Effect of Caloric Restriction on Bone Mineral Density and Bone Turnover in Overweight and Obese Individuals with Differing Calcium Intake Levels

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EFFECT OF CALORIC RESTRICTION ON BONE MINERAL DENSITY AND BONE TURNOVER IN OVERWEIGHT AND OBESE INDIVIDUALS WITH DIFFERING CALCIUM INTAKE LEVELS

by

Timothy L. Radak

A Dissertation in Partial Fulfillment of the Requirements for the Degree of Doctor of Public Health in Nutrition

August 2004
Each person whose signature appears below certifies that this dissertation, in his/her opinion, is adequate in scope and quality as a dissertation for the degree Doctor of Public Health.

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ABSTRACT OF THE DISSERTATION

EFFECT OF CALORIC RESTRICTION ON BONE MINERAL DENSITY AND BONE TURNOVER IN OVERWEIGHT AND OBESE INDIVIDUALS WITH DIFFERING CALCIUM INTAKE LEVELS

by

Timothy L. Radak

Doctor of Public Health Nutrition
Loma Linda University, Loma Linda, California, 2004
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The purpose of this multi-center controlled study was to determine the effects of supplemental calcium, dairy products, and dietary calcium on bone metabolism during energy restriction. A total of 107 overweight or mildly obese males and premenopausal females between ages 18-35 years were recruited from the faculty, staff and student populations of each of the four participating institutions (University of Tennessee, Purdue University, Ohio State University and the University of California-Davis). Subjects were randomized to the following outpatient dietary regimens for 12 weeks: Low Calcium (LC) a control diet providing a 500 kcal/day deficit, 0-1 servings of dairy products/day,
≤ 500 mg calcium per day, and a daily placebo supplement; **High Calcium (HC)** a calcium-supplemented diet identical to the control diet, with the placebo replaced by 900 mg calcium; or **High Dairy (HD)** a high dairy diet (placebo supplemented) with a 500 kcal/day deficit and containing ≥ 3 daily servings dairy products (milk, cheese and/or yogurt), for a total calcium intake of 1400 mg/day. Analysis of results revealed that all groups had significant weight loss (p<0.001). After adjusting for weight loss, LC had a significant decrease (1.201±.08 to 1.193±.07) in total body bone mineral density (BMD) (p<0.05), with a marginally significant increase (1.210±.12 to 1.227±.12) in lumbar BMD (p=0.076) for HC, and a significant increase (1.096±.12 to 1.100±.12) in femur BMD for HD (p<0.05). Lumbar bone mineral content (BMC) for HC had a significant increase (62.60±10 to 63.74±11) (p<0.05) while total body and femur BMC remained unchanged for all groups. A significant difference between groups (p<0.01) was seen for bone alkaline phosphatase (BAP). Post-hoc analysis indicated a greater decline in BAP for HC (28.43±13.5 to 24.18±12.3), compared to LC (p<0.01) or HD (p<0.03); suggesting a suppression of bone turnover. No change in bone resorption (n-teleopeptide) occurred in any group. Our results suggest that a 12-wk, 500 kcal energy restriction diet with either HC or HD suppresses bone turnover which over time may result in preservation of bone mineral density. The current calcium intake in the general population already falls short of recommendations. Implications for public health professionals for the recommending of adequate intake levels of calcium during weight loss are discussed.
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CHAPTER 1
INTRODUCTION

A. Statement of the Problem

Obesity in recent years has reached epidemic proportions in the US with two out of every three adults classified as obese or overweight (Manson & Bassuk, 2003). Caloric restriction is commonly attempted for weight loss (Serdula et al., 1999). While obese men and women are thought to be at decreased risk for osteoporosis because of influences on bone such as increased body weight, a reduction of body weight has been correlated with a reduction in bone mass (Baran, 1994; Van Loan et al., 1998; Salamone et al., 1999; Ramsdale & Bassey, 1994; Ryan, Nicklas, & Dennis, 1998; Pritchard, Nowson, & Wark, 1996; Compston et al., 1992; Skov et al., 2002; Hyldstrup et al., 1993; Fogelholm et al., 2001). For example, total body Bone Mineral Density (BMD) was assessed after a 10 week caloric restriction intervention and decreased from 1.205 ± 0.056 to 1.17± 0.058 (p< 0.005) (Compston et al., 1992). Another six month caloric restriction intervention showed a 0.7% loss in total body BMD, and 0.5% for lumbar spine (Ramsdale & Bassey, 1994). This reduction could be problematic for obese individuals undergoing weight loss and may act as a catalyst for osteoporotic disease. It has not been fully elucidated whether this reduction is due to the weight loss, other physiologic and behavioral changes, measurement protocol or error, or from inadequate calcium intake.

Most studies have positively correlated calcium intake with bone mass and preservation of bone. During caloric restriction, many calcium rich food sources such as...
dairy are perceived as high fat, and therefore minimized or avoided. As current calcium intake in the general population already falls short of recommendations, there is a need to investigate reasons for bone loss during caloric restriction and to determine adequate amount of calcium needed.

**B. Purpose of the Study**

The purpose of the study was to investigate how weight loss from voluntary caloric restriction affects bone metabolism. The study is unique in that two groups will have sufficient calcium during caloric restriction while the control group will have limited calcium intake. The last group is representative of a typical dieting pattern where calcium food sources are typically minimized in use. The results of this study will potentially help to provide public health recommendations for calcium intake during caloric restriction and provide explanation for the effect of caloric restriction on bone metabolism. A secondary purpose was also to provide insight for determining if dairy sources of calcium provide additional benefit over supplemental calcium.

This study is important in that it addresses risks for two major chronic diseases, namely obesity and osteoporosis. It is well known in the public health profession that obesity carries significant co-morbidities. A significant proportion of the population undergoes caloric restriction between the ages of 18 to 35, a period of time when the laying down of bone for the remainder of the life cycle occurs. Determining the effects on bone metabolism during this time may provide clues on optimizing calcium intake during weight loss that will be beneficial in later years when bone preservation is of utmost importance to minimize osteoporotic related fractures.
C. Research Questions

Question #1: Does the level and type of calcium intake during a weight reduction intervention affect bone mineral density and content as determined by duel-energy x-ray absorptiometry?

Question #2: Does the level and type of calcium intake during a weight reduction intervention affect bone metabolism as determined by serum bone markers?

Question #3: Does the level and type of calcium intake during a weight reduction intervention affect bone metabolism as determined by urinary excretion of calcium and other bone metabolism markers?
CHAPTER 2
REVIEW OF THE LITERATURE

A. Obesity and Osteoporosis in the United States

Obesity, a largely preventable disease, has reached epidemic proportions in recent years and results in numerous costs, both in health and financially. Approximately two out of every three adults in the US are classified as obese or overweight (Manson & Bassuk, 2003). National Health and Nutrition Examination Survey (NHANES) data show an increase in the age-adjusted prevalence of obesity from 22.9% in years 1988-94 to 30.5% for the years 1999-2000 (Flegal et al., 2002). The prevalence of overweight individuals among the same time periods also increased from 55.9% to 64.5%. Other research indicates a steady increase in obesity or overweight across all states (Mokdad, 1999). Increases in mortality attributable to obesity are approximately 280,000 per year (Allison et al., 1999) and include numerous co-morbidities such as cardiovascular disease, type 2 diabetes, osteoarthritis, hypertension, certain cancers, gall bladder disease, and others (Must et al., 1999; Manson et al., 1995; Bray, 1987).

Obesity ranks as the second leading cause of preventable death in the U.S. (National Institutes of Health\textsuperscript{1}, 1998; WHO, 2002), and is rapidly replacing undernutrition as the most common form of malnutrition in the world (Kushner, 2002). Years of life lost from obesity have been calculated to be as great as 13 and 8 years for Caucasian men and women respectively (Fontaine et al., 2003). The enormous health costs related to obesity have been estimated to be approximately $99.2 billion dollars for the year 1995 (Wolf & Colditz, 1998) though that figure has been revised downward to
account for the increased mortality among the obese (Allison, Zannolli, & Narayan, 1999). The care and prevention of obesity is becoming a primary focus of adult health care (Layman, 2002). However, psychological or social stigma, more than for health status seems to drive the public’s efforts to reduce weight.

Osteoporotic related diseases also carry significant personal and societal health costs. Osteoporosis is related to low bone mass and results in an increased susceptibility to various fractures. It has been estimated that over a woman’s lifetime about half of her trabecular and one third of her cortical bone will be lost (Nguyen et al, 1998). Direct medical costs related to osteoporotic fractures accounted for approximately $13.8 billion for the year 1995 (Ray, 1997). Indirect costs and intangible costs like quality of life are surmountable as well. Occurrence of hip fracture in the US is only second to Europe (Lau, 2001). Relative to fracture, roughly 40% of women and 13% of men at age 50 years will present a clinically diagnosable fracture over their lifetime (Melton LJ, 1995). Mortality statistics estimate that for hip fracture alone, estimated survival is reduced from between 12 –20% (Sexson, 1987). Prevalence of the disease is expected to increase over the next number of decades (Wehren, 2003) and is likely to become the most common disorder in the aging population (Raisz, 2000).

Among the approaches to reduce excess weight, typically some form of caloric restriction is employed and a significant proportion of the public that are obese or overweight claim to have attempted weight loss strategies (Serdula, 1999). Weight control or management is recommended to address the health risks associated with obesity (WHO, 2000; National Institutes of Health\textsuperscript{2}, 1998; Fujioka, 2002). The positive effects on chronic disease from the resultant reduction in body weight have been well
documented both in health and in health costs (Gorsky, 1996; National Institutes of Health\textsuperscript{1}, 1998).

**B. Obesity and Bone Health**

Relative to bone metabolism or osteoporosis, the negative effects of obesity lend itself to controversy, as a positive relationship exists between body weight, as well as body mass index (BMI), and bone mass or bone mineral density (BMD). This relationship has been shown for both total and regional sites i.e. spine and femur in pre, peri, and postmenopausal women (Rico et al., 2002; Lindsay et al., 1992; Pocock et al., 1989; Cifuentes et al., 2003; Albala et al., 1996; Edelstein, 1993; Szejnfeld, 1993; Chao et al., 2000). Body weight is a chief determinant for bone mass in women (Rico et al., 2002) and low body weight is an independent predictor of low bone mass later in life (Hawker et al., 2002). Percentage of body fat in premenopausal women is also significantly related with total body bone mineral content (BMC) (Lindsay et al., 1992). In older women, percentage fat correlate well with BMD (Ensrud et al., 1997). Possible explanations for the protective effect of obesity besides mechanical load may be from the additional stimulation of estrogen in relation to additional adipose tissue via androstenedione, and a reduction in sex hormone binding globulin (Albala, 1996; Rose, 2002; Kirschner, 1991; Cleland, 1985; Holbrook, 1993).

As obesity tends to increase bone mineralization, it would decrease the risk for osteoporotic diseases. It is certain that low bone mass increases risk for fractures. However, when looking at actual outcomes related to bone mass, a decrease in hip fractures as a result of greater body weight has not been supported in all studies (Ensrud et al., 1997; Farmer et al., 1989; Cummings et al., 1995, Cumming et al., 1994). It is clear
that osteoporotic fracture risk is higher in lower weight women than in heavier women (Cifuente et al., 2003; Albala, 1996). But differences in fracture rate have not been found in all studies between overweight and average weight women (Ensrud et al., 1997).

C. Effect of Caloric Restriction on Bone Mass

Bone mineral density is an established factor in relation to future osteoporotic disease and low BMD and low peak bone mass are one of the strongest risk factors for hip fractures (Jordan 2002; Heaney et al., 2000; Hawker et al., 2002). Bone mass decreases in women after peak attainment in the thirties (Baran, 1994; Sowers, 2003; Krall, 1999). Accordingly, preservation of bone mass is of interest through menopause. One six year prospective study determined that bone loss at the femoral neck begins as early as the mid twenties (Bainbridge, 2002). Approximately 99% of peak total body BMD occurs in women between ages 19.6 and 24.6 years and 99% of total body BMC was attained between ages 22.5 and 29.9 years (Teegarden et al., 1995).

Observational studies have indicated an increased chance for hip fracture for men and women who experience weight loss during early or middle adulthood and later in life (Cummings et al., 1995, Cumming et al., 1994; Langlois, 2001; Langlois, 1996; Mussolino, 1998; Meyer, 1998; Ensrud et al., 1997). Numerous studies indicate that with weight loss a concomitant loss of bone mass occurs, either for the total body or regional sites (Anderson, 1997; Jensen, 1994; Compston, 1992; Ramsdale, 1994; Jensen, 2001; Ricci, 1998; Skov, 2002; Ricci, 2001; Hyldstrup, 1993). Weight loss regimens of these studies ranged from 10 to 24 weeks in duration and one lasting 12 months (Pritchard, 1996). One study assessed total body BMC six months post completion and found that subjects who lost additional weight also lost additional bone mineral content
while subjects who regained weight, also regained bone mineral content (Jensen, 1994). Compston et al. (1992) also found total body BMC approached initial levels when reassessed ten months after a weight loss intervention with subsequent weight gain. Avenell et al. (1994) did not find this when subjects regained weight in their study.

It is not entirely clear exactly what is responsible for the loss in bone mass during weight loss. Relative to exercise or adiposity, Jensen et al. (1994) as well as Salamone et al. (1999) believe this is due in part to the decrease in weight applied to bone which influences bone remodeling. In some studies, the effect of weight loss is greater on weight bearing rather than non weight bearing bones (Rico et al., 2002). Anderson et al. (1997) tested this hypothesis to see if exercise could preserve the loss by adding a resistance training component to one of the diet groups but did not find a significant bone sparing effect compared to the diet only group. Svendsen et al. (1993) found that decreases in lumbar spine BMD actually were greater in the diet and exercise group than in the diet only group. Another study however, did find benefit to preserving regional BMD (Ryan et al., 1998).

Relative to hormonal influences, McLean et al. (2001) looked at cognitive change among dieter’s and implicate the production of cortisol as a contributing factor to bone loss during weight loss. Another factor possibly could be a decrease in adipose cells resulting in a reduction in estrogen or estrone levels as confirmed by Ricci et al. (2001). Parathyroid hormone levels tend to be altered during weight loss and the slight increase may also be a contributor to bone loss (Roberts et al., 2001; Ricci, 2001).

Relative to calcium intake, reduced absolute calcium intake has been correlated with loss of bone during weight loss (Ramsdale, 1994). However, Salamone et al. (1999)
found that BMD loss at the hip was significantly greater in a weight loss with activity treatment versus the control group who were weight stable and had significantly less calcium intake.

There have also been methodological issues raised when the analytical method used for determining bone loss is dual-energy x-ray absorptiometry (DXA) (Nguyen et al., 1997; Van Loan et al., 1998; Tothill, 1999; Van Loan et al., 1995). The increased thickness of soft tissue in obese subjects may interfere with bone edge detection and affect accuracy of measurements (Compston, 1992; Nguyen, 1998). Measurements are also expressed in areal density as g/cm² and do not factor in the dimension of depth (Laskey & Prentice, 1999; Prentice, Parsons, & Cole, 1994).

Relative to adiposity, obese subjects tend to have increased bone size resulting in a possible overestimation of BMD. Another potential consideration may be that women with greater bone mass also lose bone mass at a faster rate than women of lower bone mass (Davis, 1992). Bone mass is higher in obese women than normal weight women and the bone loss seen from weight loss may only serve to bring those women back to within the “normal” range (Anderson et al., 1997). Leptin levels have been shown to decrease during weight loss and may play a partial role in negatively influencing the rate of bone turnover (Wadden, 1998). It has also been suggested that leptin regulates bone formation independent of its influence on body weight (Macdonald et al., 2004).

Lastly, there are many who undergo caloric restriction repeatedly, and the effect of weight cycling has also been investigated to determine any additional impact on loss of bone mass but without conclusive determination (Fogelholm et al., 1997; Gallagher, 2002; Bacon et al., 2004).
D. Calcium and Bone Mass

As one of the major minerals in bone making up the skeleton, calcium has been well researched in relation to bone growth, preservation, and health. The adult human body contains roughly 1000 to 1500 g of calcium, making it the most abundant mineral of which 99% is contained in bone (Ilich, 2000). The 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) estimated mean intake of calcium in women aged 18-50 to be approximately 640 mg/d (Institute of Medicine, 1997), and 770 mg/d for women aged 20-59 according to a more recent NHANES 1999-2000 survey (Wright et al., 2003). Both averages fall significantly short of current recommendations. A NIH Consensus Conference some 10 years ago highlighted that calcium is one of two nutrient deficiencies in the United States that warrant a national effort to increase average intake levels (National Institute of Health Consensus Conference, 1994).

Low calcium intake can limit bone formation in early life and cause bone loss in maturity (Heaney, 1999). Two epidemiological studies confirm this finding along with increasing risk for hip fractures in women, but this has not been consistently shown for men (Matkovic, 1979; Hu et al., 1993; Owusu, 1997). Supplemental calcium has been shown to reduce the risk of hip fractures (Bendich, 2001). While variations in calcium intake during youth have been estimated to affect peak bone mass by only 5 to 10%, the influence of hip fracture risk later in life may account for 25 to 50% of risk (Heaney et al., 2000). While other vitamins and minerals have been looked at, calcium is clearly the most well researched (Reid, 2001).
A meta analysis of eight calcium intake studies indicate that increased bone mass or accretion occur among children and adolescents from both supplement and dietary sources (Wosje & Specker, 2000). However, a 15 year prospective study of adolescent and young adults found weight bearing activity and not calcium intake to be a predictor of BMD (Welten et al., 1994). In an editorial, Heaney refutes this anomalous finding by suggesting that calcium is a threshold nutrient and implies that the study’s European subjects had higher levels of calcium than their US counterparts (Heaney, 1995). This would be significant because if baseline levels of calcium are already at the threshold level, additional calcium would not be expected to improve bone health (Ilich, 2000). An earlier prospective study here in the US found calcium intake to have an insignificant influence on BMD or BMC after two years of evaluation (Mazess, 1991).

A meta analysis of 33 cross-sectional, longitudinal, and intervention studies found a small but significant positive correlation between calcium intake from either supplement or diet, and either BMD or BMC in groups aged 18 to 50 years (Welten et al., 1995). Mean calcium intakes were between 436 to 1437 mg/d. Another meta analysis looking at early postmenopausal women also found a positive correlation between calcium intake and bone mass in the 49 studies investigated (Cumming, 1990). More recent studies have (Reid et al., 1995) and have not confirmed this finding (Ravn et al., 2000; Hosking et al., 1998). A recent meta analysis of 13 Randomized Controlled Trials (RCT) in postmenopausal women suggest that while there is a positive effect seen by calcium during the first year, the effects became similar to that seen in control groups by the second year and that short term interventions might overestimate the benefit of calcium on bone (Mackerras, 1997). Heaney (2000) performed the largest meta analysis
to date using calcium supplements or diet (dairy products), investigating 139 studies. The positive relation of calcium on bone was reaffirmed in all but two RCT and 21 observational studies. Around the same time, another meta analysis investigated 46 studies using only dairy products and found mixed results concluding that there is inadequate evidence to support a recommendation for daily intake of dairy foods for bone health. (Weinsier & Krumdiek, 2000). This prompted an editorial by Heaney and one in return discussing categorical decisions in Weinsier’s meta analysis i.e. classifying an observational study in a strength category that included RCT’s (Weaver & Heaney, 2001; Weinsier & Krumdiek, 2001). In relation to calcium supplements or dairy, and impact on bone, two of the six dairy studies in Heaney’s analysis showed supplement use fared significantly better in comparison to dairy though was not noted as such. One in a clinical trial (Storm et al., 1998), and one in a retrospective study which did not find a significant relation to dairy and bone mass but did for supplement use (Ulrich et al., 1996), of which was also noted in another review (Gueguen & Pointillart, 2000). Lastly, one more recent study not included in the above analysis’s showed a protective role on bone for dietary calcium by lowering the rate of bone loss in premenopausal women aged 25 to 30 years as measured by BMC (Uusi-Rasi et al., 2002).

Overall, it appears that calcium intake has a statistically significant influence on bone accretion and bone preservation in the vast majority of studies. And while research suggests that other nutrients are also important, like vitamin D intake, it is calcium that has the greatest bone preserving and building effect (Heaney, 2000). As median calcium intakes among females fall below recommended levels after childhood even when
supplemental calcium intakes are included, the issue of calcium intake, regardless of source, is of paramount importance (Looker, 2003).

**E. Role of Calcium on Bone Mass During Energy Restriction**

As loss of bone mass occurs during energy restriction, and calcium intake has been demonstrated to increase or preserve bone mass, it has been recommended that calcium supplementation be given during weight loss to reduce the risk of bone loss (Westerterp, 2002). Studies have been conducted to determine the effect of calcium intake on bone mass during energy restriction and will be discussed.

Supplementation in obese postmenopausal women during caloric restriction randomized to 1 g of calcium or placebo for six months showed decreased bone turnover and greater preservation of BMD in the supplemented group as compared to the control group (Ricci et al., 1998). Another caloric restriction study randomized pre and postmenopausal obese women to 1 g of calcium or nothing (no placebo) for three months, and found significant differences in whole body and spine BMC, with the treatment group losing less BMC (Jensen, 2001). A follow up three months post completion showed no change in weight in the calcium supplemented group while the unsupplemented group gained weight. However, the unsupplemented group continued to lose more BMC, while the supplemented group had no change in BMC suggesting continued bone preservation in calcium supplement users.

Another caloric restriction study randomized obese premenopausal women to 1 g of calcium or placebo along with a control group which did not undergo caloric restriction for 6 months (Shapses et al., 2001). While BMD of spine tended to increase in the supplemented group as compared to the two other groups which lost a non significant
amount, total body or lumbar spine BMD or BMC did not significantly change between the supplement and placebo group nor control group. Markers of bone turnover also were not significantly different between groups. These findings are in contrast to the above two studies suggesting that calcium supplementation did not improve bone status during caloric restriction. It also suggests that low calcium intakes (mg/d) in the weight loss with placebo group ($u = 459 \pm 145$) or the weight maintenance control group ($u = 795 \pm 244$) did not lose significantly more bone than in the supplementation group and generally indicate that bone mass was not significantly affected by moderate weight loss. This prompted an editorial by Barker and Blumsohn who, in reanalyzing the data, suggest that the overall change in lumbar spine BMD was not significantly different from either of the other two groups (Barker & Blumsohn, 2002). And that in post hoc testing, only a difference was seen between the calcium and control groups suggesting that there is no support for determining that the calcium group tended to increase lumbar BMD. They also suggest however, that when extrapolating the change seen in lumbar BMD in the placebo group to a year, the 95% confidence interval would increase to a 3.5% loss in bone and would represent a significant loss for premenopausal women who undergo caloric restriction, supporting aforementioned findings.

Lastly, a recent 12 week study looking at bone turnover during caloric restriction showed that low intakes of calcium ~500 mg/d, resulted in significant increases in bone turnover and bone formation, as compared to the other diet group which had ~1400 mg/d (Bowen, Noakes & Clifton, 2004). This study however, did not show any significant changes in total body BMD in either group. These results are somewhat expected as changes in short term studies tend to be seen in bone markers more than in bone mass,
particularly when bone remodeling is believed to act in cycles, known as the “bone remodeling transient” (Heaney, 2001).

**F. Markers of Bone Metabolism During Weight Loss**

Consideration should be given if making determinations solely from bone markers as most bone marker enzymes also assess other tissues in addition to bone (Seibel, 2002). Also, a recent study giving a bone marker sample to numerous labs showed that interlaboratory results can have significant coefficients of variation due to the various assay methods available (Kleerekoper, 2001). A summary of above mentioned studies using bone markers shows that caloric restriction in obese pre and postmenopausal women resulted in an increase in bone formation and resorption (Sartorio et al., 1990; Ricci et al., 2001; Bowen, Noakes & Clifton, 2004) or no change (Svendsen et al., 1993). The addition of calcium, either from supplement or dairy, either suppressed markers of bone resorption or formation (Ricci et al., 1998; Bowen, Noakes & Clifton, 2004), or showed little effect overall (Jensen et al., 2001; Shapses et al., 2001). It is suggested that when interpreting effects of weight loss or calcium on bone metabolism, that DXA results, and mineral excretion be combined along with serum or urinary bone markers to assess changes for more representative determinations.

**G. Calcium Intake as an Independent Regulator of Weight and Body Composition**

There is now heightened interest from epidemiological as well as animal and human experimental data to suggest that calcium may play a role in weight regulation, which could add to the above reasons to ensure adequate calcium intakes during a weight loss regimen. Epidemiologic studies have indicated a strong inverse correlation between adiposity and calcium intake (Parikh & Yanovski, 2003). Zemel, who first proposed the
idea of calcium and effect on body weight looked at NHANES III data. The Relative Risk for being in the highest quartile of adiposity was highest among those with lowest calcium intake, a result which was controlled for activity and energy intake (Zemel et al., 2000). Davies et al. (2000) explored the relationship further by retrospectively analyzing five clinical trials of pre, peri, and postmenopausal women. A significant negative correlation between calcium intake and body weight was observed. For each age group the odds ratio for women being overweight was 2.25 for young women in the lower half of calcium intakes within their respective groups. Heaney et al. (2002) extended the analysis by adding an additional RCT and using multiple regression to predict BMI on the basis of calcium and other selected macronutrients. The regression coefficient for calcium intake was significant at \(-0.003\) (\(p<0.001\)) which was determined to equate to average BMI being 0.3 kg/m\(^2\) lower for each intake of 100 mg of calcium.

Zemel first became aware of the relationship from reanalyzing a previous study determining the effect of calcium intake on hypertension in obese African-Americans (Zemel, 2002). He found that increases in dietary calcium from roughly 400 to 1,000 mg/d over a year resulted in a 4.9 kg reduction in body fat. Two additional servings of yogurt were used compared to a control group. Research has been conducted to determine the mechanism responsible for how dietary calcium exerts its ‘anti-obesity’ effect. Animal studies first attempted to ferret out explanations by studying the agouti obesity gene found in human adipocytes. The agouti protein stimulates the influx of calcium into adipocytes and stimulates fatty acid synthase (FAS). FAS is involved in lipogenesis and inhibits basal and agonist-stimulated lipolysis in human and murine
adipocytes via a Ca\(^{2+}\) dependent mechanism (Zemel et al., 2000). Exogenous high calcium intake suppresses 1,25-(OH\(_2\))D and decreases calcium influx to the adipocyte.

**Figure 1:** [Ca\(^{2+}\)] mediated mechanisms and regulation of adiposity. (Modified and adapted from Zemal;2003)

Increasing adipocyte intracellular calcium promotes triglyceride storage and exerts control over stimulating lipogenesis and inhibits lypolysis. The inhibitory effect of intracellular calcium is also believed to be partially from its ability to inhibit phosphodiesterase (PDE) (Xue et al., 2001). Trangenic mice expressing agouti in adipose tissue were placed on low and high calcium diets with the former exhibiting increases in lipogenesis, inhibition of lypoysis and accelerated increases in body weight and fat mass (Zemel, 2003). In the same mouse model another similar experiment added a caloric restriction component in addition to calcium to see whether additional fat loss could be
created secondary to caloric restriction. As hypothesized, the low calcium treatment caused a two fold increase in adipocyte intracellular calcium, a weight gain of 29% and increase in pad fat mass while the high calcium treatments showed a 50% decrease in intracellular calcium and greater decreases in weight loss and fat pad mass. FAS was reduced significantly by high calcium and almost twice as much when the calcium was derived from dairy.

Human clinical trials were next conducted to assess the effect of calcium on weight loss and body composition as direct endpoints during caloric restriction. Subjects were randomized to (0-1 servings of dairy/d and 400-500 mg calcium with placebo, high calcium using control diet with 800 mg calcium supplement, or high dairy: 3-4 low fat dairy servings/d for total calcium of 1200-1300/d. All groups had a balanced deficit diet (-500kcals). All groups lost weight with the control losing 6.4±2.5% of body weight, which was increased by 26% in the high calcium group, and 70% in the high dairy group. (Zemel, 2003). Fat loss as assessed by DXA followed a similar trend.

The above animal and human studies support a beneficial role for calcium on weight loss and body composition with the benefit being greatest in dairy products than from supplemental calcium. Zemel suggests one of the additional components responsible for dairy’s increased effect is found in the whey fraction of milk (Zemel, 2003). To test the suggested results on calcium and dairy on body composition and weight loss, Barr (2003) performed a Medline search locating all randomized studies using calcium or dairy which had data on change in body composition or weight. Nine studies were found using dairy of which seven showed no significant differences in change in body weight or composition between control and treatment groups. The two
others showed a significantly greater increase in weight for the dairy supplemented group versus controls.

The author notes that whether or not these results are in conflict with calcium eliciting increased energy utilization depends on whether compensation occurred by the subjects for the additional calories brought about from the dairy supplements.

Seventeen other studies using calcium supplements showed again, no significant differences between calcium supplemented and control groups except for one study which actually showed greater weight loss in the calcium supplemented group. It should be noted that the studies were not caloric restricted and did not have body weight or composition as primary endpoints. Teegarden et al. (2003) and Zemel note that in order for an effect of calcium to be seen, caloric intake must be factored in. Additionally, most of the trials assessed were on normal weight populations. Clearly, more studies are needed to assess calcium’s impact on body composition and weight during caloric restriction and to determine if dairy calcium exerts an additional positive effect.

H. Considerations Among Choices of Calcium

Those who try to reduce weight via caloric restriction commonly strive to consume less fat (Serdula et al., 1999; Bennett et al., 1991; Levy & Heaton, 1993). Dairy products, a major source of dietary calcium for the public, are sometimes perceived as fat containing foods and typically not emphasized during periods of caloric restriction (Novotny et al., 2003; Miller et al., 1995; Teegarden, 2003; Gorbach et al., 1990). For a public already striving to meet calcium intake recommendations, this may have a deleterious effect on bone health and metabolism as studies involving calcium intake and bone mass have suggested. This can be added to the risks already suggested from weight
loss relative to a decrease in bone mass. Calcium has been identified as one of the nutrients at risk in diets used for weight control (Kant, 2003; Ritt, Jordan, & Levitz, 1979; Gentile et al., 2001). There is also concern for non-overweight individuals as they too engage in caloric restriction (Neumark-Sztainer et al., 2000).

Various issues have been raised surrounding the sources of calcium available. Supplements can be easier to take than dietary sources, less expensive, better absorbed, and can meet recommendations with one pill, as well as being more accepted by a sizable amount of the population whom are lactose intolerant (Savaiano, 2003; Keller et al., 2002; Werner et al., 1999). Those who follow a strict plant based diet might benefit from supplements especially during weight loss. Those who refrain from any dietary source of calcium derived animal products also share some decreases in chronic diseases than their omnivorous counterparts (Jenkins et al. 2003; ADA, 2003) and calcium intake or balance may not be inadequate as originally perceived (Davey, 2003, Kohlenberg-Mueller & Raschka, 2003). While many plant based sources of calcium generally have good fractional absorption (Weaver & Plawecki, 1994; Heaney & Weaver, 1990; Weaver et al., 1991), with some better than dairy sources (Recker et al., 1988; Weaver, Proulx, & Heaney, 1999), their intake alone falls short of meeting calcium requirements for the majority of the population. The challenge with plant sources being sufficient quantities of intake, and dairy products remain the most significant calcium source for the public (Miller, Jarvis, & McBean, 2001).

Pill supplementation comes without the addition of cholesterol and saturated fats, known risk factors for CVD, and has been shown to have favorable effects on blood lipids though in general has no significant additional effect (Jacqmain et al., 2003).
Though both are found in dairy products, studies using dairy products did not find significant increases but rather decreases in plasma lipid and lipoproteins related to cardiovascular risk (Jacqmain et al., 2003). The CARDIA study indicated a reduction in cardiovascular and type 2 diabetes risk factors with increased dairy intake (Pereira et al., 2002). Some forms of cancer have been associated with dairy consumption (Willett, 2003; Jarvinen et al., 2001) while many have not or have been found protective (Knekt et al., 1996; Shin et al., 2002; Hirose et al., 2003; Radosavljevic et al., 2003; Miller, Jarvis, & McBean, 2001). Other studies and a review has suggested dairy consumption can be related to an elevated insulinaemic index (Bjorck et al., 2000; Liljeberg & Bjorck, 2001; Solomons, 2002). Others note reductions in hypertension and homocysteine levels in diets containing low fat dairy such as the DASH diet regimen (Craddick et al., 2003). Other components of dairy such as conjugated linoleic acid have been suggested to be potentially protective of certain diseases and have been shown to exhibit antitumor properties (D'Orazio et al., 2003; Eynard & Lopez, 2003) though one epidemiological study found conjugated linoleic acid intake to have a weak but positive relation with breast cancer incidence (Voorrips et al., 2002). Dietary sources of calcium have been shown to decrease risk of renal stones while supplemental calcium has been shown to increase risk and could possibly interfere with other minerals (Curhan et al., 1997; Looker, 2003). Lastly, some follow up studies showed that the positive effect of calcium didn’t persist years later for pill supplements (Looker, 2003) and dairy calcium (Merrilees, 2000), though one study did suggest the effects persisted with dairy (Barker, Lambert et al., 1998). Regardless of the decision to choose between calcium sources, the
most important factor is having chosen one as a source or addition to one’s total calcium intake, particularly during caloric restriction.

I. Conclusions and Significance

It is prudent to suggest that despite obesity being identified as a protective factor for osteoporotic disease, because of obesity-related co-morbidities, weight loss is desirable and outweighs the potential risks on bone health which are now being investigated. As well, the influence of calcium intake for additional weight loss effects as well as for preservation of bone mass warrants additional investigation. As calcium intake and weight loss appear to effect bone mass during the time up to peak accretion, and that the window shuts from peak bone mass onward, it is of importance for additional studies to determine risks and benefits of both concurrently, in hopes of preserving bone mass, reducing osteoporotic risk, and addressing the obesity epidemic. Very few studies have been conducted specifically to examine the effect of calcium on body composition, particularly for the overweight of obese populations. There is a need for more studies to evaluate the combined effect of weight loss and varying levels of calcium on bone metabolism. A study proposed such as ours where all subjects undergo caloric reduction with two treatment groups having sufficient calcium while another with restricted calcium intake will help provide insight as to the effect of weight loss with varying levels of calcium on bone metabolism. It will allow for examination of the ‘anti-obesity’ effect of calcium as proposed by Zemel.
In addition, it will also help in determining the cause of a decrease in bone mass, if any, by assessing DXA measurements along with urinary mineral excretion and markers of serum bone turnover and formation. Lastly, it will help to determine any difference between dairy and pill supplement forms of calcium on the above measures of interest.
CHAPTER 3
METHODS

A. Design

Analytic, double blinded (except for dairy group), randomized, controlled, parallel experimental design. Free-living setting with weekly visits to the USDA Western Human Nutrition Research Center (WHNRC).

B. Subject Characteristics

Overweight or mildly obese otherwise healthy young females and males (n=27) aged 18-35 were recruited from the faculty, staff and student populations in the Davis/Sacramento area via news stories and advertisements in the local press and approved flyers distributed to medical complexes, churches, and on campus. A drop-out rate of 25% was factored due to attrition. Recruitment and selection of candidates was expected to take about two months but was completed in 4 weeks. Subjects were informed of the study requirements and written informed consent was obtained in accordance with the Committee for Protection of Human Subjects. A sample flyer can be seen in Appendix A. A flowchart for screening can be seen in Appendix B.

Inclusion criteria were as follows: Body Mass Index (BMI) 25-34.9 kg/m², age 18-35 years, low calcium diet (determined by food frequency and diet history): less than 600 mg calcium per day from non-calcium-fortified foods and less than 800 mg calcium per day total, no more than 3 kg weight loss during past three months, negative pregnancy test at entry; women of childbearing potential may be enrolled if they have had a tubal ligation or use one of the following means of contraception: condom, diaphragm,
oral or implanted contraceptives, or intrauterine device. Women in exclusive relationships with male partners who have had a successful vasectomy were not be required to use any additional means of birth control.

Exclusion Criteria was as follows: BMI $\leq 25$ or $\geq 35$, type II diabetes requiring the use of any oral antidiabetic agent and/or insulin (because of confounding effects on body weight regulation), adverse response to study foods (lactose intolerance, dairy intolerance, dairy allergy or aversion to the placebo product) determined by self-report, high calcium diet (determined by food frequency and diet history): $\geq 600$ mg calcium per day from non-calcium fortified foods and $\geq 800$ mg total calcium per day, history or presence of significant metabolic disease which could impact on the results of the study (i.e. endocrine, hepatic, renal disease), history of eating disorder, presence of active gastrointestinal disorders such as malabsorption syndromes, pregnancy or lactation, use of obesity pharmacotherapeutic agents within the last 12 weeks, use of over-the-counter anti-obesity agents (e.g. those containing phenylpropanalamine, ephedrine and/or caffeine) within the last 12 weeks, use of calcium supplements in the past 12 weeks, recent (past four weeks) initiation of an exercise program, recent (past twelve weeks) initiation of hormone replacement therapy or change in HRT regimen, and recent (past twelve weeks) initiation of hormonal birth control or change in hormonal birth control regimen.

C. Methods

Subjects meeting the inclusion and exclusion criteria, and who had successfully passed the screening and medical questionnaires, were studied for a two-week lead-in period with an initial calcium intake of approximately 500mg/day and a dairy intake of
<1 serving/day to establish their current caloric requirements and provide an opportunity for baseline dietary and physiological assessment, and then randomized to the following outpatient dietary regimens for 12 weeks: (1) a control diet providing a 500 kcal/day deficit, 0-1 servings of dairy products/day, 500 mg calcium per day, and a daily placebo supplement; (2) a calcium-supplemented diet identical to the control diet, with the placebo replaced by 900 mg calcium; or (3) a high dairy diet (placebo supplemented) providing a 500 kcal/day deficit and containing three daily servings of dairy products (milk, cheese and/or yogurt), to bring the total calcium intake from 500 to 1400 mg/day. The first two arms of the study were conducted in a placebo-controlled, blinded fashion, while the third arm (dairy) out of necessity was unblinded. However, subjects on the high dairy diet also received a placebo supplement and all groups were treated as active-treatment arms, with pill counts serving as a component of the compliance measurement, as indicated below.

The diets for the treatment arms were constructed to provide comparable levels of macronutrient and fiber, to approximate the average consumption in the U.S. (~35% energy from fat, ~49% energy from carbohydrates, ~16% energy from protein, 8-12 g/day fiber). Nutritional supplements were not permitted, and caffeine intake was maintained at a constant level (individualized for each patient, based on baseline assessment). Subjects were provided with weekly individual instruction, counseling and assessment from the study dietitian regarding dietary adherence and the development and reinforcement of strategies for continued success. Although the diets were individualized to achieve a 500 kcal/person/day deficit, comparable advice was given to subjects in all treatment groups. All subjects maintained complete diet diaries, and compliance was
assessed by weekly subject interview and review of the diet diary and pill-counts. Subjects in the high dairy group were permitted to utilize both full-fat and low-fat milk, cheese and yogurt, with the fat accounted for in the exchange lists given with each individual diet prescription. Physical activity and tobacco use was assessed using standard questionnaires and subjects were encouraged to maintain pre-study (baseline) levels throughout the study.

Body weight was measured weekly with a calibrated scale and height measured with a wall-mounted stadiometer with subjects in street clothes with no outerwear or shoes. Body mass index (BMI) was calculated via standard equation (kg/m²). Waist circumference was also measured weekly in the standing position, with measurements obtained midway between the lateral lower rib margin and the ileac crest. The measurement was taken mid-exhalation, and the average of two readings were recorded. Bone mineral indices, lean mass, and percent fat mass were assessed using a LUNAR Prodigy DXA system version 2.26.002 (Lunar Corp., Madison, WI). A spine phantom and standard calibration was done before each test day to determine if any drift had occurred. All sites used identical DXA equipment. Whole body and regional site BMC and BMD were measured at the beginning of the study and at week 12 using DXA by staff trained and certified in DXA assessment.

Fasting blood samples were collected twice by a phlebotomist at baseline and week 12 for markers of bone metabolism as well as 24 hour urine samples for assessing urinary metabolites.

As this study was part of a larger multi-center trial, the overall methods, distinct from my original research outcomes will be mentioned here. Additional blood samples
were collected for circulating glucose, insulin, lipids and calcitrophic hormones. Anatomical distribution of fat was assessed by DXA. A subset of subjects also had the effects of the dietary treatments measured for energy expenditure (via doubly labeled water), resting metabolic rate, and thermic effect of foods.

D. Biochemical Analysis

Urine samples were frozen and sent to University of California-Davis (UCD) and were assessed for minerals Ca, P, K, Na, Mg, and Cr by inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) (Vista AX CCD Simultaneous ICP-AES, Varian Analytical Instruments, Walnut Creek, CA). Serum bone markers N-telopeptide (N-Tx) (Ostex Intl. Corp., Seattle, WA) and bone alkaline phosphatase (BAP) (Quidel Inc, San Diego, CA), were also analyzed at UCD. The enzyme-linked immunosorbant assays provided quantitative measures of cross-linked N-Tx of type I collagen and BAP activity in serum. N-Tx is an indicator of bone resorption and BAP is an enzyme produced by osteoblasts, the cells responsible for bone formation.

All samples were analyzed in duplicate with sample variance of > 20% repeated. Overall coefficient of variation (CV) was 7.6% and 5.3 % for N-Tx and BAP, respectively.

E. Data Collection and Statistical Analysis

Anthropometric data was collected at each weekly visit and entered into spreadsheets by staff. Nutritionist IV (First Data Bank, San Bruno, CA) was used for input of food and beverage intake from food diaries.

Differences between treatment groups in outcome variables were compared by analysis of variance (ANOVA). Post hoc pair-wise comparisons were examined using
Fisher's Least Significant Difference for significant interaction effects. Analysis of covariance (ANCOVA) was used to adjust for weight loss. Students paired T test was used for assessing significant differences within groups. Statistical significance was set at the 0.05 level of probability. SPSS for Windows 11.5.0 (SPSS Inc, Chicago, IL) was used for all analyses.

F. Potential Risks

1. Risks

   a. Venous blood draws pose potential risk of bruises, and very rarely, infection.

   b. DXA measurements expose participants to a small amount of radiation.

   c. The duration of the study is 3 months and visits to the laboratory may coincide with busy times in the subject's lives. This could be stressful to some individuals.

   d. The content of the questionnaires (medical and reproductive history, etc) includes items of personal nature and the intrusion of privacy could make subjects uncomfortable.

2. Protection From Risks

   a. Proper and sterile techniques by a phlebotomist will be used when collecting blood to minimize chance of bruising and infection.

   b. The use of DXA does expose participants to minimal radiation doses. X-ray radiation exposure doses 10,000 times greater than that of this study would be needed prior to attaining a detectable increase.
in the risk of cancer. Therefore, the radiation exposure of this study is of negligible health risk to participants. The State of California requires all DXA operators to be examined and licensed by the State Department of Health Services, Radiologic Health Branch. Laboratory personnel at WHNRC have been trained and certified for DXA operation. Additionally, the State of California Health Services requires that all DXA measurements be prescribed by a physician. The requirement was satisfied by the attending physician at the WHNRC. As an added precaution, subjects completed a pregnancy test immediately prior to each of the two DXA measurements. An additional test was performed mid way through the study as a precaution in case of accidental pregnancy.

c. Personal information was kept confidential in secure files and used only by staff who needed access to help properly complete the study. All data entry was coded and the participants’ identities concealed.
CHAPTER 4

BONE METABOLISM AND CALCIUM INTAKE PUBLISHABLE PAPER

(to be submitted to American Journal of Clinical Nutrition)

Effect of Caloric Restriction on Bone Mineral Density and Bone Turnover in Overweight and Obese Individuals With Differing Calcium Intake Levels

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Running Title: Bone Turnover During Weight Loss

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ABSTRACT

**Background:** Bone mineral density (BMD) has been shown to decline with weight loss. Whether the decline is due to weight loss or inadequate calcium intake is unknown.

**Objective:** To determine the effects of supplemental calcium, dairy products, and dietary calcium on bone metabolism during energy restriction.

**Design:** Multi-center 12 wk. randomized trial with 74 overweight individuals. Caloric need was assessed using food diaries. Diets included: 500 kcal/d energy deficit and either low calcium (LC: ≤ 600 mg/d), high calcium (HC: ≥ 1400mg/d), or high dairy (HD: ≥3 dairy servings, total calcium 1400 mg/d).

**Results:** All groups had significant weight loss (p<0.001). After adjusting for weight loss, LC had a significant decrease (1.201±.08 to 1.193±.07) in total body BMD (p<0.05), with a significant increase (1.096±.12 to 1.100±.12) in femur BMD for HD (p<0.05) and marginally significant increase (1.210±.12 to 1.227±.12) in lumbar BMD (p=0.076) for HC. Lumbar bone mineral content (BMC) was significantly increased for HC (p<0.05) while total body and femur remained unchanged. A significant difference between groups (p<0.01) was seen for bone alkaline phosphatase (BAP). Post-hoc analysis indicated a greater decline in BAP for HC (28.43±13.5 to 24.18±12.3), compared to LC (p<0.01) or HD (p<0.03); suggesting a suppression of bone turnover. No change in bone resorption (n-teleopeptide, N-Tx) was observed.
Conclusions: Our results suggest that a 12-wk. 500 kcal energy restriction diet with HC suppresses bone turnover; which may result in preservation of BMD with weight loss.

A longer intervention is needed to evaluate benefits of high calcium intake during weight loss.

KEY WORDS: weight loss, bone mineral loss, bone mineral density, calcium supplement, caloric restriction
Obesity, in recent years, has reached epidemic proportions in the US with two out of every three adults classified as obese or overweight (1). Corresponding to the increase in body weight there has been an increase in dieting. In this regard, calcium from dairy sources has received attention beyond its importance to bone. Specifically, calcium from dairy products has been examined for its role in regulating body composition, i.e., body fat (2,3). Epidemiologic studies have indicated an inverse relationship between adiposity and calcium intake (4). Zemel and colleagues (2) have shown a greater reduction in body weight and body fat in individuals consuming dairy foods compared to individuals with high calcium intakes or controlled intakes with normal calcium intake. Animal studies used to determine the “anti-obesity” effect of dairy products have suggested that the agouti gene found in human adipocytes is down regulated by exogenous calcium intake resulting in decreases in intracellular calcium in the adipocyte, thereby stimulating lipolysis and inhibiting lipogenesis (5). Barr (6) reviewed all randomized studies using calcium or dairy foods and changes in body weight or composition and discovered that the majority of the studies found no significant differences in changes in body weight or composition between control or treatment groups. Two studies, however, showed a significant increase in body weight for the dairy supplemented group versus the control group. Although these results were in conflict with other findings that suggested calcium stimulated increased energy expenditure, not all the studies examined the possible increase in caloric intake associated with the inclusion of dairy foods in the diets. Other studies using calcium supplementation showed no significant differences between calcium supplemented and control groups in changes in body weight, but one study
demonstrated greater weight loss in the calcium supplemented group. It should be noted that these studies did not involve caloric restriction and did not have body weight or composition as primary endpoints. Teegarden and Zemel (7) noted that in order for an effect of calcium to be seen caloric intake must be included in the analysis. Additionally, most of the trials that examined changes in body weight and or composition with increased calcium intake were conducted on normal weight individuals.

Controlling food intake and caloric restriction are commonly used as a means to reduce body weight. Little thought is given, however, to the potential consequences of caloric restriction on other body systems. Specifically, obese women are thought to be at decreased risk for osteoporosis because of influences such as increased body weight on bone. However, a reduction in body weight has been correlated with a reduction in bone mass (8-15). This is problematic for women and may act as a catalyst for osteoporosis. Recently Bacon and colleagues (16) have shown that in obese women with histories of chronic dieting approximately 1/3 of the women had osteopenia or osteoporosis. It has not been fully elucidated whether this reduction is due to the weight loss, other physiologic and behavioral changes, measurement error, or from inadequate calcium. Studies have positively correlated calcium intake with bone preservation in premenopausal (17), and early postmenopausal women (18), though, not all studies have found a positive relationship (19, 20). It has been suggested that short-term interventions might overestimate the benefit of calcium on bone (21). However, during caloric restriction, increased calcium intake decreased bone turnover and increased bone mineral density in postmenopausal obese women (22), and increased bone mineral content in pre and postmenopausal obese women (23). However, these results have not been seen in all
studies (24). Therefore, it was our purpose to examine the effect of caloric restriction on indices of bone metabolism with different levels of intake and types of calcium e.g. food or supplementation, in a multi-center controlled trial. The study was unique because two groups had sufficient calcium intake during caloric restriction while a third control group had limited calcium intake. This last group is representative of a typical dieting pattern where calcium food sources are perceived as high fat, and therefore minimized or avoided (25-28). This study also examined whether dairy sources of calcium provided additional bone related benefits over supplemental calcium during weight loss. Thus, this information will be useful for establishing recommendations for calcium intake during weight loss.

SUBJECTS AND METHODS

Subjects

Overweight and mildly obese premenopausal females and males (n=107) between ages 18-35 years were recruited from the faculty, staff and student populations of each of the four participating institutions (University of Tennessee, Purdue University, Ohio State University, and the University of California-Davis). News stories and advertisements in the local press were used for recruitment. Selection criteria included a body mass index (BMI) between 25-34.9 kg/m², ≤ 3 kg weight loss during past three months, not currently pregnant or pregnant within the last year, consume a low calcium diet of < 800 mg calcium per day (determined by food frequency questionnaire and two 7-day diet histories). Exclusion criteria included: a history or presence of significant metabolic disease including Type 2 diabetes, presence of active gastrointestinal disorders, history of eating disorder, use of obesity pharmacotherapeutic or over-the-counter agents within the
last 12 weeks, use of calcium supplements in the past 12 weeks, recent (past twelve weeks) initiation of hormone replacement therapy, hormonal birth control, or change in HRT or hormonal birth control regimen, recent (past four weeks) initiation of an exercise program, or adverse response to study foods (lactose intolerance, dairy intolerance, dairy allergy or aversion to the placebo product).

Study Design

Subjects who met criteria for entrance into the study participated in a two-week lead-in period to establish current caloric requirements (using 2 - 7 day food diaries) and to obtain baseline dietary and physiological data. Following the lead-in period individuals were randomized to the following outpatient dietary regimens for 12 weeks: Low Calcium (LC) a control diet providing a 500 kcal/day deficit, 0-1 servings of dairy products/day, ≤ 500 mg calcium per day, and a daily placebo supplement; High Calcium (HC) a calcium-supplemented diet identical to the control diet, with the placebo replaced by 900 mg calcium; or High Dairy (HD) a high dairy diet (placebo supplemented) with a 500 kcal/day deficit and containing three or more daily servings of dairy products (milk, cheese and/or yogurt), for a total calcium intake of 1400 mg/day. The first two groups were placebo-controlled and blinded to the investigators. The third group (HD) because of the use of dairy foods was not blind. Individuals on the high dairy diet also received a placebo supplement. Subjects were informed of the study requirements and written informed consent was obtained in accordance with the Committee for Protection of Human Subjects at each institution.

At baseline and week 12, fasted blood samples were collected for markers of bone formation and resorption; bone-specific alkaline phosphatase (BAP) and N-
telopeptide (N-Tx), respectively. Additionally, total body bone mineral density (TBBMD), and bone mineral content (TBBMC) were assessed, as well as lumbar spine (L1-L4) and femur bone mineral density (BMD) and bone mineral content (BMC). Lean mass and percent fat along with bone parameters were all determined using dual energy x-ray absorptiometry (DXA, Lunar Prodigy). Twenty-four hour urine collections were obtained for analysis of urinary minerals and creatinine.

**Diet**

During the two-week lead in period, diet records (2-7 day) were kept and analyzed by a registered dietitian to provide an initial estimate of caloric intake (Nutritionist IV; First Data Bank, San Bruno, CA). This was further refined by calculating energy needs using World Health Organization equations for calculation of basal metabolic rate, which was then adjusted for activity level to provide an estimate of total daily energy expenditure (TDEE). TDEE was calculated as (1.3 x BMR) for obese patients engaged in mild daily activity and (1.5 x BMR) for those engaged in strenuous daily activity. Discrepancies between estimated TDEE and baseline caloric intake were resolved, if necessary, by repeat diet records reviewed by the project dietitian. Based on this initial estimate of caloric needs, a food exchange-based diet was prescribed to result in a caloric deficit of approximately 500 kcal/day. The diets for the treatment arms were constructed to provide comparable levels of macronutrient and fiber, to approximate the average consumption in the U.S. (~35% Kcal from fat, ~49% Kcal from carbohydrates, ~16% Kcal from protein, 8-12 g/day fiber). Nutritional supplements were not permitted, and caffeine intake was maintained at a constant level (individualized for each patient, based on baseline assessment). Subjects were provided with weekly individual instruction,
counseling and assessment from the study dietitian regarding dietary adherence and the
development and reinforcement of strategies for continued success. Although the diets
were individualized to achieve a 500 kcal/deficit, comparable advice was given to
subjects in all treatment groups. All subjects maintained 3-day food intake diaries and
compliance was assessed by weekly subject interview and review of the food diary and
pill-counts. Subjects in the high dairy group were permitted to utilize both full-fat and
low-fat milk, cheese and yogurt, with the fat accounted for in the exchange lists given
with each individual diet prescription. Physical activity and tobacco use were assessed
using standard questionnaires (29). Subjects were encouraged to maintain pre-study
(baseline) levels throughout the 12-week intervention.

**Laboratory Methods**

Body weight was measured to the nearest 0.1 kg on a calibrated scale and height
was measured to the 0.1 cm on a wall-mounted stadiometer. Subjects were in street
clothes with no outerwear or shoes. Body mass index was calculated as kg/m². Bone
parameters, lean mass, and percent fat were measured using a LUNAR Prodigy DXA
was done before each test day to determine if any drift had occurred. All sites used
identical DXA equipment. Urinary metabolites were frozen and sent to University of
California-Davis (UCD) and were analyzed using an inductively coupled plasma-atomic
emission spectrophotometer (ICP-AES) (Vista AX CCD Simultaneous ICP-AES, Varian
Analytical Instruments, Walnut Creek, CA). Serum bone markers of n-teleopeptide (N-
Tx; Osteomark, Ostex Intl Corp., Seattle, WA) and bone specific alkaline phosphatase
(BAP; Quidel Inc, San Diego, CA) were also analyzed at UCD. All samples were
analyzed in duplicate with sample variance of >20% repeated. Overall coefficient of variation (CV) was 7.6 and 5.3% for N-Tx and BAP, respectively.

Statistics

Descriptive statistics were used to examine the physical characteristics of each group. Students paired T-test was used for assessing significant differences within groups. Differences between treatment groups in outcome variables were determined by analysis of variance (ANOVA). Post hoc pair-wise comparisons were examined using Fisher’s LSD. Analysis of co-variance (ANCOVA) was used to adjust for varying amounts of weight loss. Statistical significance was set at the 0.05 level of probability. SPSS for Windows 11.5.0 (SPSS Inc, Chicago) was used for all analyses.

RESULTS

A total of 107 men and women were enrolled in the study. Nineteen did not meet compliance either for calcium intake, pill intake, dairy serving intake or from uncollected data; 14 withdrew from the study for personal reasons. A total of 74 remained for analysis: LC (n=27), HC (n=22), HD (n=25).

Body Composition

As expected, weight loss was significant within each group (p<0.001). Between group differences indicated that percent fat and fat mass decreased significantly in the HD group (p<0.05) as compared to HC (p<0.05) and LC (p<0.05), but no significant within group changes were observed in the LC and HC groups Table 1. Lean mass did not change, however, as a percentage of total weight the HD group had a significant within group increase from 55.2% to 58.4%.
**Bone Parameters**

A significant within group decline in TBBMD was observed for the LC group only (p<0.01). A significant between group decline in TBBMD was observed in the LC group (p<0.05) with post hoc analysis revealing a greater decline in the LC group as compared to the HC group (p=0.016), but not compared to the HD group. After adjusting for weight loss, the between group decline in TBBMD for the LC group persisted (Table 1 and Figure 1). Lumbar BMD (L1-L4) showed a slight within group increase (p=0.08) for the HC group with HD and LC groups remaining unchanged. A significant increase in BMD of the femur was observed in the HD group (p<0.05) with no significant changes in either HC or LC groups. Lumbar BMC was significantly increased for HC group only (p<0.05). No other significant changes were seen in BMC for total body or femur sites, for any of the groups.

**Serum Bio-Markers**

No significant changes were seen for any of the groups for markers of bone resorption, N-telopeptide (p=0.74). Bone-specific alkaline phosphatase, a marker of bone formation, was significantly reduced within the HC group (p<0.01) and a between group difference was observed in the HC group only (p<0.01). Post hoc analysis revealed a greater decline for HC as compared to HD (p<0.001) or LC (p<0.05). Further adjustment for weight loss did not affect the result (Table 2 and Figure 2).

**Urinalysis**

Twenty-four hour urine collections for mineral excretion and creatinine were analyzed by ICP-AES. Due to freezer malfunction, three samples were not analyzed thus total samples for mineral analysis were 71 (LC=27, HC=20, HD=24). Analyses were
corrected for dilution factors, and expressed as a ratio of the specific mineral to creatinine. Within group changes were observed with increases in calcium excretion in the HC and LC groups (p<0.05), a significant increase in magnesium excretion for the LC group (p<0.05), a significant decrease in sodium in the HD group (p<0.05), and a significant decrease in creatinine for HC and LC groups (p<0.05). Calcium and magnesium excretion between groups approached statistical significance (p=0.078; p=0.077) with the HD group having a lower excretion of both minerals at 12 weeks compared to LC and HC, but the trend disappeared after adjusting for weight loss (p=0.102; p=0.109) (Table 2). No other significant changes were seen observed for any of the treatment groups.

*Dietary Calcium Intake and Physical Activity Levels*

Dietary calcium intake was determined from food diaries. Baseline calcium intake was an average of 6 days from 2-7d food diaries. During intervention, calcium intake was assessed weekly from 3d food diaries. Dietary calcium levels remained under 500mg/d for HC and LC groups, as advised by the study dietitian. Intake for the HD group fell short of the recommended 1400mg/d, averaging 1221 mg/d. A significant increase in the overall baseline to week 12 intake was seen for HD (p<0.001) and HC (p<0.001), and a significant decrease for LC (p<0.001) (Table 2).

Inclusion criteria required that subjects not initiate a new exercise program and were encouraged to maintain current levels of exercise throughout the 12 week study period. Though subjects were encouraged to remain at pre baseline physical activity levels, subjects were asked to write down any increase or decrease in activity in their weekly record book. Subjects were also asked to keep a 24-hour record of all activity
and inactivity for 2 weekdays and 1 weekend day for each of the 2 lead-in weeks up to Baseline, and for the last week of the study (Wk 12). There were a total of 9 activity levels to use, ranging from sleeping (activity level 1) to intense manual work (activity 9). Subjects recorded their level of activity in 15 minute intervals (96 per day = 24hr). Activity records were collected and analyzed by computing the activity level type and time for each subject. The 2 lead-in weeks were averaged while the average for the last week of the study took into account any increase or decrease in activity for weeks 1 through 11. Differences in activity level were assessed between the groups and within each group over time. No significant differences were found between or within the treatment groups over the study duration (p=0.88).

DISCUSSION

Our results showed a decline in TBBMD (with an average calcium intake of 512 mg/d) in the low calcium group. This persisted even after adjusting for weight loss. Few studies have investigated the influence of calcium on TBBMD in obese or overweight pre-menopausal women and men. Our findings concur with Jensen et al. (23) in which a non placebo control group with a caloric restricted formula diet containing 800mg/d for 12 weeks also lost TBBMD. However, the Jensen et al. results and ours, are in contrast with a longer 6 month intervention by Shapses et al. (24) who showed little bone loss in the placebo group with an average calcium intake of 459 mg/d. A recent similar study by Bowen, Noakes & Clifton (28) also showed no change in TBBMD in the low calcium group (509 mg/d) when measured 4 weeks after a 12 week intervention.

Our results also showed a significant increase in femur BMD in the HD group and a modest increase in lumbar BMD in the HC group. Shapses et al. (24) also reported that
lumbar BMD tended to increase in their supplemented group. However, this result was refuted by Barker & Blumsohn (30), who reanalyzed the data, and found no significant difference between any of the groups for lumbar BMD. They also suggest that when extrapolating the change seen in lumbar BMD in the placebo group out to one year, the 95% confidence interval would increase to a 3.5% loss in bone and represent a significant loss for premenopausal women who undergo caloric restriction. Lastly, in a 3 year weight stable trial with supplemented dairy products, starting at the 30th month, the intervention group (additional 610mg Ca/d) preserved significantly more vertebral bone density significantly than control group (p<0.03)(31).

Our findings of increased bone density occurred with a corresponding change in BMC only at the Lumbar site for HC. These findings are in contrast to Fogelholm et al. (32) where initial weight loss (first 3 months) in obese premenopausal women did not significantly change BMC. However, there was a significant decline in BMC during 33 months of follow up in which 62% of the weight lost was regained. Bacon et al. (16) have shown that obese premenopausal women with a history of weight cycling have significant negative correlations between BMC and the number of weight loss episodes (r=−0.250) with no correlation observed for BMD. Previous weight cycling history was unknown in our study, but inclusion criteria included no more than 3 kg of weight loss during the previous 3 months, and no prior history of eating disorder behavior.

Our results also showed a significant decline in BAP for the HC group with a maintenance of N-Tx in all groups. This suggests a suppression of bone turnover in the HC group. Since bone turnover is a slow process, a long term suppression of bone turnover during weight loss could amount to preservation of bone density over time.
Caloric restriction studies in obese pre and postmenopausal women have shown either an increase in bone formation and resorption when weight loss occurred, (34, 35) or no change (36). The addition of calcium in weight loss studies either suppressed markers of bone metabolism (22, 28), or showed little effect overall (23, 24). Interestingly, no decline in BAP was seen for the HD group in our study. The HD group in our study averaged ~115 mg/d less of calcium than the diet prescription and markers of bone formation and bone turnover remained unchanged. The lack of a significant change in bone markers for the HD group was unexpected. Perhaps the 115 mg Ca/d represents the threshold at which bone turnover is impacted during weight loss. This amount adds support in suggesting that additional calcium beyond standard recommendations is required during weight loss regimens. This concurs with a recent 6 week calcium and weight loss study which found that 1800 mg Ca/d provided adequate intestinal absorption (348± 118) that was ~56% greater than with a calcium intake of 1000 mg/d (195± 49), based on an average estimated need of ~240 mg Ca/d for postmenopausal women (37).

Increased urinary mineral excretion in the LC group suggest that extended periods of dieting and inadequate intake of calcium may impact BMD. Though calcium excretion increased in the HC group, it seemingly did not come at the expense of bone, since a marginal increase in Lumbar BMD and significant decline in bone formation (BAP) occurred. In the HD group, urinary results support the results seen for BMD, BMC, and serum bone markers; suggesting a reduction in bone turnover for this group.

It is still largely unknown why bone loss occurs during caloric restriction, though our study provides support for the role of high calcium intake in maintaining bone. A number of possible reasons have been explored. Jensen et al. (38) as well as Salamone et
al. (39) and others believe this is due in part to the decrease in weight applied to bone which influences bone remodeling. However, the effect of weight loss on bone was greater on weight-bearing rather than non weight-bearing bones (8). Svendsen et al. (8) found that decreases in lumbar BMD were greater in the diet and exercise group than in the diet only group. However, Ryan et al. (40) did find a benefit in preserving regional BMD with exercise. McLean et al. (41) looked at cognitive change among dieter’s and implicated the production of cortisol as a contributing factor to bone loss during weight loss. Leptin levels may also influence the rate of bone turnover and has been shown to decrease during weight loss (42). There may also be some hormonal implications such as a decrease in adipose cells resulting in a reduction in estrogen or estrone levels (35). Furthermore, parathyroid hormone levels tend to be altered during weight loss and the slight increase may also be a contributor (35, 43).

There have also been methodological issues raised when the analytical method used for determining bone loss is DXA (44-46). The increased thickness of soft tissue in obese subjects may interfere with bone edge detection and affect accuracy of measurements (47, 48). Measurements are also expressed in areal density as g/cm² and do not factor in the dimension of depth (49, 50). Obese subjects tend to have increased bone size resulting in a possible overestimation of BMD. Another consideration may be that women with greater bone mass lose bone mass at a faster rate than women of lower bone mass (51). Anderson et al. (52) noted that because bone mass is higher in obese women than normal weight women, the bone loss seen with weight loss only serves to bring the BMD levels back to within “normal” range. Finally, there are many who undergo caloric restriction repeatedly, and the effect of weight cycling has also been
investigated to determine any potential additional impact on loss of bone mass but without conclusive determination (53, 54).

As most weight reduction typically is regained, it remains to be determined whether a concomitant increase in bone density and content follows (54). One study assessed TBBMC six months post completion and found that subjects who lost additional weight also lost additional bone mineral while subjects who regained weight, also regained bone mass (38). Compston et al. (48) also found that TBBMC approached initial levels when reassessing ten months after a weight loss intervention with subsequent weight gain. Avenell et al. (55) did not find this when subjects regained weight in their study.

The observed associations between high calcium intake and a reduction in bone turnover, whether from dairy foods or supplements, are encouraging and provide further support for maintaining adequate levels of calcium during caloric restriction. However, there are several limitations most notably being the absence of data on vitamin D, leptin, and parathyroid hormone levels as well as the short duration of this study. Longer interventions are needed to evaluate the findings seen from this study for a positive effect of calcium on bone metabolism during caloric restriction. Attention to the above mentioned limitations in future studies will assist in determining more precisely, the impact of caloric restriction on bone metabolism.
Acknowledgements

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Author Contribution

TL Radak completed this work as partial fulfillment for a doctoral degree, served as project manager, and contributed the bone aspects of the project. E Gertz was instrumental in the bone turnover data for the project and was involved in the development of the manuscript. MB Zemel was Co-PI for the project and site director for the University of Tennessee. D Teegarden was Co-PI for the project and site director at Purdue University. RM Lyle was co-investigator at Purdue University and involved in manuscript development. BA Craig and Y Liu were the study statisticians for the project. V Matkovic was co-investigator at Ohio State. MD Van Loan was co-investigator at the USDA-WHNRC at the University of California, Davis and also research director for the bone turnover and involved in manuscript development.
REFERENCES


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### TABLE 1. Anthropometric and Total and Regional Bone Mineral Characteristics at Baseline and 12 Weeks¹

<table>
<thead>
<tr>
<th></th>
<th>Low Calcium (n=27)</th>
<th>High Calcium (n=22)</th>
<th>High Dairy (n=25)</th>
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<tr>
<td></td>
<td>Baseline Wk 12</td>
<td>Baseline Wk 12</td>
<td>Baseline Wk 12</td>
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<tr>
<td>Height (cm)</td>
<td>166.4±7.6</td>
<td>165.5±10.9</td>
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<td>Body weight (kg)</td>
<td>79.4±11.6</td>
<td>80.9±14.9</td>
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<td>76.6±11.1²</td>
<td>78.0±14.0²</td>
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<td>BMI (kg/m²)</td>
<td>28.6±2.7</td>
<td>29.3±2.5</td>
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<td></td>
<td>27.6±2.8²</td>
<td>28.3±2.6³</td>
<td>26.7±2.4²</td>
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<td>Fat Mass (kg)</td>
<td>31.6±4.5</td>
<td>30.7±6.2</td>
<td>32.3±5.9</td>
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<tr>
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<td>29.1±5.0³</td>
<td>28.2±6.5³</td>
<td>28.2±5.6³</td>
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<tr>
<td>Fat Mass %</td>
<td>40.4±5.6</td>
<td>38.5±6.4</td>
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<td>38.6±5.8³</td>
<td>36.7±6.7³</td>
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<td>Lean Mass (kg)</td>
<td>44.6±10.2</td>
<td>46.9±12.0</td>
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<td>44.2±9.2</td>
<td>46.5±11.0</td>
<td>43.8±8.6</td>
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<td>TBBMD (g/cm²)</td>
<td>1.201±0.08</td>
<td>1.201±0.08</td>
<td>1.210±0.09</td>
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<tr>
<td></td>
<td>1.193±0.07⁴</td>
<td>1.205±0.07</td>
<td>1.208±0.09</td>
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<tr>
<td>BMD-Lumbar</td>
<td>1.211±.13</td>
<td>1.211±.13</td>
<td>1.210±.12</td>
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<tr>
<td></td>
<td>1.227±.12⁵</td>
<td>1.227±.12</td>
<td>1.244±.14</td>
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<tr>
<td>BMD-Femur</td>
<td>1.110±.12</td>
<td>1.093±.12</td>
<td>1.096±.12</td>
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<tr>
<td></td>
<td>1.106±.12²</td>
<td>1.092±.12²</td>
<td>1.100±.12²</td>
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<tr>
<td>TBBMC (g)</td>
<td>2773±391</td>
<td>2716±425</td>
<td>2863±378</td>
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<tr>
<td></td>
<td>2764±408</td>
<td>2708±470</td>
<td>2845±426</td>
</tr>
<tr>
<td>BMC-Lumbar</td>
<td>64.64±11</td>
<td>62.60±10</td>
<td>66.8±11</td>
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<tr>
<td></td>
<td>64.40±11</td>
<td>63.74±11⁵</td>
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</tr>
<tr>
<td>BMC-Femur</td>
<td>34.14±6</td>
<td>33.95±6</td>
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<td></td>
<td>34.16±6</td>
<td>33.94±6</td>
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</tr>
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</table>

¹±SD. TBBMD, Total Body Bone Mineral Density; BMD-lumbar, Bone Mineral Density at L1-L4; TBBMC, Total Body Bone Mineral Content. Values with different letters indicate significant differences between groups.

²Significant decline within group (p<0.01).

³Significant decline within group (p<0.05).

⁴Adjusting for weight loss, significant decline (p<0.05).

⁵Adjusting for weight loss, marginally significant increase (p=0.076).

⁶Significant increase (p<0.05).
TABLE 2. Serum Bone Markers, Urinary Minerals, and Calcium Intake at Baseline and 12 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Low Calcium (n=27)</th>
<th>High Calcium (n=22)</th>
<th>High Dairy (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Wk 12</td>
<td>Baseline</td>
</tr>
<tr>
<td>N-Tx (nmol/L)</td>
<td>16.05±5.7</td>
<td>16.76±5.8</td>
<td>14.88±6.0</td>
</tr>
<tr>
<td>BAP (U/L)</td>
<td>26.97±9.0</td>
<td>27.80±10.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.43±13.5</td>
</tr>
<tr>
<td>Calcium/Cr (mg/d)</td>
<td>0.90±.61</td>
<td>1.30±.74&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1.06±.76</td>
</tr>
<tr>
<td>Potassium/Cr (mg/d)</td>
<td>16.89±7.2</td>
<td>19.87±10.6</td>
<td>18.26±9.7</td>
</tr>
<tr>
<td>Sodium/Cr (mg/d)</td>
<td>29.42±17.6</td>
<td>32.24±21.4</td>
<td>33.85±24.6</td>
</tr>
<tr>
<td>Magnesium/Cr (mg/d)</td>
<td>0.49±.17</td>
<td>0.66±.28&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.72±.39</td>
</tr>
<tr>
<td>Phosphorus/Cr (mg/d)</td>
<td>5.98±3.2</td>
<td>6.97±4.5</td>
<td>7.28±3.5</td>
</tr>
<tr>
<td>Creatinine (mg/d)</td>
<td>127.1±45.1</td>
<td>108.3±53.0&lt;sup&gt;i&lt;/sup&gt;</td>
<td>136.3±51.3</td>
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<tr>
<td>Calcium intake (mg)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>691±319</td>
<td>456±72&lt;sup&gt;i&lt;/sup&gt;</td>
<td>682±210</td>
</tr>
</tbody>
</table>

<sup>1</sup>μ±SD. N-Tx, n-teleopeptide; BAP, bone alkaline phosphatase; Cr=Creatinine expressed as a ratio for each mineral.

Values with different letters indicate significant differences between groups.

<sup>2</sup>Significant within group decline (p<0.01).

<sup>3</sup>Significant within group decrease (p<0.05).

<sup>4</sup>Significant within group decline (p<0.05).

<sup>5</sup>Significant within group decline (p<0.001).

<sup>6</sup>Significant within group increase (p<0.001).

<sup>7</sup>Dietary calcium intake at baseline is average from lead-in week and Wk 12 is average of all 12 treatment weeks.
Figure 1: Mean (±SD g/cm²) total in serum total body bone mineral density (TBBMD) at baseline and week 12 between the 3 treatment groups in overweight and obese men and women. HD = high dairy; LC = low calcium; HC = high calcium. A significant decline after adjustment for total weight loss was seen in LC group (* p<0.05). Baseline values are displayed in black.
**Figure 2:** Mean (±SD) total for serum bone alkaline phosphatase (BAP) at baseline and week 12 between the 3 treatment groups in overweight and obese men and women. HD = high dairy; LC = low calcium; HC = high calcium (significant decline * p<0.05). Baseline values are displayed in black.
CHAPTER 5
BONE METABOLISM AND CALCIUM PUBLISHABLE REVIEW PAPER
(to be submitted to Journal of the American Dietetic Association)

Caloric Restriction and Calcium’s Effect on Bone Metabolism and Body Composition in Overweight and Obese Pre-Menopausal Women – A Review
by
Tim L Radak, DrPH, RD

Key Words: weight loss, caloric restriction, calcium, bone mineral density
Word Count for Abstract: 249
Word Count for Text: 5567

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Caloric Restriction and Calcium’s Effect on Bone Metabolism and Body Composition in Overweight and Obese Pre-Menopausal Women – A Review

Abstract

Obesity results in numerous preventable deaths and co-morbidities. Caloric restriction is commonly attempted for weight loss. While obese men and women are thought to be at decreased risk for osteoporosis, because of influences on bone such as increased weight, a reduction of body weight has been correlated with a reduction in bone mass. It has not been fully elucidated whether this risk factor is due to the weight lost, other physiologic and behavioral changes, measurement error, or from inadequate calcium. The importance of maximizing peak bone mass during pre-menopausal years is well known since low bone mass is a risk factor for future osteoporotic related fractures. Most studies have demonstrated a positive relationship between calcium intake and bone mass. During caloric restriction, calcium intake has been shown to decrease bone turnover and increase bone mineral density, but this finding has not been observed in all studies. Calcium from dairy sources has received additional attention, beyond its importance to bone, for its role in regulating body weight and composition. Calcium from dairy foods are perceived as high fat, and therefore, generally minimized or avoided during caloric restriction. But additional weight and fat loss has been seen when dairy sources of calcium have been included in weight loss programs. Current calcium intake for pre-menopausal women is significantly below recommendations and this review underscores the need for maintaining at least adequate intake levels of calcium, if not more, during weight loss regimens to minimize potential long term detrimental effects on bone metabolism.
Caloric Restriction and Calcium’s Effect on Bone Metabolism and Body Composition in Overweight and Obese Pre-Menopausal Women – A Review

Introduction

Obesity has reached epidemic proportions in the US with two out of every three adults classified as obese or overweight (1). National Health and Nutrition Examination Survey (NHANES) data show an increase in the age-adjusted prevalence of obesity from 22.9% in years 1988-94 to 30.5% for the years 1999-2000 (2). The prevalence of overweight individuals among the same time periods also increased from 55.9% to 64.5%. Increases in mortality attributable to obesity are approximately 280,000 per year (3) and include numerous co-morbidities i.e cardiovascular disease, type 2 diabetes, osteoarthritis, hypertension, certain cancers, gall bladder disease, and others (4-6).

Osteoporosis and related diseases are increasing. Approximately 40% of women at 50 years of age will experience an osteoporotic fracture during their lifetime (7). Osteoporosis is a disease of low bone mass and results in an increased susceptibility to various fractures. It has been estimated that over a women’s lifetime about half of her trabecular and one third of her cortical bone will be lost (8). Prevalence of the disease is expected to increase over the coming decades (9) and is likely to become the most common disorder in the aging population (10).

Caloric restriction is a typical approach employed by individuals to reduce excess body weight; a significant proportion of the public engage in weight loss activities (13). Weight control or management is recommended to address the health risks associated
with obesity (14-16). The positive effects of weight loss on chronic diseases have been well documented both in patient health and in health care costs (17, 18).

Obese women are thought to be at decreased risk for osteoporosis because of the mechanical influence of increased load on bone mass. However, a reduction in body weight has been correlated with a reduction in bone mass, which could be a catalyst for future osteoporotic disease. It has not been fully elucidated whether this reduction in bone mass with weight loss is due to 1) the weight loss itself, 2) measurement error, or 3) inadequate calcium intake. Calcium is one of the most investigated nutrients in relation to bone health. Current calcium intake in pre-menopausal women is well below national recommendations. Major calcium food sources are perceived as high fat and, therefore, generally minimized or avoided during caloric restriction. This could have implications for bone health. However, calcium from dairy sources has received additional attention for its role in regulating body weight and composition, particularly fat mass, and simultaneously may have a positive effect on bone health (11, 12). This review will examine the areas of: 1) caloric restriction for reducing body weight and its effect on chronic disease and obesity, 2) the effect of caloric restriction on bone mass, and 3) the role for calcium on weight loss, body composition, and bone health during caloric restriction. Considerations among the various sources of calcium and effect on other diseases will also be examined. Suggestions for intake levels and calcium sources during caloric restriction will be provided based on available studies.
Obesity and Bone Health: Relationship of Body Composition and Bone Mass

The effect of obesity on bone metabolism is not understood, but a positive relationship exists between body weight, body mass index (BMI) and bone mass or bone mineral density (BMD). This relationship has been shown for both total body bone mass and for regional sites, e.g. spine and femur in pre, peri, and postmenopausal women (19-26). Rico et al. (19) assert that body weight was a chief determinant for bone mass in women. Low body weight was an independent predictor of low bone mass later in life (27). Percentage of body fat in premenopausal and older women was also significantly related with total body BMD (20). In older women, percentage fat also correlates well with BMD (28). Possible explanations for the protective effect of obesity, besides mechanical load on bone may be the additional conversion of estrogen from androstenedione in adipose tissue, or a reduction in sex hormone binding globulin (23, 29-32). As obesity increases, bone mineralization increase thereby reducing the risk for osteoporotic diseases.

Numerous cross sectional and longitudinal studies have shown bone loss in premenopausal women though not for all studies (33). When examining outcomes related to bone mass, a decrease in hip fracture, as a result of greater body weight, has not been demonstrated in all studies (28, 34-36). It is clear, however, that osteoporotic fracture risk is higher in women with lower body weight than in heavier women (22-23, 37). But differences in fracture rates between overweight and average women have not been found in all studies (28). Interestingly, a prospective study by Johansson (38) found BMD to be a better predictor of death than blood pressure and cholesterol. Furthermore, low bone mass was an independent predictor of survival.
Effect of Caloric Restriction on Bone Mass

It has been established that bone density can predict future osteoporotic disease and low BMD is one of the strongest risk factors for hip fractures (39). Low peak bone mass is also an osteoporotic risk factor (40, 27). Many studies investigating changes in bone mass of women have suggested that bone mass begins to decrease after peak attainment in the thirties and prior to menopause (33, 41-42). One recent six year prospective study determined that bone loss at the femoral neck began as early as the mid twenties (43) and another study estimated that 99% of peak total body BMD occurs in women between ages 19.6 and 24.6 years and 99% of total body BMC was attained between ages 22.5 and 29.9 years (44).

Observational studies have indicated an increased chance for hip fracture for women who experience weight loss during early or middle adulthood and later in life (35-36, 45-48). Several studies indicate that with weight loss there is a concomitant loss of bone mass either for the total body or regional sites (49-57). Weight loss protocols in these studies ranged from 10 to 24 weeks in duration and one lasting as long as 12 months (58). Additionally, in studies that examined eating behaviors of women, Van Loan and colleagues (59) found on average a 12% lower BMC in women with cognitive dietary restraint and normal to low body weight. Furthermore, Bacon et al. (60) found that in a group of premenopausal obese women with histories of chronic dieting behavior, 1/3 of the women had either osteopenia or osteoporosis. These studies clearly demonstrate that dietary restriction is negatively associated with bone health.
Role of Calcium on Bone Mass

As one of the major minerals in the skeleton, calcium has been well researched in relation to bone growth, preservation, and health. The adult human body contains roughly 1000 to 1500 g of calcium, making it the most abundant mineral of which 99% is contained in bone (61). Current recommendations for women aged 19-50 are 1000 mg/d (62). The 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) estimated mean intake of calcium in women aged 18-50 to be approximately 640 mg/d; only 64% of the recommendation. (62) In a more recent NHANES survey (1999-2000) calcium intake was about 770 mg/d for women aged 20-59; up slightly but still only about 75% of the recommended level of intake (63). Both averages fall significantly short of current recommendations and are quite distant from the upper intake level for calcium set at 2500 mg/d (62). These averages also fall below a requirement of 975 mg/d determined in a premenopausal calcium balance study (64). A NIH Consensus Conference in 1994 highlighted calcium as one of two nutrient deficiencies in the United States that warrant a national effort to increase average intake levels (65).

Low calcium intake can limit bone formation in early life and cause bone loss in maturity (66). Two epidemiological studies confirm this finding along with demonstrating an increased risk for hip fractures in women later in life (67, 68). Supplemental calcium has been shown to reduce the risk of hip fractures (69). While variations in calcium intake during youth have been estimated to affect peak bone mass by only 5 to 10%, the influence of hip fracture risk later in life may account for 25 to
50% of risk (70). While other vitamins and minerals have been looked at, calcium is clearly the most well researched (71).

A meta analysis of 33 cross-sectional, longitudinal, and intervention studies found a small but significant positive correlation between calcium intake from either supplement or diet, and either BMD or BMC in groups aged 18 to 50 years (72). Mean calcium intakes were between 436 to 1437 mg/d. A second meta analysis using 49 investigations of early post menopausal women examined the relationship between calcium intake and bone mass and also found a positive correlation between calcium intake and bone mass (73). More recent studies (74-76) report conflicting results. Heaney (40) performed the largest meta analysis to date of studies using calcium supplements or diet (dairy products) and reaffirmed the positive relationship between calcium intake and bone mass. This finding was consistent in the 139 studies examined except for 2 randomized controlled trials (RCT) and 21 observational studies; 83% of all studies showing a positive relationship. A fourth meta analysis of 46 studies in which only dairy products were used found mixed results. The authors concluded that there was inadequate evidence to support a recommendation for daily intake of dairy foods for bone health (77). This report prompted an editorial by Heaney discussing categorical decisions in Weinsier’s meta analysis i.e. classifying an observational study in a strength category that included RCT’s (78, 79). Relative to calcium supplements versus dairy foods and the impact on bone, two of the six dairy studies in the meta analysis conducted by Heaney indicated that supplement use was significantly better in comparison to dairy, however this observation was not noted. Furthermore, a clinical trial (80), and one retrospective study (81) did not find a significant relationship between dairy foods and
bone mass, but an association was observed between supplement use and bone mass. This finding was also noted in another review by Gueguen and Pointillart in 2000 (82).

Finally, one recent study not included in the above analyses showed a protective role of dietary calcium on bone mass by lowering the rate of bone loss in premenopausal women aged 25 to 30 years (83).

Overall, it appears that calcium intake has a statistically significant influence on bone accretion and bone preservation in the vast majority of studies. Although research suggests that other nutrients are also important, such as vitamin D, it is calcium that has the greatest bone preserving and bone building effect (84). The issue of calcium intake is of paramount importance particularly as median calcium intake for females fall short of recommended levels after childhood, even when supplemental calcium intakes are included (85).

**Role of Calcium on Bone Mass During Energy Restriction**

Because of a demonstrated loss of bone mass during energy restriction, and calcium intake having been shown to increase or preserve bone mass, it has been recommended that calcium supplementation be given during weight loss to reduce bone loss (86). Calcium has been identified as one of the nutrients at risk in diets used for weight control (87-89). Studies have been conducted to determine the effect of calcium intake on bone mass during energy restriction and will be discussed (Table 1). A caloric restriction study which randomized pre and postmenopausal obese women to either one g/d of calcium or a control group for three months found a significant difference between groups in whole body and spine BMC, with the calcium supplemented group losing less
A three month follow-up measurement showed no change in weight in the supplemented group, but the control group gained weight and continued to lose more BMC. The supplemented group had no change in BMC; suggesting continued bone preservation for calcium supplement use.

A six month caloric restriction study by Shapses et al. (90) randomized obese premenopausal women to either 1) one g/d of calcium, 2) placebo or 3) a control group without caloric restriction. Although no significant differences were observed, BMD of spine tended to increase in the supplemented group while the two other groups tended to lose BMD or BMC from the total body or lumbar spine. Additionally, markers of bone turnover were not significantly different between groups. These findings are in contrast to the above study by Jensen (53) and suggest that calcium supplementation did not improve bone status during caloric restriction. It also suggests that low calcium intakes during weight loss did not result in a significant loss of bone and that bone mass was not adversely affected by moderate weight loss. This prompted an editorial by Barker and Blumsohn who, in reanalyzing the data, suggested that the overall change in lumbar spine BMD was not significantly different between groups (91) and that in post hoc testing only a difference was seen between the calcium and control groups; indicating that there is no support for suggesting that the calcium group tended to increase lumbar BMD. Barker and Blumsohn also suggested that when extrapolating the change seen in lumbar BMD in the placebo group to a year, the 95% confidence interval would increase to a 3.5% loss in bone and be a significant loss for premenopausal women who undergo caloric restriction. This work of Barker and Blumsohn does support earlier findings.
A recent 12 week study conducted by Bowen, Noakes & Clifton (92) which examined bone turnover during caloric restriction in men and pre and post menopausal women aged 20 to 65 years showed that low intakes of calcium, ~500 mg/d, resulted in a significant increases in bone turnover compared to the other diet group which had ~1400 mg/d. This study however, did not show any significant changes in total body BMD in either group probably because 12 weeks is too short a time period to see changes in BMD that are assessed using dual energy x-ray absorptiometry. Recently Radak and colleagues (unpublished data) conducted a 12 wk multi center randomized caloric restriction study in overweight and obese men and premenopausal women. Two groups had total average calcium intakes (high calcium, 1334±76; high dairy, 1221±126), while a placebo control group averaged 458±71 mg/d. Results showed significant decreases in total body BMD in the placebo group and increases in femur BMD and lumbar BMC in the high calcium and high dairy groups, respectively. Serum bone alkaline phosphatase (BAP) significantly declined in the high calcium group. These results suggested that, in the short term, high calcium intakes during weight loss can suppress bone turnover and when consumed over extended periods of time may preserve bone mass. Interestingly, no decline in BAP was seen in the high dairy group. The high dairy group averaged ~115 mg/d less of calcium the diet prescription and markers of bone formation and bone turnover remained unchanged. The lack of a significant change in bone markers for the high dairy group was unexpected. Perhaps the 115 mg Ca/d represents the threshold at which bone turnover is impacted during weight loss. This concurs with a recent 6 week calcium and weight loss study which found that 1800 mg Ca/d provided adequate intestinal absorption.
(348± 118) that was ~56% greater than with a calcium intake of 1000 mg/d (195± 49), based on an average estimated need of ~240 mg Ca/d for postmenopausal women (93).

Finally, results from short term studies that assessed bone mass may be confounded by that fact that bone remodeling is believed to act in cycles, known as the "bone remodeling transient" (94). Because calcium is a threshold nutrient, the age and intake of calcium can influence bone mass (95). Most weight lost typically is regained, but whether a concomitant increase in bone density and content follows weight regain is uncertain (96). One study which assessed total body BMC six months post completion found that subjects who lost additional weight also lost additional bone mineral, but subjects who regained weight, also regained bone mass (50). Compston et al. (51) also found that total body BMC approached initial levels when assessment was ten months after a weight loss intervention with subsequent weight gain. However, Avenell et al. (97) did not observe a similar response when subjects regained weight. In summary, investigations of changes in BMD or BMC during weight reduction are not consistent in their findings. Long term studies involving placebo and control groups with a post-intervention follow up are needed to more thoroughly examine the effect of weight loss on bone metabolism. These studies should also include markers of bone turnover and not rely solely on BMD and BMC values obtained by DXA to more accurately assess changes in bone health.
Calcium Intake as an Independent Regulator of Body Weight and Composition

There is now heightened interest from epidemiological, animal, and human experimental data to suggest that calcium may play a role in weight regulation, which could provide an additional reason to ensure adequate calcium intake during weight loss. Epidemiologic studies have indicated a strong inverse relationship between adiposity and calcium intake (98). Zemel (99) examined NHANES III data and found that calcium intake was related to body weight. The relative risk for being in the highest quartile of adiposity was highest among those with the lowest calcium intake. This observation persisted when physical activity and energy intake were controlled. Davies et al. (100) explored the relationship further by retrospectively analyzing five observational and cross sectional studies of pre, peri-, and postmenopausal women. A significant negative association between calcium intake and body weight was observed. For each age group, the odds ratio for women being overweight was 2.25 for young women in the lower half of calcium intakes within their respective groups. Heaney et al. (12) extended the analysis by adding an additional RCT to the analysis and using multiple regression analysis to predict BMI based on calcium intake and other selected macronutrients. The regression coefficient for calcium intake was significant (p = −0.003) and equated to the average BMI being 0.3 kg/m^2 lower for each 100 mg increment in calcium intake.

Zemel was first aware of the relationship between calcium intake and body weight after reanalyzing a previous study which examined the effect of calcium intake on hypertension in obese African-Americans (101). Increases in dietary calcium ranged from approximately 400 to 1,000 mg/d and continued for a year. The result was a 4.9 kg
reduction in body fat. Two additional servings of yogurt were used to increase the dietary calcium intake in the experimental group compared to the control group.

Research has been conducted to determine the mechanism responsible for the "anti-obesity" effect of dietary calcium. Animal studies first studied the agouti obesity gene found in human adipocytes. The agouti protein stimulates the influx of calcium into adipocytes thereby stimulating fatty acid synthase (FAS). FAS is involved in lipogenesis and inhibits basal and agonist-stimulated lipolysis in human and murine adipocytes via a Ca\(^{2+}\) dependent mechanism (99). Exogenous high calcium intake suppresses 1,25-(OH\(_2\))-D and decreases calcium influx to the adipocyte (Figure 1).

Increasing adipocyte intracellular calcium promotes triglyceride storage and exerts control over lipogenesis and inhibits lypolysis. The inhibitory effect of intracellular calcium is also believed to be partially responsible for the inhibition of phosphodiesterase (PDE) (102). Trangenic mice expressing agouti in adipose tissue were placed on low and high calcium diets with the former exhibiting increases in lipogenesis, inhibition of lipolysis, and accelerated increases in body weight and fat mass (11). In the same mouse model another experiment added a caloric restriction component in addition to calcium to see whether additional fat loss could be created secondary to caloric restriction. As hypothesized, the low calcium treatment caused a two fold increase in adipocyte intracellular calcium, a weight gain of 29% and increase in pad fat mass while the high calcium treatments showed a 50% decrease in intracellular calcium and greater decreases in weight loss and fat pad mass. FAS was reduced significantly by high calcium with almost a 2-fold change when the calcium was derived from dairy. Other animal studies confirm the positive effect of calcium on fat and weight reduction (103) and suggest that rats on
caloric restriction and restricted calcium intake have an increased bone turnover and decrease in BMD (104).

Human clinical trials have been conducted to assess the effect of calcium on weight loss and body composition during caloric restriction. Subjects were randomized for 24 weeks to either 1) placebo pill with ≤ 1 servings of dairy/d totaling 400-500 mg calcium 2) high calcium using control diet with 800 mg calcium supplement, or 3) high dairy: 3-4 low fat dairy servings/d for total calcium of 1200-1300 mg/d. All groups had a balanced deficit diet (-500kcals). All groups lost weight with the control losing 6.4 ± 2.5% of body weight, which was increased by 26% in the high calcium group, and 70% in the high dairy group. (105). Fat loss as assessed by DXA followed a similar trend.

A similar study recently conducted by the author and colleagues included subjects randomized to diets similar to the Zemel study, specifically: placebo pill with ≤ 500 mg calcium from either non dairy or ≤ 1 servings of dairy/d, high calcium using control diet with 900 mg calcium supplement, or high dairy: ≥ 3 low fat dairy servings/d for total calcium of 1200-1300/d. All groups had a balanced deficit diet (-500kcals). All groups lost weight with the control losing 2.83 ± 2.8 kg of body weight, 2.83 ± 2.8 for the high calcium group, and 4.20 ± 3.8 in the high dairy group (Radak et al., unpublished). Fat loss, assessed by DXA, was significant for the high dairy group (p<0.05).

The above animal and human studies support a potential beneficial role for calcium on weight loss and body composition with the benefit appearing greatest in dairy products. Zemel suggests one of the additional components responsible for dairy’s increased effect is found in the whey fraction of milk (11). To examine the results of
these calcium and dairy food studies on body composition and weight loss, Barr (106) performed a Medline search of all randomized studies using calcium or dairy which had data on change in body composition or weight. Nine studies were found using dairy. Seven of these studies showed no significant differences in change in body weight or composition between control and treatment groups. Two other studies showed a significantly greater increase in weight for the dairy supplemented group versus controls. The author notes that these results were in conflict with calcium eliciting increased energy utilization, but the results may be compromised if the subjects compensated in some way for the additional calories from the dairy supplements. Seventeen other studies using calcium supplements showed again, no significant differences between calcium supplemented and control groups except for one study which actually showed greater weight loss in the calcium supplemented group. It should be noted that the studies did not include caloric restriction and did not have body weight or composition as primary endpoints. Teegarden and Zemel (107) note that in order for an effect of calcium to be seen, caloric intake must be factored in. Additionally, most of the trials included normal weight individuals in whom caloric restriction would not be appropriate. Clearly, more research is needed to assess calcium’s impact on body composition and weight during caloric restriction and to determine if dairy calcium exerts an additional positive effect above that of calcium supplementation.

Other Potential Influences on Loss of Bone During Caloric Restriction

It is not entirely clear as to the mechanism responsible for the loss of bone mass during weight loss. A number of possible reasons have been explored and will be
discussed. Jensen et al. (50), and others have suggested that the bone loss associated with weight loss may be due to the decrease in weight applied to bone, e.g. mechanical load, which influences bone remodeling. If this were the case then the effect of weight loss would be greater on weight bearing bones rather than non weight bearing bones (19). Anderson et al. (108) examined this hypothesis to see if exercise could preserve the loss by adding a resistance training component to one of the diet groups, but no significant bone sparing effect was observed. Svendsen et al. (109) found that decreases in lumbar spine BMD actually were greater in the diet and exercise group than in the diet only group. In a six year prospective study in pre and perimenopausal women, current physical activity was not correlated with BMD or bone loss (110). However, Ryan et al. (111) did find a benefit to preserving regional BMD.

McLean et al. (112) looked at cognitive change among dieter’s and implicated the production of cortisol as a contributing factor to bone loss during weight loss. Leptin levels may also influence the rate of bone turnover and has been shown to decrease during weight loss (113). It has also been suggested that leptin regulates bone formation independent of its influence on body weight (114). Another factor could be a decrease in adipose cells resulting in a reduction in estrogen or estrone levels as confirmed by Ricci et al. (56). Parathyroid hormone levels tend to be altered during weight loss and this slight increase may also be a contributor (56, 115).

There have also been methodological issues raised when the analytical method used for determining bone loss is dual-energy x-ray absorptiometry (DXA) (116-119). The increased thickness of soft tissue in obese subjects may interfere with bone edge detection and affect accuracy of measurements (8, 51). Measurements are also expressed
in areal density as g/cm$^2$ and do not factor in the dimension of depth (120, 121). Obese subjects tend to have increased bone size resulting in a possible overestimation of BMD. Another potential consideration may be that women with greater bone mass also lose bone mass at a faster rate than women of lower bone mass (122). Anderson et al. (108) noted that because bone mass is higher in obese women than normal weight women, the bone loss seen from weight loss only serves to bring those women back to within the "normal" range.

Many individuals undergo repeated episodes of caloric restriction. Therefore, the effect of weight cycling on bone mass and turnover has also been investigated. Results from these studies are not conclusive (60, 95, 123). Recently, however, Bacon et al. (60) examined a group of obese women, each with a history of chronic dieting, and found that 1/3 of the women had either osteopenia or osteoporosis.

While other nutrients play a role in bone metabolism, most are within adequate intake levels compared to levels of calcium intake. However, it is unclear whether other nutrients are compromised during caloric restriction to the degree of calcium. This is of particular importance for individuals who engage in repeated weight loss episodes. Nutrients found in fruit and vegetables have been suggested as having a positive association with BMD for late premenopausal women (114). Vitamin D is needed for calcium absorption in the intestine and also plays a role in bone turnover (124). Positive effects of calcium on bone have been reported both with and without the inclusion of vitamin D (125). This may be explained partially because the major source of vitamin D is cutaneous production via sunlight exposure, which is geographically variable. Trials looking to differentiate the effect of vitamin D or calcium have shown that the
preservation of bone is due primarily to calcium and not vitamin D (126), though a
deficiency of vitamin D could promote negative consequences for bone metabolism (95).
Perhaps the largest contribution to bone loss during caloric restriction is due to a reduced
calcium intake.

**Considerations for Choices of Calcium Source**

While available studies suggest preservation of bone with adequate calcium
during caloric restriction, and that dairy sources of calcium may yield additional
weight/fat loss during caloric restriction, there are other factors to consider between
supplementation or dietary sources (Table 2). Those who try to reduce weight via caloric
restriction commonly strive to consume less fat (13, 127-128). Dairy products, a major
source of dietary calcium for the public, are sometimes perceived as fat containing foods
and typically not emphasized during periods of caloric restriction (129-132). As
mentioned, this may have a deleterious effect on bone health for premenopausal women
already striving to meet calcium intake recommendations.

Various issues have been raised surrounding the sources of calcium available.
Supplements can be easier to take than dietary sources, less expensive, better absorbed,
and can meet recommendations with one pill, as well as being more accepted by a sizable
portion of the population that are lactose intolerant (133-135). Those who follow a strict
plant based diet might benefit from supplements (136), particularly during weight loss.
While many plant based sources of calcium generally have good fractional absorption
(137-139), with some better than dairy sources (136, 140), their intake alone falls short of
meeting calcium requirements for the majority of the population. The challenge with
plant sources is getting sufficient quantities of intake. Dairy products remain the most significant calcium source for the public (141). The National Health Interview Survey in 1989 quantified calcium supplement use at roughly ~25 percent for women (142). Pill supplementation comes without the addition of cholesterol and saturated fats, known risk factors for CVD, and has been shown to have some favorable effects on blood lipids though in general has no significant additional effect (143).

Dairy products, as a source of dietary calcium and other nutrients, have been proposed over supplements for additional reasons beyond their effect on bone health (140). The CARDIA study indicated a reduction in cardiovascular and type 2 diabetes risk factors with increased dairy intake (144). Others note reductions in hypertension and homocysteine levels in diets containing low fat dairy such as the DASH diet (145).

Other components of dairy such as conjugated linoleic acid have been suggested to be potentially protective of certain diseases and have exhibited antitumor properties (146, 147) though one epidemiological study found conjugated linoleic acid intake to have a weak but positive relation with breast cancer incidence (148). Other forms of cancer have been associated with dairy consumption (149, 150), but many studies give conflicting results with some showing dairy consumption to be protective and others studies indicting a lack of protective effect with dairy foods (141, 151-154). Other studies as well as a review have suggested dairy consumption can be related to an elevated insulinaemic index (155-157). While dairy products contain cholesterol and saturated fats, studies using dairy products did not find significant increases but rather decreases in plasma lipid and lipoproteins related to cardiovascular risk (143). However, in hypercholesterolemic individuals that had been on a lipid lowering diet, the addition of
dairy for 6 weeks increased LDL and decreased HDL, while also increasing lipid peroxidation (158).

Dietary sources of calcium have been shown to decrease risk of renal stones while supplemental calcium has been shown to increase risk and could possibly interfere with other minerals (85, 159). Lastly, some follow up studies showed that the positive effect of calcium didn’t persist years later for pill supplements (85) and dairy calcium (160), though one study did suggest the effects persisted with dairy (161).

Regardless of the decision to choose between calcium sources, the most important factor is to choose one as a source or addition to one’s total calcium intake, particularly during caloric restriction.

**Conclusions and Applications**

It is prudent to suggest that weight loss during premenopausal years is desirable and outweighs the potential risks to bone health which are now being investigated. The effects on bone metabolism during this time may impact bone health in the future, when bone preservation is of utmost importance to minimize osteoporotic related fractures. Available premenopausal studies show a decline in bone mass with weight loss and low calcium intakes. However, there are still many unknowns relative to the cause of bone loss during weight loss. Findings from studies that examine bone health during weight loss suggest that high calcium intake during energy restriction may attenuate the loss of bone. Intake levels for calcium during caloric restriction studies range from 1000-1800 mg/d. As most women’s calcium intake is well below 1000 mg/d, adding an additional 500-1000 mg/d from dietary or supplemental sources would not exceed the upper intake
level and could provide a benefit to bone metabolism, weight loss and fat loss during caloric restriction. The influence of high calcium intake beyond standard recommendations warrants additional investigation for additional weight loss, fat loss as well as for preservation of bone mass. If supplements are recommended, the addition of vitamin D is suggested. At this time, unless there is a known medical condition or heredity history, DXA scans to assess bone status are not performed in premenopausal women.

The stage appears set for possible future osteoporotic disease as a combination of already low calcium intake, normal bone loss due to aging, the negative influence of weight loss on bone mass, and potentially an additional insult from weight cycling, all together represent a risk to bone health and may predispose premenopausal women to future osteoporotic events. Because calcium intake and weight loss appear to effect bone mass it is important for additional studies to determine the risks and benefits of both concurrently. Longer term interventions are needed to evaluate whether the influence of calcium persists during and after weight loss and to also evaluate other potential influences on bone loss.
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soy milk and non-fat cow milk on lipid profile and lipid peroxidation in patients


Figure 1: $[\text{Ca}^{2+}]$ mediated mechanisms and regulation of adiposity.
<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Intervention</th>
<th>Duration</th>
<th>Outcome</th>
</tr>
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<tr>
<td>Jensen, 2001</td>
<td>62 pre and post-menopausal women</td>
<td>1 g Ca or nothing (no placebo)</td>
<td>3 months</td>
<td>Significant differences in whole body and spine BMC with the treatment group losing less BMC. Follow up three months post completion showed no change in weight in the supplemented group while the unsupplemented group gained weight. The unsupplemented group continued to lose more BMC, while the supplemented group had no change in BMC suggesting continued bone preservation for supplement use.</td>
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<td>Shapses et al., 2001</td>
<td>38 premenopausal women</td>
<td>1 g Ca, placebo, or control group with no caloric restriction</td>
<td>6 months</td>
<td>No significant differences, but spine BMD tended to increase in supplement group, &amp; other 2 groups tended to lose BMD and BMC from total body or spine.</td>
</tr>
<tr>
<td>Bowen et al., 2004</td>
<td>50 pre and post menopausal women and men</td>
<td>2.4 g Ca or .5 g</td>
<td>12 weeks and 4 weeks energy balance</td>
<td>Low Ca group had 40% increase in bone resorption (urinary Dpr) and significant increase in bone formation marker (serum osteocalcin) as compared to High Ca group. No change in TBBMD in either group.</td>
</tr>
<tr>
<td>Radak et al., 2004</td>
<td>74 premenopausal women and men</td>
<td>High Dairy (1221 mg Ca)</td>
<td>High Ca (1334 mg Ca)</td>
<td>Low Ca (456 mg Ca) with placebo</td>
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<tr>
<td>Cifuentes et al., 2004</td>
<td>57 postmenopausal women</td>
<td>1 or 1.8 g Ca in weight loss or weight maintenance groups</td>
<td>6 weeks</td>
<td>For 1.8 g vs 1 g Ca: Serum markers of bone turnover only; Marker of resorption (N-Tx) showed no significant changes. Marker of bone formation (Serum Oc) showed a decrease and significantly prevented a rise in levels.</td>
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<tr>
<td>Ricci et al., 1998</td>
<td>31 postmenopausal women</td>
<td>1 g Ca or placebo</td>
<td>6 months</td>
<td>Decreased bone turnover and greater preservation of BMD in the supplemented group as compared to the control group.</td>
</tr>
</tbody>
</table>

BMC = bone mineral content, BMD = bone mineral density, TBBMD = total body bone mineral density, BAP = bone alkaline phosphatase, Dpr = deoxypyridinoline, N-Tx = N-telopeptide, Oc = Osteocalcin.
<table>
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<tr>
<th>Supplemental</th>
<th>Dairy</th>
<th>Plant based</th>
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<td>Less expensive, slightly better absorbed than dairy, can meet recommendation with one or two pills, good for lactose intolerant, increase risk for renal stones</td>
<td>Sometimes perceived as high fat, additional benefit to fat loss during caloric restriction, contains other vitamins and minerals, source of cholesterol and saturated fat, mixed results relative to chronic disease</td>
<td>Excellent fractional absorption, contains vitamins and minerals as well as other phytonutrients, challenging to meet calcium intake recommendations solely from plant products</td>
</tr>
</tbody>
</table>
A. Summary and Implications of Findings

The observed associations in our study between higher calcium intake and bone preservation, whether from dairy or supplements, are encouraging and provide further support for maintaining at least recommended levels of calcium during caloric restriction. Public health professionals need to emphasize adequate calcium intake when prescribing weight loss regimens. The majority of studies examined used ~ 1 gram calcium in their treatment groups. A small number of studies utilized levels of dietary calcium intake beyond current recommended levels, and showed significant benefit. While many more studies are needed to support these findings, it sheds insight for the role calcium plays in maintaining bone and could result in advisement for going above current recommendations during episodes of weight loss. However, it is not fully known whether other factors could have influenced the findings such as other dietary nutrients, level of activity etc and would refrain from saying calcium is the “magic bullet” for bone preservation during caloric restriction. Our study also had several limitations most notably being the absence of data on Vitamin D, leptin, and parathyroid hormone levels though these were original outcomes for the principal investigator of the parent study and will be reported in a different article.
B. Future Research

Longer term interventions, perhaps two years in duration, are needed to evaluate if the findings seen from this study for a positive effect of calcium on bone metabolism during caloric restriction remain. More research is also needed to further evaluate other mentioned theories that may influence or be responsible for an effect on bone metabolism during weight loss. The inclusion of the above mentioned limitations in future studies, and particularly the use of a combination of calcium and vitamin D when administering a supplement in trials, will assist in determining more precisely the impact of caloric restriction on bone metabolism and appropriate recommendations and guidelines for attenuating its negative influence on bone metabolism.
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Appendix A: Sample Flyer

Earn $300 for participating in a Weight Loss Study
Overweight Men and Women Volunteers Needed

The USDA Western Human Nutrition Research Center is seeking healthy men and women to participate in a weight loss program to determine if calcium increases weight loss when on a weight loss diet.

Volunteers will be placed on a personally individualized 12 week diet, and will receive weekly professional guidance, counseling, and food and nutrition information.

14 campus visits will be required. Volunteers will be paid $300 compensation and will receive valuable test results showing cholesterol and triglyceride levels, blood pressure, body composition etc.

Volunteers are:
☆ healthy and between the ages of 18 and 35
☆ not currently on a diet regimen
☆ not currently taking prescription or over the counter diet supplements or pills
☆ not currently taking calcium supplements or be allergic to dairy products
☆ if a women, not be pregnant or plan to get pregnant over time of the study
☆ within the height and weight chart below

For more information about this exciting study, call (530) 752-4168 and press #3
You can also find more information at the USDA-WHNRC website
http://www.whnrc.usda.gov/volunteering.html

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<td>4'10&quot;</td>
<td>120 - 167 lb.</td>
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<td>124 - 173 lb.</td>
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<td>160 - 223 lb.</td>
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<td>128 - 179 lb.</td>
<td>5' 8&quot;</td>
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<td>132 - 185 lb.</td>
<td>5' 2&quot;</td>
<td>137 - 191 lb.</td>
<td>5' 10&quot;</td>
<td>174 - 244 lb.</td>
<td>5' 3&quot;</td>
<td>141 - 198 lb.</td>
<td>5' 11&quot;</td>
<td>179 - 251 lb.</td>
<td>5' 4&quot;</td>
<td>146 - 204 lb.</td>
<td>6' 0&quot;</td>
<td>184 - 258 lb.</td>
</tr>
<tr>
<td>5' 5&quot;</td>
<td>150 - 210 lb.</td>
<td>5' 6&quot;</td>
<td>155 - 217 lb.</td>
<td>5' 7&quot;</td>
<td>160 - 223 lb.</td>
<td>5' 8&quot;</td>
<td>164 - 230 lb.</td>
<td>5' 9&quot;</td>
<td>169 - 237 lb.</td>
<td>5' 10&quot;</td>
<td>174 - 244 lb.</td>
<td>5' 11&quot;</td>
<td>179 - 251 lb.</td>
</tr>
</tbody>
</table>
Appendix B: Screening Flowchart

**Dairy Study SCREENING AND RECRUITING FLOW CHART**

Screen for:
1. Application Information Eligibility Sheet
2. Calcium Phone Screening Instruction Sheet
3. If have time to see that they pass both, then schedule Screening Appt (we’ll have ~3 group info screening sessions, the potential subject will pick one that works best with their schedule)
4. See Tim for Screening appt dates or tell subject we will get back to them for appt.
5. Fill in Telephone Log

**Screening Appointment**
1. Explain study and give consent form. Get signed when thoroughly reviewed and understood.
2. Use “Case Report Form-Enrollment Visit” and fill out as go along (wt, ht, bp, rhr). If pass weight then continue on. If not, disqualify.
3. Give Calcium Food Frequency. If pass, continue
4. Give Medical Questionnaire. If pass criteria, continue to blood draw.
5. Blood draw given for basic health profile. This will be done throughout the meeting.
6. Schedule Appointment #2 (-2 wk) Lead In Period and check availability for 164 L and Blood Drawing (Rm 164J).
7. Explain and Give Food Record Booklet. Give general info and welcome letter.
8. Give calendar for all subsequent visits with requirements at each visit.

(-2 wk) Lead In Period
1. Do pass Blood draw for healthy?
2. Check (wt, ht, bp, rhr)
3. Get Food Record and Activity Record, review both for completion
4. Give 3 Day activity record Questionnaire to take home
5. Get list of food preferences from subject
6. Give Food Record Booklet
7. Schedule Appointment #3 (-1 wk) Lead In Period

Appointment #3 (-1 wk) Lead In Period
1. Check (wt, ht, bp, rhr)
2. Get Food Record and Activity Record, review both for completion
3. Give 3 Day activity record Questionnaire to take home
4. Prescribe diet to Subject
5. Prepare for Baseline (explain upcoming procedure, DXA, Blood, Fasting, Urine)
6. Give out Urine bottle, carrying case, funnel, instruction sheet
7. Schedule Appointment for Baseline Visit

Baseline
1. Get Food Record and Activity Record. Fill out “Case Report Form-Baseline”
2. Counsel subject about diet instruction and compliance, pills (administer pills)
3. Subject undergoes anthropometric (waist, wt, ht, bp, rhr), blood, DXA, urine, and preg testing.
4. Schedule Appointment for Week #1