were rotated, relatively large forces of acceleration would be detected by 2 of the 3 internal sensors. This would tend to attenuate any interunit differences attributable to poor calibration a single internal sensor. However, additional differences in study design and statistical methods may have contributed to the variance in ICC values.

Kochersberger et al. (1996) calculated their ICCs from the minute by minute values recorded during rotation at variable speeds. We used total frequency counts from many testing sessions in which machines were accelerated and decelerated at a consistent frequency along one axis. Although neither testing situation is entirely representative of acceleration/deceleration forces encountered in field use, the choice of comparison by total frequency counts is more applicable to the type of field use and data collection planned for our substantive study.

It is also possible that the coefficients reported by Kochersberger were higher than ours because of a greater range of acceleration tested, or because the range was limited to relatively lower accelerations. We found that discrepancies between some machines became more apparent during more vigorous testing (high accelerations). Kochersberger et al. rotated the machines at various speeds, starting "at a level where the units just detected motion". Although this implies that quicker speeds were also used for testing, details were not provided, so we are uncertain if movement counts registered during bench testing in this manner fell within a typical range recorded during field use. It is therefore important to bench test at accelerations within the range expected during field use. The

movement counts generated during our bench testing sessions were similar to those recorded when accelerometers were worn by subjects during steady leisurely or brisk walking. Kochersberger also reported high test-retest reliability of Tritrac readings for repeated 5-minute sessions of sitting, and treadmill walking, but again the walking was limited to slow speeds (1 and 2 mph; $r = 0.97$).

We found that the Tritracs were able to distinguish between different intensities of ambulatory activities when worn by study subjects in a structured setting. The instruments are not able to predictably distinguish between different low intensity activities in lying and sitting. No attempt was made in this study to measure energy expenditure directly and relate Tritrac scores to small variations in energy expenditure during different low intensity activities.

We developed a set of ranges of activity values reflective of different intensities of non-weight bearing and weight bearing activities, and tested them in a small group of subjects for their predictive capabilities. We were able to correctly classify these activities with 85% accuracy. As might be expected, most misclassification appeared to occur during activities of low intensity, although due to the small sample, this did not reach statistical significance. Also, this testing is further limited by the fact that the subjects were observed, and movement monitored, during only a small selection of activities. These Activity Categories are promising, however, in that they may provide a method of describing the habitual activity patterns of subjects monitored objectively, without the burden and subjectivity of activity diaries, and the intrusion of observation. As a further step to development of this method of categorizing accelerometer results, cross validation against an established observational technique is recommended.

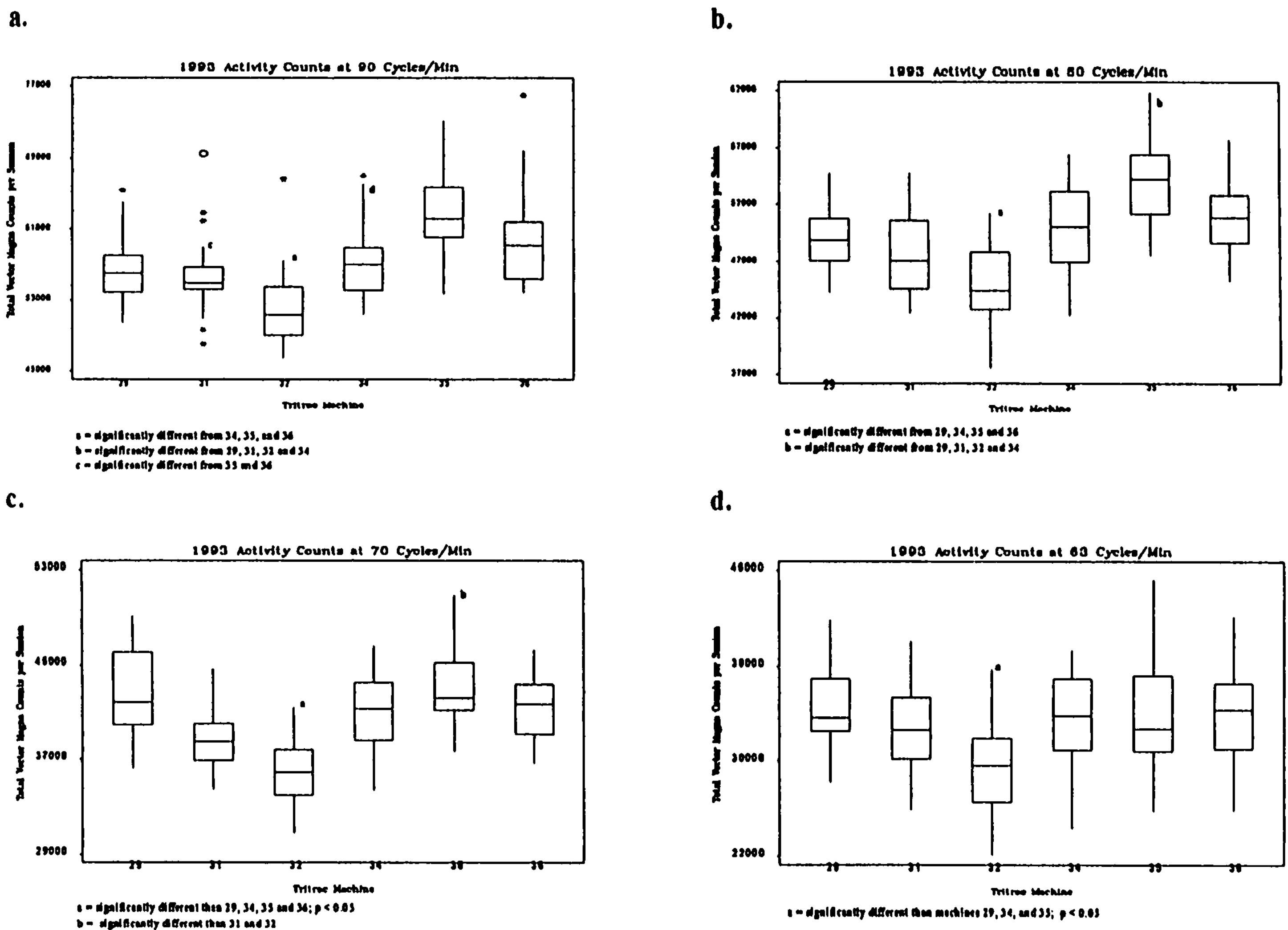

Figure B1. Box and whisker plots of total activity counts (Vector Magnitude counts) recorded during all testing sessions in 1993. The relative differences between the machines are illustrated.

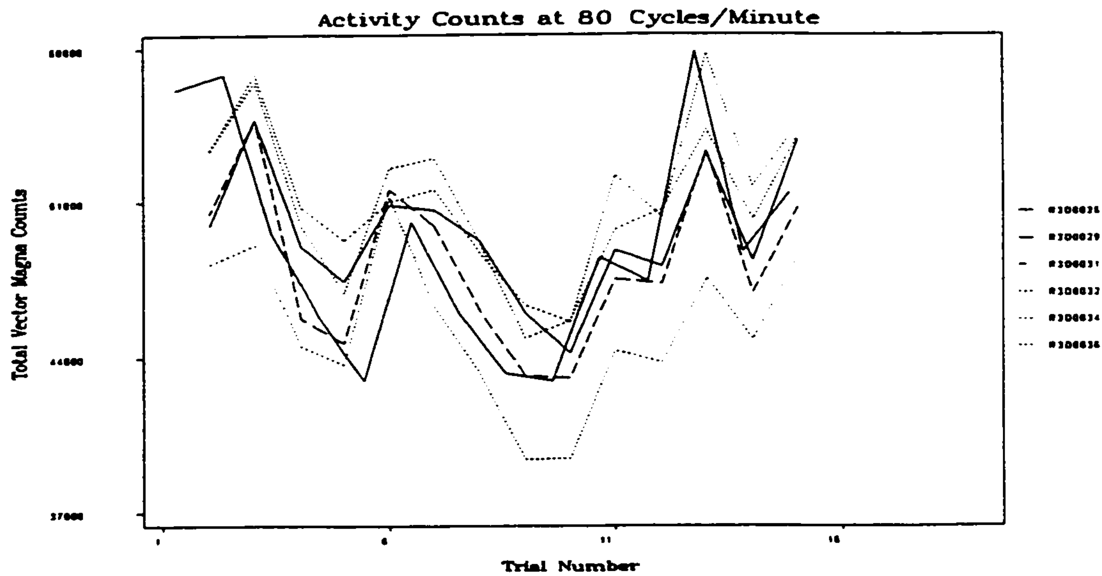

Figure B2. Time series plot of total Vector Magnitude counts recorded per testing session during 1993 trials of mechanical agitation at a frequency of 80 cycles per minute.

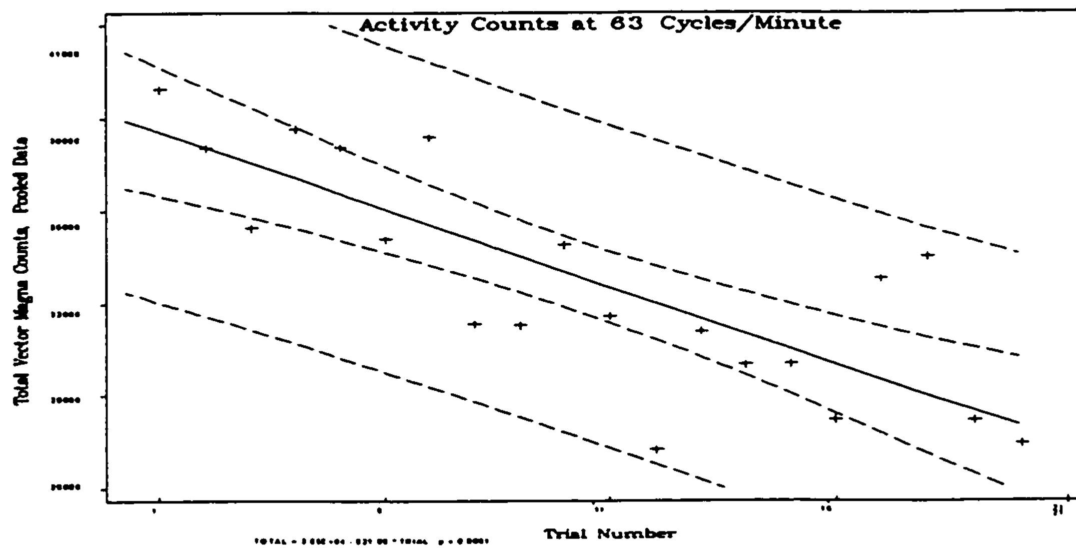

Figure B3. Apparent drift in measurement of low frequency movement over a series of 20 testing sessions in 1993.

APPENDIX C

VALIDITY AND STANDARDIZATION OF THE FOOD FREQUENCY QUESTIONNAIRE

C.1 Introduction

To evaluate the importance of daily calcium intake to the rate of bone loss in postmenopausal women, there is a need to assess the usual dietary habits of these women. The method used must be practical for a large number of women, effect little change in behaviour, and be capable of reflecting diet during a specified period of interest. Food frequency questionnaires (FFQs) target food habits during specified weeks or months, rather than isolated days as captured in food diaries. They may therefore be more representative of long-term habit. Unlike detailed dietary records, however, FFQs sample only a selected number of the foods ingested by a subject, and provide only an estimation of portion size. FFQs may tend to underestimate or overestimate the intake of certain nutrients (Block et al. 1986; Liu et al. 1996). It has been recommended, therefore, that studies using FFQs examine a sample of participants who also keep detailed dietary records (Kushi 1994). The results may be used to quantitatively calibrate the nutrient estimates from the FFQs. Although this may not change the ranking of individuals, it may be of benefit if an ultimate goal is to provide quantitative recommendations for nutrient intake. Criterion validity is also assessed in this manner, because the true dietary intake of subjects over a protracted period of time is essentially unknowable.

Comparison of FFQs to dietary records rather than dietary recall methods has been recommended because, although FFQs and recall both rely on memory of intake patterns,

food records rely strictly on recording. For this reason, comparison of FFQs to food records is less likely to produce an overestimate of the validity of the instrument (Block and Hartman 1989).

Block et al. (1986) have developed an approach to an FFQ design which is semi-quantitative in terms of portion sizes. The design includes foods based on their contribution to total population intake of energy and specified nutrients according to the National Health and Nutrition Examination Survey (NHANES II)(1985). Portion sizes and nutrient content for the selected food are also those reported by NHANES II and based on United States Department of Agriculture data tapes from 1980 (Cummings et al. 1987). The instrument has demonstrated good reproducibility with interclass correlation coefficients of 0.7 (Block and Hartman 1989). When piloted among approximately 1,000 volunteers and cancer trial participants in 3 states, the FFQ yielded higher estimates of the intakes of a few nutrients, including calcium, when compared with national averages (Block et al. 1986). These differences may, however, be attributable to changing public dietary habit, or differences in the demographics of the study population when compared with the national average.

One version of the Block style FFQ contains 34 foods that represent 85% of the intake of calcium in the adult American diet. The questionnaire was validated by comparison with the results of 7-day food records in 37 women over the age of 65 (Cummings et al. 1987). Daily calcium intake measured by the Block FFQ correlated well with calcium intake calculated from seven-day food records ($r = 0.76$). The apparent overestimation of daily calcium intake was a modest 25 mg or 4%. The questionnaire correctly identified greater than 90% (31 of 32) of women with low calcium intakes (< 800 mg per day).

Using the format described by Block et al. (1985; 1986) and Cummings (1987) we developed an FFQ to estimate the daily dietary intake of calcium, phosphorus, and protein in postmenopausal women in our geographic area. Initially, foods were selected on the basis of their contribution to the dietary intakes of these nutrients according to the NHANES II survey. Some foods were added after informal discussion with postmenopausal women concerning their eating habits, discussion with an experienced dietitian, and review of publicly available pamphlets and food lists describing recommended sources of calcium.

Foods were grouped according to major food groups or categories (dairy, breads and cereals, meats, vegetables, desserts and snacks), and some food types were subdivided to simplify the reporting of usual portion sizes, when these portion sizes may differ greatly in common use (i.e. a glass of milk versus milk in tea or coffee). A standard medium portion size was listed for each food on the FFQ, and subjects were requested to report the size of their usual portion (small, medium, or large) compared with the medium portion specified. A small portion was 0.5 times this amount, and a large portion 1.5 times the medium amount. Instructions directed the subjects to complete the FFQ based on their dietary habits during the preceding 6 months, and to indicate the frequency of consumption of specific food items in number of times per day, per week, per month, or in the past six months (for foods infrequently consumed). Following development, the survey was piloted in a small group of women to assess the clarity of instructions and readability.

Portion sizes and nutrient values were obtained from the Nutrient Analysis Program (Elizabeth Warwick, 1991) based on the 1991 Canadian Nutrient and Condensed Files. The results were entered into a database computer program (Paradox, version 3.5) designed to yield average estimates of the daily intake of each nutrient.

C.2 Testing of Criterion Validity

A group of women were identified who were participants in the Diet and Breast Cancer Prevention Trial, a large multicentre trial of the influence of dietary fat on breast cancer. As part of this trial study subjects regularly complete diet records. Researchers conducting the trial were contacted, and it was agreed that women who were part of the control wing of the trial, in Toronto, might be approached for their willingness to complete an FFQ. The study was approved by the ethics board of the University of Toronto.

Diet records were routinely completed for the Ontario Cancer Institute (OCI) in Toronto in the following manner. Women were randomly assigned 3 non-consecutive days to monitor their diet prior to each annual clinic visit. During these days, women recorded the quantity of all foods and beverages consumed. To the extent possible, quantities of foods were measured, weights recorded from packages, or weights directly determined on scales supplied by the study organizers. Diaries were submitted to the OCI on a regularly scheduled annual visit. If appropriate, brand names, methods of food preparation, or recipes of homemade dishes were included. Nutrient calculations from the dietary records were performed using the Minnesota Nutrient Data System (NDS) software, developed by the Nutrition Coordinating Center at the University of Minnestota, MN.

On this annual visit, or by mail, women who had completed diet records within the previous six months were invited to complete an FFQ. Women were provided with written instruction on how to complete the FFQ, recording the frequency and amounts of foods consumed. FFQs were returned by mail to MUMC and analyzed to give estimates of daily intakes of calcium, phosphorus, and protein based on a Canadian database.

In order to determine the effect of the different databases on the daily averages, the nutrient values from the NDS database were substituted for the Canadian nutrient values for each food item in the individual FFQs, and daily averages calculated for each nutrient based on the American database. Care was taken to ensure that the food types and portion sizes were similar.

C.3 Statistical Analysis

Sample size for the study was determined by the following method (Edwards 1976). If the expected correlation between the food records and FFQs is anticipated to be 0.7 (Cummings et al. 1987), an acceptable confidence range for this correlation was set at approximately $0.6 < r < 0.8$. A sample size of 80 will yield comparable confidence intervals as described by the following calculations:

$$z_r \pm t_{\alpha/2,n-2} \times \sigma_{z_r}$$

$$z_{0.7} = 0.867$$

$$\sigma_{z_r} = \frac{1}{\sqrt{n-3}} = \frac{1}{\sqrt{80-3}} = 0.114$$

Therefore the confidence interval around the z-transformed correlation is

$$0.867 \pm 1.96 \times 0.114$$

or

$$0.644 < z_r < 1.090$$

The r's corresponding to 0.644 and 1.094, are 0.568 and 0.797 respectively. Thus the 95% confidence interval for a correlation of 0.7 with a population size of 80 is

approximately 0.57 to 0.80. In anticipation of an 80% response rate, target enrollment was set at 100.

The values obtained for each nutrient, from the dietary records and FFQs, were compared using Pearson product moment correlations. Paired *t*-tests were used to compare the nutrient levels from the Canadian and American databases, as well as to determine the extent of underestimation or overestimation of nutrient intakes by the FFQ relative to the dietary records.

The effect of the choice of nutrient database on the strength of the correlations between the FFQ and food records for each nutrient, was tested by converting *r*-values to *z*-scores, then dividing the difference between *z*-scores by the standard error of that difference. The result was compared with a standard table of *z*-values to determine the level of significance.

C.4 Results

FFQs were mailed or distributed to eligible OCI subjects. Of 110 FFQs mailed, 73 (66%) were completed and returned. Of those returned, 1 subject had incomplete food records, and 6 FFQs were incomplete or were unusable because they were completed using tick marks instead of numerical values to indicate frequency of foods ingested. The remaining 66 were analyzed. Three cases were omitted for implausible values reported on either food records or FFQs (2 FFQs and 1 food record; values in excess of 4 standard deviations [SD] above the mean values for protein intake in spite of body mass indexes [BMI] of 25 or less and weight within 1 SD of the mean). A total of 63 subjects were included in the final analysis. Characteristics of those subjects are presented in table C1.

Table C1. Characteristics of 63 OCI subjects completing usable food records and FFQs.

	Mean	SD
Age (yrs)	53	6
Weight (kg)	64	8
Energy Intake (kcal/day)	1760	392
BMI	24	3

There were no significant differences in the estimated daily intakes of calcium or phosphorus as assessed by the FFQ or dietary records. Protein intake was slightly but significantly underestimated by the FFQ (table C2).

Table C2. Average daily intakes of selected nutrients by each of the methods of assessment (means ± standard deviation).

	Calcium (mg)	Phosphorus(mg)	Protein (gm)	Energy
Food Records	828 (± 293)	1219 (± 281)	71 (±15)	1760 (±392)
FFQ (Canadian Database)	856 (± 409)	1187 (± 409)	65 (± 21)*	---
FFQ (American Database)	825 (± 390)	1094 (± 376)†	65 (± 21)*	---

* significantly different from estimated intake from Food Records, $p < 0.05$
† significantly different from FFQ (Canadian Database) $p < 0.001$

FFQs and food records were significantly correlated for all nutrients, with the strongest correlations for estimated daily intake of calcium ($r = 0.60$)(table C3).

Table C3. Pearson Product Moment Correlations (r) of the Diet Record nutrient values with those estimated from the FFQ.

	FFQ (Canadian Database)	FFQ (American Database)
Calcium	0.60‡	0.56‡
Phosphorus	0.38†	0.40†
Protein	0.32*	0.35†

* $p < 0.05$, †$p < 0.01$, ‡$p < 0.0001$

The correlations of table 3 were compared statistically by first converting to z-scores by the following formula (Edwards 1976):

$$z_r = 1/2\,[\log_e(1 + r) - \log_e(1 - r)]$$

$$z_{0.60} = 0.693$$

$$z_{0.56} = 0.633$$

then the difference between the z scores is tested with the formula:

$$z = \frac{(z_{0.60} - z_{0.56})}{\sigma_{z0.60-z0.56}}$$

where

$$\sigma_{0.60} = 1\big/\sqrt{63-3} = 0.129 \quad \text{and} \quad \sigma_{0.56} = 1\big/\sqrt{62-3} = 0.130$$

therefore the standard deviation of the difference between the two z-scores is

$$\sigma_{z0.60-z0.56} = \sqrt{\sigma^2_{z0.60} + \sigma^2_{z0.56}} = 0.1833$$

The test of the difference between the two correlations is therefore given by

$$z = \frac{(0.693 - 0.633)}{0.1833}$$

$$z = 0.3273$$

Using a table of "z", we find that the probability of $z > -0.3273$ and $z < 0.3273$ is 0.74. Also 0.3273 does not exceed the critical value 0.4750. The null hypothesis is tenable, and we conclude that the correlations are not significantly different.

The correlations for the other nutrients were similarly tested and were not found to be significantly different. We can therefore conclude that the use of a different database for calculating the nutrient contents of the recorded foods did not significantly affect the strength of the association between the FFQ and food records.

C.5 Discussion

We found that an FFQ developed to estimate the daily intake of calcium, phosphorus, and protein in southern Ontario women, compared acceptably with nutrient estimates determined from 3-day food records. There was no difference in daily calcium or phosphorus intakes according to method, although daily intake of protein may be slightly underestimated by the FFQ. This may be due to inadequate representation of non-meat proteins in the FFQ. Study subjects were completing dietary records as part of their involvement in a study of the influence of dietary fat on breast cancer. Although the 63 subjects completing FFQs were part of the control group of the breast cancer study, and therefore not involved in any dietary intervention, they may have exhibited greater health-conscious behaviours than the general population, and have attempted to reduce dietary fats by substituting non-meat proteins for meat products. There was some indication that health conciousness increased in the study group due to study involvement. Sequential dietary records indicated a 1% per year decline in fat intake.

The correlations between the estimated intake of calcium from the FFQ and food records fell withing the range anticipated. Perfect correlations are not expected because

of the variability in daily dietary habits which may influence food records, and the estimation of portion sizes (as opposed to stringent measurement) in the FFQs. Also, the average time between receipt of food records for the cancer trial during a regularly scheduled visit, and completion of FFQs, was approximately 44 days. Because food records were completed prior to the regularly scheduled visit date, the actual time between completion of the two forms of survey would have been greater than 44 days, but was not accessable at the time of preparation of this report. The extended time between surveys would tend to reduce any recall bias which may be introduced when instruments are delivered within a short period of time. Cummings et al. (1987). reported higher correlations, but they did not report the time interval between completion of the food records and FFQs. Our correlations may also be slightly lower because our women completed only 3 days of dietary records whereas those studied by Cummings et al. (1987) completed 7 days of records. Our nutrient estimated from the dietary records may have therefore demonstrated increased variability, and have been somewhat less representative of usual dietary habit. This is not anticipated to be problematic for the larger study of diet and cancer, because dietary records are repeated annually over a number of years. For this comparison, however, we analyzed only those records completed within the 6 months preceding the administration of the FFQ.

Correlations between the instruments were lower than expected for the daily nutrient intakes of phosphorus and protein. Phosphorus contents of approximately 5% of food represented in the FFQ were missing from the Canadian database. Approximately 4% of phosphorus contents were missing from the NDS database. Omissions such as these may have contributed to a lower correlation between instruments for this nutrient, particularly if these foods were frequently reported. Correlations were not significantly altered by substituting values from the American database into the FFQ. This suggests

that differences in nutrient content reported by the two databases did not contribute significantly to the lower correlation. Problems due to missing data would not, however, have been corrected by this substitution procedure, because the Canadian nutrient values were converted to the American values mathematically for each FFQ, and missing values in the original FFQ database would continue to be perpetuated after conversion.

The modest correlation between the FFQ and food records for protein and phosphorus may again have resulted from dietary habits, such as the substitution of vegetable proteins for meat products, which were not captured by the FFQ. Many meat-based protein-rich foods are also high in phosphorus. Our FFQ was developed using the major contributors of calcium, phosphorus and protein in the American diet according to the NHANES II survey. Canadian dietary habits may be similar in terms of calcium-rich foods, but may otherwise differ in terms of the contributors of protein and phosphorus. Finally, although the correlations for these nutrients were not as high as that for calcium, the mean values were statistically equivalent for phosphorus, and only slightly different for protein. This may suggest that the food items are reasonably representative in terms of sources of protein and phosphorus, but that higher daily variation in intake exists for these nutrients when compared with calcium. Longer dietary records would reduce this variation and result in higher correlations.

In conclusion, a 130 item FFQ compared favourably with 3-day food records in estimating daily intake of calcium, phosphorus, and protein. Correlations between the methods were strongest for calcium. This may indicate that calcium habit exhibits less day to day variation that either phosphorus or protein. The average intake of dietary calcium in this sample of southern Ontario women was less than 860 mg per day, and was not different between methods. The Osteoporosis Society of Canada has set the recommended calcium intake for adult women at 1000 mg per day. As participants in a

dietary study, these subjects were probably more health conscious than the average population. This suggests that the average adult women falls short of the recommended intake of calcium.

APPENDIX D

PREPARATION OF STANDARDS FOR SCINTILLATION COUNTING

1. Prepare non-radioactive stock by adding 322.8 mg of calcium gluconate (MW 430.38) to 300 ml of deionized water to create a 0.0025 Molar solution of dissolved calcium gluconate.

2. To prepare radioactive stock, 0.02 MBq of ^{45}Ca as ^{45}CaCl solution is diluted to100 ml with non-radioactive stock. This solution represents 100% of administered dose per litre; i.e. 0.2 MBq ^{45}Ca per litre.

3. 10 ml standards of 1% to 5% of oral dose per litre are prepared in duplicate by the following dilutions:
   5% standard = 500 μλ of 100% solution; add 9.5 ml calcium gluconate solution
   3% standard = 300 μλ of 100% solution; add 9.7 ml calcium gluconate solution
   2% standard = 200 μλ of 100% solution; add 9.8 ml calcium gluconate solution
   1% standard = 100 μλ of 100% solution; add 9.9 ml calcium gluconate solution

4. In duplicate, transfer 1 ml of each standard solution to a scintillation vial. Add 10 ml of scintillation fluid (Ecoscint H, National Diagnostics, Atlanta, GA).

5. Add 10 ml of scintillation fluid to 1 ml of calcium gluconate stock in duplicate as blanks. These blanks are used to determine background radiation during counting.

6. Blanks, standards, and unknowns are counted in a liquid scintillation counter, for 100 minutes.

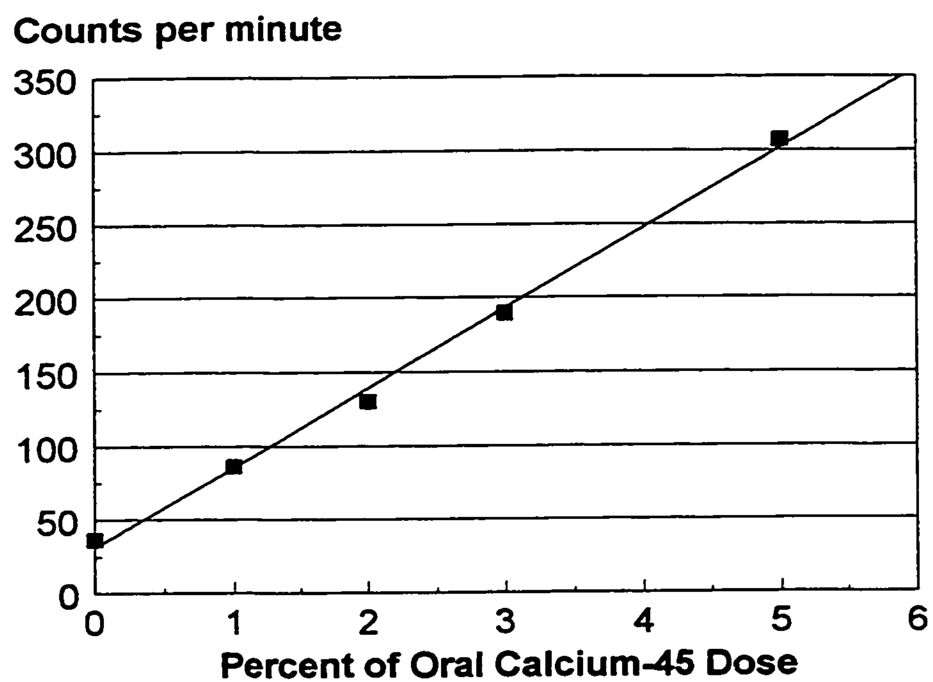

Figure D1. Example of standard curve of the regression of radiation counts on percent of oral dose of calcium-45, determined on August 23, 1993.

APPENDIX E

FAT AND BMD STUDY

E.1 Introduction

Positive associations between body weight and BMD measured at a number of sites are common in the literature. BMD, a measure of areal density rather than true density, is influenced by the size of the skeleton, however. Obesity, or relative body fatness, which is independent of stature, has also been reported to be a positive predictor of BMD suggesting that this is a relationship of some physiological importance. Clinically, the relationship between body weight and bone mass has broad implications. There is an inverse association between body weight and the risk of fracture (Ribot et al. 1994).

However, relationships between bone mass and body weight may be largely established during bone acquisition, with these associations persisting into adulthood. There is some evidence, however, to suggest a protective effect of obesity on the preservation of bone mass after menopause (Tremollieres et al. 1993), although the effects of weight changes on remodeling are less well studied. A relationship between change in bone mass and weight loss has been demonstrated (Compston et al. 1992b; Jensen et al. 1994; Pritchard et al. 1996; Ramsdale and Bassey 1994; Ryan et al. 1998) in several groups of dieters, but bone changes appear to be transient (Compston et al. 1992b). Rapid changes in bone mass could be attributed to increases or decreases in activation of remodeling cycles, thus altering the amount of remodeling space. The impact, with respect to fracture risk, of short term gains or losses in weight in adulthood is not well characterized.

Dutch and British studies have reported significant changes in total body BMD in response to relatively rapid weight loss in overweight women (Jensen et al. 1994; Ramsdale and Bassey 1994). Ramsdale and Bassey (1994) reported significant correlations between change in body mass and change in total body and lumbar BMD (r = 0.34 and 0.28 respectively) for 45 premenopausal women on a 6 month modest weight loss program (4800 kjoules/day or 1100 kcal). The magnitude of the response was a 1.4% loss in total body BMD per 10 kg drop in body weight. Compston et al. (1992b) reported a decrease in total body BMD of 1.6% per 10 kg weight loss in only a 10 week period.

In our sample of 62 postmenopausal women, a very strong correlation was noted between 2-year change in body weight and 2-year change in whole body BMC ($r = 0.76$, $p < 0.01$). There was a 176 g or 8% decline in whole body BMC per 10 kg decline in body weight. A moderate correlation was also noted between change in lumbar BMD and change in body weight ($r = 0.37$, $p < 0.05$). There was a 0.029 g/cm^2, or 3% decline in lumbar BMD per 10 kg weight loss. The strength and magnitude of the association between changes in whole body bone mineral and changes in body weight suggest that systematic error may contribute to the relationship.

The influences of soft tissue composition and thickness on the accurate measurement of BMD has been considered by many investigators. Tissue thickness, except perhaps at the extremes of the possible physiological range, appears to have little influence on the measurement of bone mass when tested in *in vitro* systems. Mazess et al. (1989) found no effect of differences in "tissue" thickness on BMD of bone phantoms, scanned in water depths of 10 - 24 cm (Lunar DPX). Addition of lard to the phantom, however, caused a progressive decrease in BMC. Also using the Lunar DPX densitometer, Johnson and Dawson-Hughes (1991) measured aluminum spine phantoms in water, or water and oil (70:30), at thickness ranging from 15.2 to 27.9 cm. BMD of the

aluminum phantom was significantly lower at 15.2 cm thickness than at higher thicknesses, but there was little effect in mid range (18-26 cm). A higher proportion of fat in the mixture caused a reduction in measured BMD at the higher thicknesses. Overall BMD varied up to 2.4% as a result of differences in thickness.

Morita et al. (1993) scanned cylindrical spine phantoms using the DPX-L and QDR-2000 densitometers, in baths of either water or water and oil (0-50% oil). The scan mode was not identified (lumbar or whole body). Results indicated that the influence of body thickness on BMD was less than 2% for thicknesses ranging from 15 cm to 25 cm (direction of change and actual values not reported), although precision decreased with increasing thickness. Variation due to changes in fat content were less than 3% (values not reported). There is insufficient information in the article to determine directional trends or significance of the changes.

Svendsen et al. (1993) demonstrated larger influences on bone mass of combined changes in tissue thickness and relative tissue composition. Using the Lunar DPX, 6 women were scanned with and without 8.8 kg of porcine lard (3.5 cm thick) placed on the ventral side of the body. BMC increased 7%, as did bone area, with no change in measured BMD.

Apart from the effects of tissue thickness and composition, individual differences in the distribution of fat and lean tissue components within the scanning region may also influence the accurate measurement of bone mass. When bone mass is measured by dual X-ray absorptiometry (DXA), the transmission of photons is attenuated by both bone and the soft tissue overlying bone. Calculation of bone mass requires correction for the attenuation attributable to the soft tissue above and below (which cannot be directly measured). If soft tissue adjacent to bone is similar in composition, the attenuation of the X-ray beam in these sites may be used to calculate the correction factor required to

estimate bone mass. This approach is reasonable provided such bone-free soft tissue sites are available for scanning, and are representative of the tissues overlying the bone. This assumption has, however, been challenged by Formica et al. (1995) who used QCT to show that the fat content anterior to the 3rd lumbar vertebrae differed from that on either side of bone. Fat content in the soft tissue region lateral to the vertebrae generally exceeded that anterior to the vertebrae, with greater differences and therefore greater error in the measurement of bone mass occurring in postmenopausal women relative to premenopausal women. The ratio of the fat content overlying bone to the fat content adjacent to bone also differed depending on the total amount of fat present (i.e. the degree of obesity).

Although these differences may contribute to the population variance in bone mass and lessen the accuracy of single assessments of BMD, they may be considered inconsequential for short term prospective studies which intend to follow individual changes in BMD in response to pharmacological interventions or pathological processes. If the ratios of fat above and adjacent to bone change during weight gain or loss, however, the effects on measured BMD may be of clinical significance. If such body mass changes result in changes in relative fat distribution as described above, the result would be an overestimation of bone mass following weight gain (i.e. artifactual increase in BMD), and an underestimation (artifactual decrease in BMD) following weight loss.

Inhomogeneities in fat distribution are most likely due to visceral fat, which is not uniformly distributed throughout the body. Farrell and Webber (1989) examined the relative proportions of fat in regions overlying and adjacent to lumbar vertebrae using QCT, simulating the typical field of view for a dual energy absorptiometry scan. In most individuals, the regions adjacent to the vertebrae contained greater amounts of fat than the regions containing the vertebrae, a median deviation of 4.1%. Of perhaps greater interest

is the fact that the greatest deviations in fat distribution occurred in individuals with more than 30% fat at the vertebral site. Because the effect of this pattern of inhomogeneity is to artifactually increase BMD, it is suggested that those with higher body weight are more likely to have artificially elevated values, and that weight gain may cause an apparent gain in BMD in some individuals.

We determined to investigate the contribution of systematic error to the observed relationships between change in body weight and change in whole body and lumbar bone mass and density. The effect on bone mass and density of changes in the homogeneity of fat distribution in the abdomen was also investigated at the lumbar spine.

E.2 Sample Size Calculations:

Longitudinal data from 2-yr study of postmenopausal women demonstrated the following relationship between whole body BMC and change in body weight (ΔBW) in kg.

$\Delta BMC = 17.65 * \Delta BW - 12.79$ This is a change of approx. 0.8 % per kg.

The relationship between ΔBW and ΔBMC was apparent in all quartiles of weight. Measurement error for whole body BMC was conservatively assumed to be 1.5%. With an error (Type I) of 5% and 80% power to detect a true difference, the sample size was calculated as 12 subjects to detect a change in BMC of 2% using a simulated weight gain of 2.5 kg. The weight simulation was subsequently increased to 5 kg to also allow the data to be used for ongoing investigations of the relationship between ΔBW and ΔBMD. Continued recruitment to a total of 33 subjects, and a reduction in measurement error to 1%, would permit detection of a 0.8% change in whole body BMD per 5 kg change in body weight (based on the observations of Compston et al. 1992b) with 80% power.

For the lumbar spine, sample size calculations were based on the assumption of an anticipated 3% change in lumbar BMD for each 10 kg change in body weight, and that at least 20% of the increase in body fat might be deposited regionally in subcutaneous and visceral deposits in the abdominal region. This assumption was based on the observation from regional body composition of whole body scans that 25 to 55% of total body fat was deposited in the torso. The calculated sample size was 15.

E.3 Method

Volunteers were recruited through word of mouth among university staff, students and hospital staff. Recruitment occurred separately for the study of each anatomical region i.e. the influence of simulated changes in body weight on either whole body or lumbar spine BMC and BMD. The studies were approved by the McMaster University Faculty of Health Sciences and Affiliated Institutions Ethics Committee. At the time of this report, 12 subjects have completed the whole body weight gain simulation study, and 12 have completed the lumbar spine weight gain study.

For the whole body study, 2 whole body scans were performed without repositioning using the QDR 1000W densitometer. For the second scan, 5 kg of porcine lard encased in soft plastic bagging as 30 cm^2 slabs was placed over the torso and proximal portions of the extremities. The increase in thickness attributable to the slabs was no greater than 4 cm in any one area. The lard was distributed such that approximately 0.5 kg was added to each extremity and 3 kg was added to the upper and lower torso. Whole body scans were performed according to the manufacturer's specifications and analysis was done using the compare mode of the analysis program to ensure similar demarcation of body regions.

For the lumbar study, 3 scans were performed without repositioning. For the second scan, a 2 kg 30 cm^2 slab of lard was placed centrally over the abdomen. For the third scan, the slab was moved to the side attempting to avoid overlap of the central abdominal line overlying the lumbar spine. Analyses were done using the compare mode of the densitometer to ensure identically sized regions of interest.

E.4 Statistical Analysis

The influence of simulated weight gain on bone area, BMC and BMD of the whole body was tested using paired *t*-tests. Similar tests were also performed for each body segment to determine if systematic error might be region specific. At the lumbar spine, the influence of symmetrical and asymmetrical weight gain on lumbar BMC and BMD was testing by repeated measures ANOVA.

E.5 Results

Baseline characteristics of the 12 subjects included in the whole body study are presented in table E1. A broad representation of age, bone mass, bone density, and percent body fatness was present in the study group. All subjects were able to cooperate with the protocol and no scans required elimination due to movement artifact.

Table E1. Baseline characteristics of subjects scanned in whole body scan mode with simulated weight gain.

	Mean	SD	Min	Max
Age	41.6	18.5	25	78
Weight (kg)	65.3	9.8	45	90
% Body Fat	21.0	10.7	6	41
Whole Body BMC	2465.6	551.3	1567	3446
Whole Body BMD	1.1091	0.1192	.8770	1.322

Significant increases occurred in bone mass, bone area and bone density when weight gain was simulated by the addition of the fat slabs (table E2). Although bone area increased by a modest 0.8%, the change in BMC was greater (2.6%) and the ratio between bone mass and area was sufficiently altered to cause a significant 1.8% increase in BMD.

Table E2. Results of paired *t*-test of whole body measurements performed with and without 5 kg of lard.

	N	Baseline	With Fat	Difference	Percent Difference	Significance
BoneArea (cm2)	12	2296.0	2313.8	17.719	0.81	**0.0237**
BMC (gm)	12	2600.3	2666.0	65.715	2.59	**0.0001**
BMD (gm/cm^2)	12	1.1230	1.1427	0.0197	1.75	**0.0001**

When analyzed by region, there were regional differences in the magnitude of systematic error in the measurement of BMD (table E3). Significant errors at the head, ribs, lumbar spine, pelvis and legs. Interestingly the direction of the error, in response to weight gain, differed by region. Decreases in BMD were apparent at the lumbar spine and pelvis, whereas increases occurred in the legs, ribs and head, with the largest increase in the head region.

In order to estimate the relative impact of each region on the cumulative error in BMD measurement for the whole body, the size of the error was adjusted for the percentage of total body area contributed by each region. The results are presented in table E4. When adjusted for total bone area, the legs and the head contributed equally to the percent error occurring in whole body BMD due to simulated weight gain.

Table E3. Differences by region in BMD, with and without 5 kg of lard.

MEASUREMENT	N	Mean Difference	Percent Difference	Significance
Head BMD (gm/cm^2)	12	0.0932	4.9	**$p < 0.001$**
Ribs	12	0.00917	1.3	**$p = 0.045$**
Thoracic Spine	12	-0.00325	-0.2	$p = 0.719$
Lumbar Spine	12	-0.0147	-1.6	**$p = 0.031$**
Pelvis	12	-0.0198	-2.0	**$p = 0.017$**
Legs	12	0.0184	1.5	**$p = 0.004$**
Arms	12	0.0035	0.3	$p = 0.712$

Table E4. Influence of site (region) on percent difference in total BMD with and without added fat. Difference weighted by percentage contribution to total area scanned.

	N	Percent Difference in BMD	Percent of Total Bone Area	Weighted Influence on BMD
Total Body BMD	**12**	**1.8**	**100**	-
Head BMD	12	**4.9**	12	**4.1**
Ribs	12	1.3	14	1.2
Thoracic Spine	12	-0.2	7	0.1
Lumbar Spine	12	-1.6	3	-0.3
Pelvis	12	-2.0	9	-1.3
Legs	12	1.5	**39**	**4.1**
Arms	12	0.3	16	0.3

Baseline characteristics for the 12 volunteers for the lumbar spine study are presented in table E5. Because percent body fat was not available from whole body scans for all individuals, relative fatness is presented as body mass index (BMI).

Table E5. Baseline characteristics of the 12 volunteers participating in repeat scanning of the lumbar spine.

	Mean	SD	Min	Max
Age	45	18	25	78
Weight (kg)	60.5	6.9	48	69
BMI	25	9	18	55
Lumbar BMC	59.91	12.55	47.59	88.53
Lumbar BMD	1.0160	0.1720	0.7630	1.3630

Bone area was not significantly affected by the addition of fat in either a symmetrical or asymmetrical distribution (table E6). Symmetrical addition of fat also failed to significantly influence the measurement of BMC or BMD. Asymmetrical addition of fat slabs, however, resulted in a significant increase in BMC without affecting bone area. This in turn resulted in a significant increase in measured BMD.

Table E6. Results of lumbar measurements performed with and without 2 kg of lard.

	N	BMD With Fat	Difference from Baseline	Percent Difference	*p*
Lumbar Area	12	59.07	-0.0825	0.1	0.47
Lumbar BMC	12	60.04	0.1242	0.2	0.67
Lumbar BMD	12	1.0197	0.00375	0.4	0.27
	With Asymmetrical Fat				
Lumbar Area	12	58.80	-0.0164	0.02	0.93
Lumbar BMC	12	61.66	0.6573	1.11	**0.03**
Lumbar BMD	12	1.0507	0.0117	1.11	**0.04**

E6. Discussion

We found that simulated weight gain caused a systematic increase in both BMC and BMD during whole body scanning. Our findings are in partial agreement with those

of Svendsen et al. (1993) who noted an increase in BMC but not BMD during simulated weight gain experiments. The increase in BMC reported by Svendsen (0.8% change in BMC per kg of fat) was larger than that observed in the present simulated weight gain study, but similar what we observed among postmenopausal women during true weight gain and loss (0.84% change in BMC per kg of body weight). Different results during simulation may be attributable to dissimilar distribution of the added fat during scanning, differences in body mass or BMI of the subjects, or to differences in densitometer software.

We noted regional differences in the direction and magnitude of the change in whole body BMD following the addition of fat. To our knowledge, this has not been previously reported. Regional differences may occur because of the difficulty in determining bone mass in body regions lacking non-bony soft tissue reference sites. This may explain the large error occurring in the head region. It may not, however, explain the increment in BMD occurring in the leg regions in response to added fat. It is possible that suitable adjacent soft tissue references are lacking from the ankle and foot regions, and that addition of fat elsewhere in the legs or body affects the soft tissue reference used for these areas. Further software development by densitometer manufacturers may reduce these problems. Hologic Inc. has changed the manner of referencing soft tissue in the head region (Hologic, Inc. 1996). It may be appropriate to repeat these studies using the new software. However, because older model densitometers and software will continue to be in use, clinicians should be aware of the effect of changes in weight on whole body BMC and BMD if changes in weight occur during patient or subject follow-up.

At the lumbar spine, symmetrical placement of added fat failed to significantly influence the measurement of BMD. This is in agreement with a number of previous investigations of the accuracy and precision of DXA (Johnson and Dawson-Hughes 1991;

Mazess et al. 1989; Morita et al. 1993; Svendsen et al. 1993). However, we have also demonstrated that alterations in the distribution of fat during simulated weight gain, significantly influence the measurement of BMD. This supports the work of Farrell and Webber (1989), Formica et al. (1995) and the simulation model recently presented by Bolotin (1998). In this design, greater deposition of fat in regions adjacent to bone during weight gain will cause apparent increases in BMD.

We suggest that the clinically observed association of change in lumbar BMD with change in body weight is at least partially attributable to measurement artifact. In our substantive study, a 5 kg change in body weight was associated with a 1.5% increment in lumbar BMD. Using a simulated weight gain of 2 kg at the abdomen, a 1% increment in BMD was observed. It is difficult to estimate the actual weight gain simulated by the addition of 2 kg of lard at the abdomen. The thickness of this slab was approximately 2 cm, and although substantial, likely represents a biologically plausible increase in abdominal girth.

The modest increase in BMD observed in the simulation study may also be the result of difficulties in determining the proximity with which to place the slabs to the central line of the abdomen. Theoretically, overlap of the lumbar spine could attenuate or reverse the influence of increase in fat proportion in the soft tissue reference area on one side of the spine. An attempt was may to avoid overlap of the spine using surface landmarks, but verification of placement was not possible using the present system. This distribution of fat is, however, arbitrary. It is important, given the present knowledge, for changes in abdominal fat distribution to be substantiated *in vivo* during planned weight loss or intentional weight gain.

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