Weight Reduction and Bone Loss in Postmenopausal Women: Follow up at 2 years

by

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ABSTRACT OF THESIS

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Short-term weight loss (WL) is accompanied by bone loss in postmenopausal women, but the longer-term impact is unclear. The goal of this study was to determine whether weight regain compared to maintenance of WL in reduced obese/overweight women has an impact on bone mineral density (BMD) and content (BMC). It was hypothesized that weight regain in reduced obese women would result in partial recovery of bone, as compared to women who maintain their lost weight. We measured hip, spine, forearm, and total body BMD and BMC by dual energy x-ray absorptiometry. Hormones, markers of bone turnover, and soft tissue were assessed. We recruited postmenopausal women (n=40, BMI of 28.2 ±2.9 kg/m^2; 60.6 ± 5.6 years) 1.5 years after a 6 month weight loss program who were categorized as regaining (>75%) or maintaining their weight lost. After 6 mo of WL, both groups lost -9.1 ± 3.1% body weight with no significant difference between groups. At 23 ± 6 mo after baseline, the weight-loss maintainers (WL-M, n=22) and weight-loss re-gainers (WL-R, n=18) lost -10.4 ± 4.3 % and -2.6 ± 3.6 % of their body weight. Participants averaged 1228 ± 529 mg/d during the follow-up period, which did not differ significantly between the groups. The WL-M group showed greater BMD loss at the trochanter ($p<0.05$), 1/3 radius ($p<0.05$), and total body ($p<0.02$) compared with the WL-R group. WL-M also showed a trend to decrease serum osteocalcin ($p<0.08$) and increase cortisol ($p<0.09$) more than the WL-R group, as measured in a subset of women (WL-M n=10, WL-R n=8). There

were positive relationships between the change in fat mass and 1/3 radius ($r=0.047$, $p<0.01$) and total body ($r=0.519$, $p<0.001$) in the entire group of women ($n=40$) while changes at the trochanter and 1/3 radius correlated with leg fat ($r>0.422$, $p<0.01$), but not trunk fat, suggesting that regional fat tissue may have a differential influence on bone. These data in postmenopausal women show that bone continues to decline at the trochanter and femoral neck over a 2 year period with 10% weight loss, and with weight regain there is partial recovery of bone.

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INTRODUCTION

Obesity and overweight have become major health issues for the entire population of the United States. Obesity is defined as a Body Mass Index (BMI) of >30 kg/m^2 while overweight encompasses those with a BMI of 25- 30 kg/m^2 (NIH, 1998). The prevalence of both obesity and overweight has steadily increased since 1980 (National Center for Health Statistics, 2006). The National Health and Nutrition Examination Survey 2001-2004 reveals that approximately two-thirds of adult Americans are overweight or obese, with greater than 30% falling into the obese range (National Center for Health Statistics, 2006). One third of adult American women were obese during the time period between1999-2004 (Ogden, 2006). The increasing incidence of overweight and obesity is associated with many health problems including type II diabetes mellitus, cardiovascular disease, hypertension, asthma, and arthritis in the US population (Mokdad, 2003). Public health messages encourage overweight and obese individuals to decrease their weight to avoid these health concerns.

Health Concerns for Postmenopausal Women

Figures from the National Health and Nutrition Examination Survey estimate the prevalence of obesity in women aged 65-74 years of age to be 36% from 1999-2004, an increase of ~9% compared to data collected from 1988-1994 (Rosamond, 2008). Also after menopause, there is an increased risk of a number of chronic health concerns including cardiovascular disease, breast and cervical cancer, and osteoporosis (Rao, 2008). Given the overall increase in overweight and obesity, it is important to examine the relationship between weight and these health issues in this population.

Body Mass and Chronic Disease

There is a particular concern for weight management in older women. Many women experience an increase in total body weight during the menopausal transition. One study found that there was an average gain of 2.25 +/- 4.19 kg over a 3 year period (~1.65 lbs per year) in women aged 42-50 at baseline, which was associated with a significant increase in blood pressure, total cholesterol, triglycerides, and fasting insulin (Wing, 1991). There is also a shift towards central adiposity around menopause (Koskova, 2007) and an overall decrease in lean body mass (Sternfeld, 2005). The etiology of these changes are not fully understood, but several factors have been implicated including hormonal changes including the decrease in estrogen, DHEA, and GH-IGF-1 (Milewicz, 2001), decreases in physical activity and/or increased sedentary behaviors (Simkin-Silverman, 2000; Sternfeld, 2005; Blanck, 2007), and changes in diet composition due to altered appetitive hormones (Ritland, 2008).

The elevation of BMI in older adults has been shown to increase the risk of developing cardiovascular disease, diabetes, arthritis, and breast and uterine cancer as well as leukemia (McTigue, 2006). In particular, the metabolic syndrome, a series of risk factors including abdominal obesity, dyslipidemia, hypertension, and insulin resistance that significantly increases risk of developing cardiovascular disease and diabetes (Kaaja, 2008; Schenieder, 2006) is of great concern in this population, especially given their predispositon towards abdominal adiposity. The metabolic syndrome is estimated to affect up to 40% of postmenopausal women at present (Lobo, 2008), with women comprising ~53% of cardiovascular disease mortality in 2004 (Rosamond, 2008). However, it is known that weight reduction can have a beneficial effect on these chronic health issues (Bales, 2008;

Rivlin 2007, McTigue, 2006; Simkin-Silverman, 2000) and current recommendations encourage women to decrease caloric intake to lose weight (Dubnov-Raz, 2007), specifically 5-15% of initial body weight to reduce risk of co-morbid conditions (Zamboni, 2005; Hainer, 2008).

Menopause and Bone

It has been well established that peak bone mass is achieved early in adulthood (Walsh, 2008). Little bone is lost during pre-menopause and early perimenopause, but there is a correlation between the rate of change and the interval since the start of menopause (Pouilles, 1995). BMD declines at the greatest rate during late perimenopause and early menopause (Finkelstein, 2008). The mechanism of action of this post-menopausal bone loss is not fully understood, but it is thought that the decrease in circulating estrogen plays a role. Estrogen, when present, helps to induce apoptosis of osteoclasts, keeping the rate of bone turnover low. When estrogen production decreases during the menopausal period, there is an increase bone resorption (Kameda, 1997). While the rate of bone loss during the menopausal period is still being elucidated, there are a number of citations in the literature that present an annualized rate of loss in the weight stable postmenopausal population (Table 1).

There are several factors that increase risk of developing osteoporosis. These include gender, menstral status, lifetime calcium and vitamin D intakes, and exercise (NIH, 2000). Furthermore, the literature suggests that body weight has a considerable influence on bone (Finkelstein, 2007, Zhai 2008, Sirola J, 2003), which may cause a conflict between the need to decrease the risk for metabolic syndrome through weight reduction while possibly increasing the risk of osteoporosis.

Table 1. Percentage annual bone loss of healthy weight stable women at different sites.

Author	Subjects	n	Sites BMD*	%Mean Annual Loss (SD)	Adjusted for
Makovey, 2008	Women (45-84 yrs) perimenopausal postmenopausal HRT > 12 mo	724 121 603 (254)	LS FN Forearm	–0.37 (1.43) −0.36 (1.56) −0.77 (1.66)	Age, smoking, alcohol intake, HRT(for use <6 mo or for use >6 mo), and physical activity
Park, 2007**	Post, not on HRT	27	Wards Spine Tot Hip FN Troch	-0.88 -1.32 -0.76 -0.91 -0.61	Age, baseline BMD, baseline wt, ht, smoking status, and sport activity change
Sirola, 2003	Perimenopausal women Early Post (< 5 yrs post-menopause) Late Post (> 5 yrs post-menopause)	116 172 108	LS FN LS FN LS FN	-1.22 (0.9) -0.87(0.8) -0.5(0.9) -0.58(1.4) -0.20(0.7) -0.57(0.9)	Age, BMI, BMD @ baseline, and duration of follow-up
Uusi-Rasi 2001*	60-65 y	128	FN BMC Troch BMC Rad BMC	-0.6(1.5) -0.5(2.0) -3.2(4.7)	
Guthrie, 1998	Postmenopausal	60	LS FN	-0.7 (0.2) -0.5 (0.3)	
Nguyen, 1998	60-69 y 70-79 y >80 y		FN FN FN	-0.6 (0.1) -1.1 (0.2) -2.1 (0.6)	
Young, 1996**	Postmenopausal (45-65 yrs)	77	LS FN Troch	-0.39 -0.51 -0.45	
Pouilles, 1995	Postmenopausal 6 mo-2yr >5 yrs post	81	FN Ward's Troch FN Ward's Troch	-1.82(1.1) -2.43(1.7) -1.12(1.7) -0.48(0.8) -0.68(2.1) 0.41(1.2)	
Harris, 1992	Healthy, 1-2 yrs Healthy, > 6 yrs 41-71 yrs		LS LS FN Rad	-2.24(2.1) -0.96(3.0) -0.24(2.6) -0.14(2.2)	Body size, dietary Ca intake, treatment group, smoking

* BMD loss is reported for all studies, except Uusi-Rasi, et al 2001 that reports loss as BMC
**no standard deviation reported

BMD-bone mineral density; BMC- Bone mineral content, Post- Postmentopaual, HRT- hormone replacement therapy, LS- lumbar spine, FN- femoral neck, Troch- trochanter, Rad- radius, W-whole body, Tot Hip- total Hip.

Weight and Bone

Increased body weight has a protective effect on bone loss (Finkelstein, 2007, Zhai 2008, Sirola J, 2003). Hypothesized mechanisms for this observation include the increased mechanical loading due to greater mass, adipocyte production of hormones such as estrogen, resistin, and interlukin-6 (Zhao, 2008) and leptin (Crepaldi, 2007). Estrogen alone cannot explain the effect of weight and bone since low BMI was negatively related to total hip BMD in older men (Meyer, 2008; Bakhireva, 2004). Both weight maintenance (Wu, 2002) and gain have been shown to protect against bone loss in postmenopausal women (Sirola, 2003; Trovas, 1999) and older men (Ensrud, 2005; Langlois, 1998). In women, interventions aimed at stopping menopausal transition weight gain or promoting modest weight loss during that time, showed an increase in hip bone loss (Park, 2007). This is of concern since many women are also confronting the development of risk factors for other diseases at this time, as described above. It may be necessary to establish a delicate balance between the needs of each individual in terms of weight loss to improve co-morbid conditions such as cardiovascular disease, diabetes, and cancer and the long-term risk of developing osteoporosis.

Fat Mass and Bone

Although there is clearly a link between bone and fat mass, the relationship between these tissues is still under investigation. It has long been hypothesized that fat mass, as a component of body weight, contributes to mechanical loading of bone, yet there may be an inverse relationship between bone and fat mass, when body mass is removed statistically (Zhao, 2007). Conversely, others have found that fat mass has a positive relationship with bone density independent of weight (Reid, 2008). Clearly, there is a complex interaction

between fat and bone, which may be due to both central hormones that act on fat and bone and/or adipocyte-derived hormones that regulate bone such as leptin

Weight Loss and Bone

Observational studies have measured the effect of weight changes on bone. Weight change has been associated with the weight-bearing femoral neck BMD loss in peri- and postmenopausal women (MacDonald, 2005) and elderly women (60-80 yrs of age) (Nguyen, 1998). The forearm, a non-weight bearing site, was studied in peri- and postmenopausal women and there was no association with weight gain, although weight loss was an independent factor for predicting loss of BMD (Forsmo, 2006). Weight variability, or changes in body weight over time, increases the risk of hip fracture in both older men and women (Meyer, 1998). Additionally, a longitudinal study has shown that a ten percent loss of maximum weight is associated with increased risk of fracture in both middle aged (50-64 years) and older women (65-74) over a 22 year follow-up (Langlois, 2001), indicating that weight changes may have a long-term impact on the hip. It should be noted that the aforementioned studies were not designed to distinguish between voluntary or involuntary weight changes, but simply measured weight variability over time, therefore making it difficult to develop strong conclusions about the impact of weight change on bone based on this epidemiologic data alone.

Involuntary Weight Loss

Involuntary weight loss is often linked with disease states while voluntary weight loss is a conscious effort on the part of an individual to reduce body mass (French SA, 1999). Involuntary weight loss in elderly women (Ensrud , 1997) and older men (Langlois, 1998) has been shown to increase the risk of frailty fractures. It is important to note, however, that

the underlying cause of the unintentional weight loss, including malignancy or gastrointestinal disorder (Lankisch, 2001), changes in dietary intake due to ingestion difficulties and decreased appetite (Westergren, 2009), and sarcopenia (Miller, 2008) may have an independent effect on bone metabolism.

Voluntary Weight Loss

There have been numerous studies of the effect of voluntary weight loss on bone loss across a variety of populations. In studies with mixed populations, an overall loss of total body BMD and bone mineral content (BMC) with variations in site-specific bone changes (Compston, 1992b; Andersen, 1997; Jensen 2001) has been seen. In older men participating in voluntary weight loss, there is an increase in bone loss at the hip (Ensrud, 2005). Studies of both obese (Shapses, 2001) and overweight (Riedt, 2007) premenopausal women have shown no change in bone during six months of dietary restriction with calcium supplementation. An older group of premenopausal women (ages 44-50 yrs) with a BMI >25 kg/m^2 were found to have a higher rate of BMD loss at the hip (Salamone, 1999). Another study showed a decrease in total body and spinal BMD in overweight premenopausal women with a decrease in calcium intake during weight reduction (Ramsdale, 1994). It is possible that the older average age of the group, the inclusion of normal weight women (Salamone, 1999) or the decrease in calcium intake (Ramsdale, 1994) explain the different outcomes found in these studies of similar populations. However, these data suggest that the presence of estrogen in the premenopausal population plays a role in protecting bone, and indeed hormone replacement therapy (HRT) prevents bone loss in a postmenopausal population during weight loss (Gozansky, 2005). Both obese (Ricci, 2001; Villereal, 2008) and overweight (Riedt, 2005; Chao, 2000) postmenopausal women show an

increase in bone turnover during moderate caloric restriction. Furthermore, there is a significant loss in BMD for the total body (Ricci, 2001), trochanter, and total spine (Reidt, 2005).

Bone loss has been shown to be attenuated in postmenopausal women with increased calcium supplementation (Ricci, 1998; Riedt, 2005). We found that energy restriction decreases total calcium absorption (Cifuentes, 2004), which may activate the calcium-parathyroid hormone axis to increase calcium release from bone. Also, it is possible that mechanical loading may play a role in maintenance of bone during weight loss; data suggest that in postmenopausal women aerobic exercise prevented bone loss at the hip but not total body BMD during weight loss (Ryan, 1998). In contrast, several other studies suggest that there is no association between exercise and bone during weight loss (Svendsen, 1993, Villareal, 2008). In addition, there was no change in BMC or BMD in men with weight loss and exercise (Pritchard, 1996).

It is important to note that there has been some debate about the accuracy of DXA (dual energy x-ray absorptometry) after significant weight changes. Researchers feel that some of the inconsistencies across different studies that are seen in similar populations may be due to weight changes and higher baseline body mass. Changes in bone may be skewed due to overlying fat tissue, causing measurement artifact in the bone densitomer (Van Loan, 1998; Jensen, 1994; Formica, 1995; Vestergaard, 2000; Tothill; 1998).

Weight Regain and Weight Cycling

After voluntary weight loss, many individuals fail to maintain that weight loss over the long-term. An analysis of available data showed that only ~20% of overweight individuals who intentionally reduce weight by 10% and are able to maintain that weight for

one year (Wing, 2005) while an analysis of a random telephone survey of American adults showed that ~47-49% of people who lost 10% of their weight are able to keep it off for 1 year while 25-27% are able to keep it off for 5 yrs (McGuire, 1999).

Few studies have investigated the effect of weight regain after voluntary weight loss on bone, and their results have been contradictory. Given the difficulty of studying human populations during a period of weight gain, it is important to note that in rodent studies, there is a reduction in bone quality and strength after weight loss that remains even after weight regain (Wang, 2000, Bogden 2008). One prospective human study examined weight loss over 3 months and partial regain (9 months) in obese, premenopausal women and found that there were small losses in total body, lumbar spine and femoral neck BMC after weight regain, but this study did not have a control group (Fogelholm, 2001). A heterogeneus group of premenopausal and postmenopausal women who experienced weight loss induced bone loss (Compston, 1992b) showed a recovery of total body BMD after weight regain, but the small sample size (n=8), mixed population, short time period (1 year), and lack of examination of individual bone sites make it difficult to see any changes in bone. The only weight loss study in postmenopausal women showed a decreased spinal BMD, but recovery at the femoral neck BMD after a six month weight loss diet followed by 6 months of regain (Avenell, 1994). The short duration of the entire study (1 year) for both weight loss and regain may be shorter than the period of time needed for bone to respond to body weight changes completely, making it difficult to determine whether these changes are permanent (Heaney, 2001). Nevertheless, the results are interesting and suggest that the lumbar spine may be more vulnerable to irreversible bone loss.

Often times, people who are unsuccessful at maintaining their decreased body weight will lose weight again throughout their lifetime, which has been defined as weight cycling (Gallagher KI, 2002). One study showed that older men with self-reported four or more weight loss/gain cycles from 25-50 yrs of age had an increase in forearm fractures (Sogaard, 2008). Another study of obese, sedentary, pre-menopausal women showed that neither magnitude or frequency of weight lost affected total body or femoral BMD, although these weight cyclers, as a group, did have lower femur BMD than women of similar weight and menopausal status with lower levels of weight cycling (Gallagher, 2002). One issue with these studies is that weight history is self-reported by subjects, although the literature suggests that self-reports of past body weight are highly correlated to actual weight history (Casey, 1991; Perry, 1995). With few studies on weight cycling in general, and none specifically aimed at postmenopausal women, it is difficult to make conclusions about the long-term effects of weight cycling on the risk of osteoporosis in women, although the above studies show the potential for an increased risk.

Rationale and Hypothesis

There is a public health movement to encourage both obese and overweight people to lose weight to reduce co-morbid conditions (Zamboni, 2005). In the setting of the menopausal transition, women tend to gain weight and visceral adiposity (Koskova, 2007), thereby making it desirable for this group to lose weight to improve health and appearance. Current recommendations encourage a 5-15% weight reduction to reduce the risk of co-morbid conditions (Zamboni, 2005; Hainer, 2008). At this same point in the lifecycle, bone mass is decreasing at a high rate (Finkelstein, 2007) and the literature suggests that weight loss may put even obese and overweight women at increased risk for bone loss immediately after weight loss (Ricci, 2001; Villereal, 2008; Riedt, 2005; Chao, 2000), but there is little long-term research on this matter.

Even though many women attempt to lose weight throughout their lives, they may not be successful in keeping it off (Wing, 2005). Although this is a common situation for the American public, as a whole, there are few data to determine the effects on bone. Previous studies suggest that weight loss has a negative impact on bone, although these changes may be attenuated with regain at some sites, while other sites do not recover (Fogelhholm, 2001; Avenell, 1994; Compston, 1992b). Other studies show lower bone mineral density at some sites with increased frequency of weight cycles throughout adulthood (Sogaard, AJ, 2008; Gallagher, 2002). This may put overweight and obese postmenopausal women in the tenuous position of having both a high risk for metabolic syndrome, cardiovascular disease, and non-insulin dependent diabetes in addition to the increased risk of having an osteoporotic fracture. Given the already increased rate of mortality after a frailty fracture, this relationship may lead to a very negative overall health status. Conversely, women who are able to

improve other health conditions with weight loss, may be putting themselves at increased risk for frailty fractures later in life.

It has been shown that calcium supplementation can attenuate bone loss during moderate weight loss (Ricci 1998, Reidt, 2005, Jensen 2001), but there are no studies that provide a calcium supplement while investigating weight regain. Given current data indicating the positive impact of supplementation on bone during weight loss, it is important to gain further understanding of the long-term consequences of supplementation and its ability to maintain bone after weight loss.

The objective of the present study was to examine the effect of weight regain in reduced obese and overweight women on bone and soft tissue composition after moderate weight loss compared with a group of women maintaining their reduced body weight. To our knowledge, no study of postmenopausal women has compared how bone responds to weight regain compared with a control group of women who did not regain their body weight. *It was hypothesized that ,reduced-overweight/obese women who regain weight would have greater recovery of bone mass as compared to women who maintain their lost body weight.*

Specific Aims

The aims of this thesis are to examine reduced obese/overweight postmenopausal women 1.5 years after a 6 month weight reduction program to determine:

- whether maintaining weight loss (WL-M) compared with regaining lost weight (WL-R) has a differential effect on bone mineral density and content. A secondary goal was to examine how greater initial weight loss (>10%) over six months influences bone 1.5 years later, independent of subsequent weight change.

- the impact of weight loss in women who maintained or regained weight on bone-regulating hormones including parathyroid hormone, 25-hydroxy vitamin D, cortisol, estrone, and estradiol and markers of bone turnover.

- whether changes in lean or fat tissue mass due to weight loss influence bone mass.

METHODS

Subjects: Eighty-eight postmenopausal women (BMI 25-36 kg/m^2 at baseline), who successfully completed weight loss protocols in the Shapses laboratory (Ricci, 2001; Reidt, 2005, unpublished pilot studies from 2002, 2005, and 2006). For this study, we attempted to contact subjects approximately one and a half years later by last known phone number and address. In order to be eligible for the follow-up, women had to be postmenopausal women (>3 years) and considered healthy. They were excluded if they took medications known to influence bone metabolism, or had evidence of metabolic bone disease, thyroid disorders, immune disease, heart attack or stroke in the past 6 months, kidney stones, diabetes, active cancers or cancer therapy within the past 12 months. Fifty subjects responded to telephone inquiries and ten were excluded (initiation of osteoporosis medications, n=5; declined to participate, n=5; Appendix A). In total, 40 postmenopausal women who had undergone previous moderate weight loss protocols were included in this follow-up study.

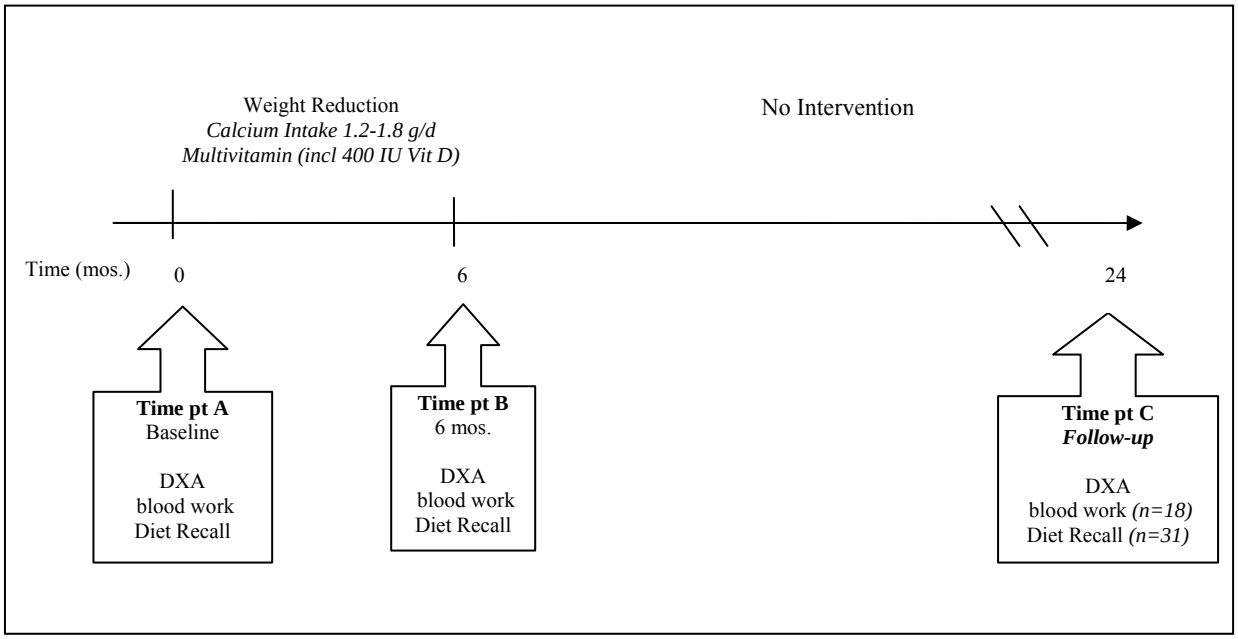


Figure 1. Timeline of Initial Study and Follow-up

Weight loss Protocol (Period A to B): All subjects underwent weight loss interventions in the Shapses laboratory that were previously reported (Ricci, 1998; Reidt, 2005) or part of unpublished pilot studies (Shapses Laboratory data, 2002, 2005, 2006). In all of these protocols, subjects were counseled to reduce usual intake by 500 kcal/d while maintaining usual physical activity levels. The subjects included in this analysis lost a mean of 9.3± 3.9% of body weight after 6 months of weight loss. During the intervention, all subjects were assigned to a calcium supplementation of either 1000 mg (High Calcium [Hi-Ca],actual intake 1494 ±313 mg C; n=16) or 200 mg (normal Ca [NL-Ca] actual intake 1057 ± 385 mg Ca n=24) (Table 2), 36 subjects were given a multivitamin with 200 mg Ca and 400 IU Vitamin D, while 4 subjects were from previous studies that did not have this supplement. Upon completion of the intervention, all subjects were counseled to consume 1.2-1.5 g Ca and at least 400 IU Vitamin D, through diet and supplementation daily.

Table 2. Calcium supplementation levels during weight loss intervention (time point A-B).

	Total (n=40)	*WL-M (n=22)*	*WL-R (n=18)*	*G-WL (n=19)*	*L-WL (n=21)*
NL-Ca (~1.1g Ca)	**24 (60%)**	**13 (60%)**	**11(61%)**	**12 (63%)**	**12(57%)**
Hi-Ca (~1.5 g Ca)	**16 (40%)**	**9(40%)**	**7(39%)**	**7 (37%)**	**9(43%)**

Normal calcium (NL-Ca), High Calcium (Hi-Ca), and No Supplement. Weight Loss Maintainers (WL-M),Weight Loss Regainers (Wl-G) Greater Weight Loss (G-WL), Less Weight Loss (L-WL), Weight Maintenance (WL-M), Weight Gain (WL-R) Time Point A to B (during weight loss intervention)

Post-Intervention Protocol (Period B to C): Subjects were contacted for a follow-up appointment approximately 2 years after baseline (range: 12-35 months). The data for this time point have not previously been reported. Subjects were excluded from follow-up if they had started osteoporosis treatments (n=5), if they had evidence of new disease states, or if

they stated they no longer consumed the recommended dose of calcium daily intake. Body weight and height were measured at baseline, after weight loss, and at follow-up. Three-day food diaries were kept at baseline, during the last month of weight loss, and at follow up in a subset of women (n=31), and analyzed by Nutritionist Pro 1.3 (First Data Bank, IN).

Fasting serum and urine specimens were taken for biochemical analysis at baseline, 6 months and at the final time point for a subset of subjects (n=18). Blood samples were centrifuged to separate serum. Both blood and urine were aliquotted into 5 ml samples and frozen. Pyridinoline (PYD, CV<8%) and deoxypyridinoline (DPD, CV<10%) were measured in urine by reverse-phase HPLC and fluorescence detection and normalized for creatinine excretion, measured from the same sample. Serum osteocalcin (OC) was measured by radioimmunoassay (RIA; BTI, Stoughton MA, USA; CV <9%). Serum N-telopeptide of type I collagen (sNTx) was measured by ELISA (Osteomark,; OSTEX International, Seattle WN, USA; CV- 4.6%). Serum 25-hydroxyvitamin D [25(OH)VitD] was measured by ^{125}I RIA (Diasorin, Stillwater MI, USA; CV <6.7%). Estrone (E_1), Estradiol (E_2), and cortisol were measured by ^{125}I RIA(DSL, Webster, TX, USA CV<9.4%, <8.9%, <8.3% respectively). Intact PTH was determined by immunoradiometric assay (DSL; CV <5.2%). BMD and BMC were measured at the spine, femoral neck, trochanter, total body, and 1/3 and ultradistal (UD) radius by DXA (DPX LUNAR/ GE LUNAR, Madison, WI, USA; CV<1%). Lean body mass, total fat mass, trunk fat and leg fat were also measured by DXA.

Weight and height were measured to nearest 0.25 kg and 0.25 cm, respectively (beam balance scale and stadiometer, Detecto, Webb City MO, USA). Subjects were categorized into the amount of weight change after the weight loss intervention to follow up (period B to

C). Weight-loss re-gainers (WL-R) were defined as those who regained weight after the weight loss intervention, (< 75% total weight loss maintained), and Weight-loss Maintainers (WL-M) were those who maintained their weight loss after the weight loss intervention (>75% total weight loss maintained). Further analyses were done by categorizing data by total amount of weight loss over the intervention period (period A to B). These groups were divided into less weight loss (L-WL) (<9.5% baseline body weight lost in six months) and greater weight loss (G-WL) (>9.5% baseline body weight lost in six months).

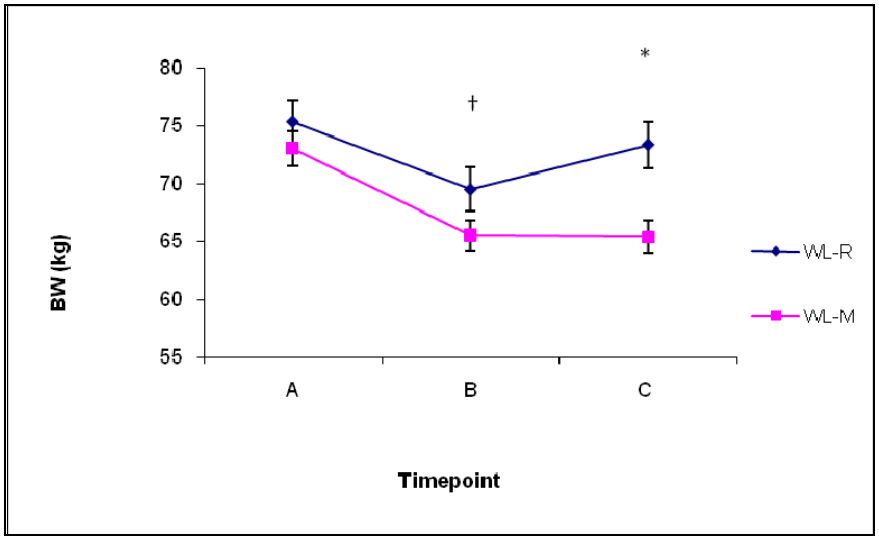


Figure 2A. Weight (kg) at baseline (time point A), the end of the weight loss intervention (time point B) and the follow-up (time point C) in the Weight Loss Maintenance (WL-M, n= 22) and Weight Loss Gain (WL-R, n=18) groups.
Data (Mean ± SEM)
*p<0.05
†p<0.09

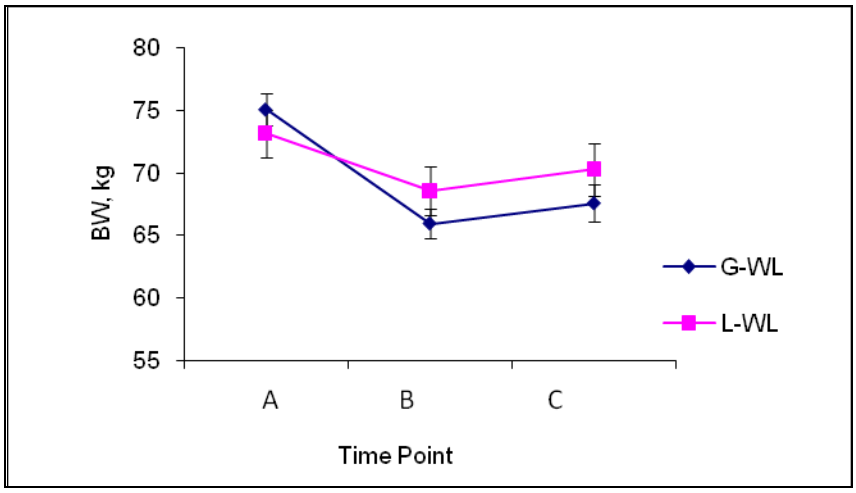


Figure 2B. Weight (kg) at baseline (time point A), the end of the weight loss intervention (time point B) and the follow-up (time point C) in the Greater Weight Loss group (G-WL, n= 19) and Less Weight Loss group (L-WL, n=21) groups.
Data (mean ± SEM)

Statistical Analysis: Values are expressed as mean ± SD. Differences in percent change from baseline in BMD, BMC, hormones, and markers of bone turnover between groups at follow up were analyzed using one-way ANOVA for 2 group assignments (either WL-M and WL-R or G-WL and L-WL, respectively). In addition, data was analyzed by one-way ANOVA to determine if initial group assignment to higher or lower calcium intake influenced bone parameters 2 years later. Paired t-tests were used to compare baseline to time point B and time point C, respectively. In addition, paired t-tests were used to compare time point B to time point C. To examine relationships between weight, lean tissue, fat tissue, bone, hormones, and markers of bone turnover, we used Pearson's product-moment correlations to assess changes between periods A to C and B to C for the entire population of 40 women. Analysis was performed with SAS statistical software (SAS Institute Inc. 9.1.3, Cary, NC).

RESULTS

Forty women were analyzed for body composition an average of 23 ± 6 months after initiation of weight loss. Five subjects from the original protocols who were contacted were excluded due to initiation of medications known to affect bone. Thirteen women who were contacted were lost to follow-up. The average weight loss for the subjects during the intervention was 8.4 ± 3.6%. At baseline, there were no significant differences in age, weight, BMI, bone, or biochemical data between groups (Table 3). When changes in bone were analyzed by level of supplementation during the intervention period (time point A to C), the only significant differences was a -4.3 ± 6.9 % change in spine BMD in the NL-Ca group compared to a -1.7 ± 4.5% loss in the Hi-Ca group ($p<0.05$). From baseline to final (time point A to C) there was a significant difference in lean body mass based on initial supplementation with a loss of 4 ± 5.8% in the NL-Ca group, and a 0.2± 4.2% loss in the Hi-Ca group.

Table 3. Baseline Characteristics of Study Participants (at time point A)

	WL-M (n=22)	*WL-R (n=18)*	*G-WL (n=19)*	*L-WL (n=21)*
Age, years	61.3 5.6	59.8 ± 5.6	59.8 ± 5.0	61.3 ± 6.1
Body Weight, kg	73.1 ± 7.1	75.3 ± 7.8	75.1 ± 5.9	73.1 ± 8.6
BMI	27.7 ± 2.6	28.9 ± 3.2	28.3 ± 2.2	28.2 ± 3.5
Lean Mass, kg	38.2 ± 5.0	38.8 ± 5.0	38.5 ± 3.7	38.5 ± 4.6
Fat Mass, kg	31.7 ± 4.8	32.7 ± 5.1	32.3 ± 4.8	31.0 ± 5.2
Calcium Intake	970 ± 417	871 ± 237	1025 ± 426	827 ± 215††
BMD (g/cm^2)				
Femoral Neck	0.86 ± 0.12	0.88 ± 0.10	0.87 ± 0.10	0.88± 0.12
Trochanter	0.73 ± 0.12	0.76 ± 0.11	0.76 ± 0.12	0.74 ± 0.11
UD-Radius	0.31 ± 0.04	0.33 ± 0.05	0.32 ± 0.05	0.31 ± 0.04
1/3 Radius	0.63 ± 0.09	0.64 ± 0.08	0.64 ± 0.09	0.63 ± 0.83
Spine	0.99 ± 0.09	1.00 ± 0.13	1.01 ± 0.09	0.98 ± 0.12
Total Body	1.12 ± 0.08	1.11 ± 0.08	1.12 ± 0.08	1.11 ± 0.08
BMC (g)				
Femoral Neck	4.40 ± 0.87	4.40 ± 0.50	4.37 ± 0.65	4.43 ± 0.78
Trochanter	8.48 ± 1.92	8.99 ± 2.06	8.86 ± 1.94	8.62 ± 2.06
UD-Radius	1.11 ± 0.17	1.15 ± 0.21	1.16 ± 0.18	1.09 ± 0.19
1/3 Radius	1.57 ± 0.22	1.57 ± 0.22	1.59 ± 0.23	1.55 ± 0.21
Total Body	2276 ± 271	2326 ± 267	2332 ± 291	2269 ± 246
Hormones				
Estradiol (pM)	54.0 ± 10.3	50.7 ± 20.9	54.0 ± 14.7	51.0 ± 16.9
Estrone (pM)	44.0 ± 20.1	55.8 ± 19.8	43.0 ± 18.4	54.1 ± 21.5
25(OH) Vitamin D (nM)	76.6 ± 22.0	79.6 ± 27.2	75.6 ± 20.7	80.1 ± 27.5
PTH (pM)	3.6 ± 1.8	4.0 ± 1.9	3.8 ± 2.4	3.5 ± 1.3
Cortisol (nM)	397.4 ± 160.1	427.8 ± 138.0	389.2 ± 121.4	438.8 ± 184.9
Markers of Bone Turonver				
PYD/creatinine (nmol/mmol)	37.9 ± 14.2	37.0 ± 14.9	43.3 ± 14.9	32.5 ± 12.1†
DPD/creatinine (nmol/mmol)	10.8 ± 5.34	12.9 ± 6.8	13.2 ± 6.6	10.5 ± 5.5
sNTx (nmol BCE)	11.6 ± 3.4	14.8 ± 5.8‡‡	13.2 ± 6.6	10.5 ± 5.5
Osteocalcin (nM)	3.3 ± 1.1	2.3 ± 1.0‡	3.0 ± 1.1	2.7 ± 1.3

Data (mean ± SD)
‡Significant difference from WL-M group ($p<0.01$); ‡‡ ($p<0.07$)
†Significant difference from G-WL group ($P<0.05$); †† ($P<0.08$)
BMD (bone mineral density), UD (ultradistal), BMC (bone mineral content)
WL-M(Weight Loss Maintenance) WL-R (Weight Loss Regain), G-WL (Greater Weight Loss), L-WL (Less Weight Loss)

Weight Loss Maintenance vs. Weight Loss Regain

After the six month weight loss protocol (period A to B), the WL-R group lost significantly less body weight (7.7 ± 3.7%; $p<0.03$) compared to the WL-M group (10.3 ± 3.2%), resulting in trends toward differences in body weight ($p<0.09$), BMI ($p<0.06$) and fat mass ($p<0.07$) (Table 4). The WL-M group lost an average of 0.1 ± 2.0 kg post-intervention while the WL-R group gained 3.8 ± 2.3 kg during that time ($p=0.0001$; Figure 2). Both BMI ($P<0.0001$) and fat mass ($p<0.01$) were significantly different between the groups by the final time point. The groups had similar intakes of Ca during the weight loss study period (time point A to B;~1.2 g/d), although individuals within each group were assigned to different levels of supplementation (WL-M group- 13 NL-Ca, 9 Hi-Ca,; WL-R group- 11 NL-Ca,7 Hi-Ca; Table 2). In the subset of subjects who had dietary recalls at the follow-up (WL-M n= 18; WL-R n= 13), the calcium intake, including both diet and supplements, was not significantly different, with intakes of 1286 ± 521 mg and 1148 ± 550 mg, respectively.

Within each group, the values at the end of the weight loss intervention (time point B) and the values at the follow-up (time point C) were compared to baseline values. After 6 months of weight loss, BMD differed significantly from baseline at the trochanter ($p< 0.001$), 1/3 radius ($p< 0.02$), spine ($p < 0.02$), and total body ($p< 0.02$), and a trend towards difference at the ultra distal radius ($p < 0.07$) in the WL-M group. BMD also decreased significantly at the trochanter ($p< 0.02$), and showed a trend towards a decrease at the spine ($p <0.06$) in the WL-R group. At the follow-up (time point C), BMD decreased significantly from baseline at the femoral neck ($p<0.001$), trochanter ($p < 0.001$), 1/3 radius ($p < 0.0001$), and total body ($p < 0.02$) in the WL-M group. In the WL-R group, BMD decreased at the

femoral neck ($p < 0.01$), spine ($p < 0.05$) and a trend towards decrease at the 1/3 radius ($p < 0.10$).

The values at the end of weight loss (time point B) and the follow-up (time point C) were also compared to one another. In the WL-M group, there was a decrease in BMD at the femoral neck ($p < 0.01$), the trochanter ($p < 0.01$), and the spine ($p < 0.05$). In the WL-R group, the BMD of the femoral neck ($p < 0.01$) was less and there was a trend towards decrease at the ultra distal radius ($p = 0.07$).

While there were no significant differences in bone composition between the groups at baseline, there was a greater loss of BMD and BMC at the UD radius and 1/3 radius ($p < 0.05$) in the weight maintenance group as well as a trend towards greater loss in total body BMD ($p = 0.06$) at the completion of the weight loss protocol (time point A to B; Figure 3A). During the follow-up period (time point B to C), the only differences seen in the rate of bone change between the groups was that the WL-M group had greater losses at the trochanter ($p < 0.03$; Figure 3B) Over the entire study period (time point A to C), the WL-M group had greater losses at the trochanter BMD ($P < 0.05$), total body BMD ($p < 0.05$), and 1/3 Radius BMD ($p < 0.01$) and BMC ($p < 0.05$; Table 6, Figure 3C).

Table 4A. Percent Change in Body Composition of Study Participants in the WL-M and WL-R groups

	WL-M (n=22)			*WL-R (n=18)*		
Time point	*A to B*	*B to C*	*A to C*	*A to B*	*B to C*	*A to C*
Duration (mo.)	6.0 ± 0	16 ± 7	23 ± 7	6.0 ± 0	15.1 ± 7	22 ± 6
Body Weight	-10.2 ± 3.2	-0.1 ± 2.9	-10.4 ± 4.3	-7.7 ± 3.7‡	5.6 ± 3.5‡	-2.6 ± 3.5‡
Lean Mass	-3.3 ± 4.0	-0.5 ± 4.7	-3.8 ± 5.3.	-2.8 ± 5.8	1.9 ± 8.8	-1.3 ± 6.1
Fat Mass	-18.2 ± 8.7	1.9 ± 7.2	-16.6 ± 10.5	-13.1 ± 6.8‡	9.8 ± 6.9‡	-4.8 ± 6.4‡
Trunk Fat	-18.2 ± 14	-1.0 ±13.9	-19.1 ± 17.4	-12.8 ± 10.9	11.1± 16.3‡‡	-4.1 ± 12.3‡
Leg Fat	-13.9 ± 11.1	-2.4 ± 15.9	-16.9 ± 13.0	-11.7 ± 7.4‡	-10. 9±9.4	-2.4 ± 9.7
Total Body BMD	-1.0±1.8	-0.5 ± 2.1	-1.5 ±1.7	0.0± 1.6‡‡	-0.4 ± 1.8	-0.3±1.8‡

Data (mean ± SD)
‡Differs from WL-M group ($p < 0.01$); ‡‡ ($p<0.07$)

Definitions: Time point A to B (during weight loss intervention); Time point A to C (baseline to final); WL-M(Weight Loss Maintenance) WL-R (Weight Loss Regain), G-WL (Greater Weight Loss), L-WL (Less Weight Loss

Table 4B. Percent Change in Body Composition of Study Participants in the G-WL and LWL groups

	G-WL (n=19)			*L-WL (n=21)*		
Time point	*A to B*	*B to C*	*A to C*	*A to B*	*B to C*	*A to C*
Duration (mo.)	6.0 ± 0	15 ± 6	22 ± 6	6.0 ± 0	16 ± 7	23 ± 7
Body Weight	-12.2 ± 2.2	2.4 ± 4.6	-10.0 ± 4.7	-6.3 ± 2.1†	2.5 ± 5.0	-4.0 ± 4.7†
Lean Mass	-4.7 ± 5.7	0.7 ± 9.3	-4.4 ± 6.5	-1.7 ± 3.6†	0.5 ± 4.1	-1.2 ± 4.8††
Fat Mass	-20.7 ± 8.5	5.5 ± 8.4	-16.2 ± 11.8	-11.5 ± 5.2†	5.4 ± 7.8	-6.8 ± 7.2†
Trunk Fat	-21.5 ± 11.7	3.5 ± 17.2	-18.9 ± 16.8	-10.4 ± 11.6†	5.8 ± 15.3	-5.9 ± 14.6†
Leg Fat	-17.2 ± 10.2	2.3 ± 17.7	-15.9 ± 16.4	-9.0 ± 7.0†	5.3 ± 11.7	-4.7 ± 7.4†
Total Body BMD	-1.0±1.7	-0.9 ± 1.6	-2.0 ± 1.6	-0.1 ± 1.7	0.02 ± 2.1	-0.1 ± 1.6†

Data (mean ± SD)
‡Differs from WL-M group ($p < 0.01$); ‡‡ ($p<0.06$)
†Differs from G-WL group ($p < 0.05$); ††

Definitions: Time point A to B (during weight loss intervention); Time point A to C (baseline to final); G-WL(Greater Weight Loss) LWL (Less Weight Loss)

Table 5A. Percent Change in Bone Measurements of Study Participants in WL-M and WL-R groups.

	WL-M (n=22)			*WL-R (n=18)*		
Time point	*A to B*	*B to C*	*A to C*	*A to B*	*B to C*	*A to C*
BMD (g/cm^2)						
Femoral Neck	-0.5 ± 3.3	-2.3 ± 3.8	-1.5 ± 2.8	0.1 ± 3.3	-2.1 ± 2.7	-2.1 ± 3.5
Trochanter	-4.1 ± 4.6	-2.8 ± 3.8	-6.8 ± 6.0	-3.1 ± 4.8	0.8 ± 6.0‡	-2.2 ± 6.8‡
UD-Radius	-1.9 ± 4.5	0.6 ± 7.4	-1.6 ± 5.6	1.3 ± 4.0‡	-2.3 ± 5.0	-1.2 ± 4.2
1/3 Radius	-2.5 ± 4.4	-1.9 ± 5.9	-4.5 ± 3.5	-0.1 ± 2.0‡	-0.9 ± 3.4	-0.1 ± 2.0‡
Spine	-3.6 ± 6.3	2.6 ± 6.1	-1.3 ± 7.2	-2.9 ± 5.8	0.7 ± 7.5	-3.7 ± 7.2
Total Body	-1.0 ± 1.8	-0.5 ± 2.1	-1.5 ± 1.7	0.0 ± 1.6‡‡	-0.4 ± 1.8	0.3 ± 1.8‡
BMC (g)						
Femoral Neck	-1.3 ± 6.6	0.3 ± 8.0	-1.4 ± 5.5	1.7 ± 5.8	-3.0 ± 4.8	-1.4 ± 6.1
Trochanter	-2.3 ± 9.3	-3.6 ± 7.8	-6.0 ± 9.5	-4.8 ± 8.5	1.1 ± 11.8	-3.9 ± 10.7
UD-Radius	-1.3 ± 9.5	-1.9 ± 5.5	-4.7 ± 13.0	3.2 ± 13.6	-0.9 ± 3.4	-10.7 ± 31.6
1/3 Radius	-2.8 ± 2.8	-1.3 ± 4.5	-4.1 ± 3.3	-0.6 ± 2.9 ‡‡	-1.0 ± 3.3	-1.6 ± 3.3‡
Total Body	-1.6 ± 5.3	-1.0 ± 4.6	-2.7 ± 5.1	-0.8 ± 3.7	-1.6 ± 4.4	-1.6 ±4.4

Data (mean ± SD)
Compared to the same time period: ‡Differs from WL-R group ($p < 0.05$) ‡ ‡ $p<0.08$
Definitions: Time point A to B (during weight loss intervention); Time point A to C (baseline to final), BMD (bone mineral density), UD (ultradistal), BMC (bone mineral content),. WL-M (Weight Loss Maintainers), WL-R (weight loss regainers)

Table 5B. Percent Change in Bone Measurements of Study Participants in G-WL and L-WL groups.

	G-WL (n= 19)			*L-WL (n= 21)*		
Time point	*A to B*	*B to C*	*A to C*	*A to B*	*B to C*	*A to C*
BMD (g/cm^2)						
Femoral Neck	0.7 ± 3.2	-3.4 ±3.0	-2.7 ± 3.9	1.1 ± 3.1 ††	-2.3 ± 4.2†	-2.3± 4.2
Trochanter	-4.2 ± 4.9	-2.8 ± 4.8	-6.9 ± 6.3	-3.1 ± 4.5	0.5 ± 5.2††	-2.5 ± 6.6†
UD-Radius	-1.6 ± 5.1	-0.6 ± 6.7	-2.4 ± 4.9	0.4 ± 3.8	-0.6 ± 6.8	-0.4 ± 5.0††
1/3 Radius	-2.7 ± 4.6	-1.8 ± 5.9	-4.7 ± 3.7	-0.3 ± 2.3 ††	-1.1 ± 3.9	-1.4 ± 2.9†
Spine	-4.5 ± 6.3	0.1 ± 6.4	-4.6 ± 5.8	-2.2 ± 5.7	2.0 ± 7.3	-0.3 ± 7.9††
Total Body	-1.0 ± 1.7	-0.9 ± 1.6	-2.0 ± 1.6	-0.1 ± 1.7	0.02 ± 2.1††	-0.1 ± 1.6†
BMC (g)						
Femoral Neck	0.7 ± 5.9	-2.6 ± 6.9	-2.2 ± 5.6	-0.5 ± 5.8	-0.1 ± 6.7	-0.7 ± 5.9
Trochanter	-1.9 ± 10.8	-2.6 ± 11.3	-5.2 ± 8.8	-4.8 ± 6.8	-0.3 ± 8.8	-4.9 ± 11.2
UD-Radius	-0.8 ± 10.4	-4.5 ± 17.9	-6.5 ± 12.9	1.8 ± 12.5	-7.9 ± 32.7	-7.8 ± 29.4
1/3 Radius	-3.0 ± 2.5	-3.0 ± 2.5	-4.7 ± 3.1	-0.7 ± 3.0 †	-0.7 ± 3.0	-1.5 ± 3.2†
Total Body	-2.7 ± 5.4	-0.4 ± 6.2	-3.2 ± 5.8	0.0 ± 3.4 ††	-1.2 ± 3.3	-1.2 ± 3.4

Data (mean ± SD)
Compared to the same time period: †Differs from G-WL group ($p < 0.05$); †† ($p \leq 0.08$)
Definitions: Time point A to B (during weight loss intervention); Time point A to C (baseline to final), BMD (bone mineral density), UD (ultradistal), BMC (bone mineral content) . GWL (greater weight loss), L-WL (less weight loss)

Figures 3 A-C. The Percent (%) Change in Bone Mineral Density (BMD) in the Weight Maintenance (WL-M, n= 22) and Weight Regain (WL-R, n=18) groups at the femoral neck, trochanter, ultradistal (UD) radius, 1/3 radius, spine, and total body.

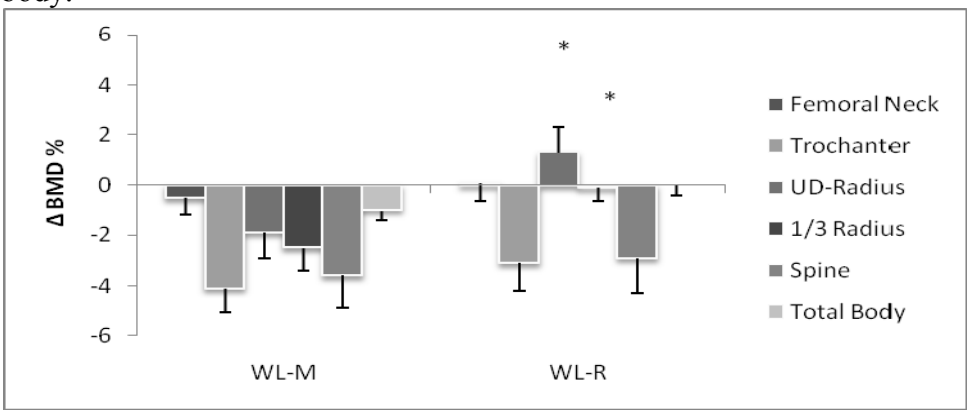


Figure 3A. BMD (% change) from time point A to B (weight-loss intervention)
Values are mean ± SEM. * Differs from WL-M group, P<0.05

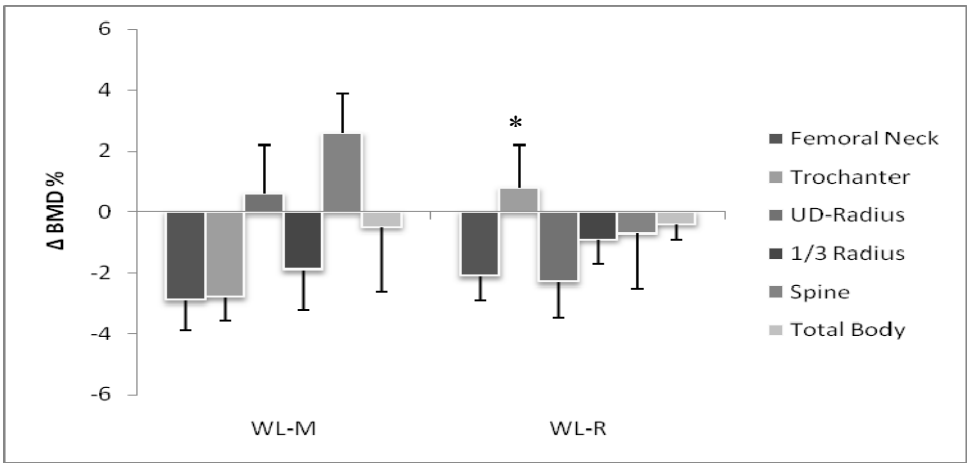


Figure 3 B. BMD (% change) from time points B to C (follow-up)
Values are mean ± SEM. * Differs from WL-M group, P<0.05

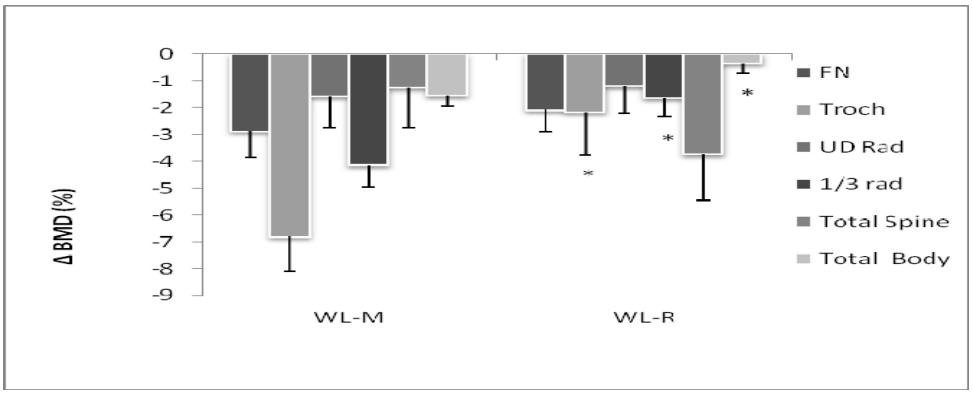


Figure 3C. BMD (% change) from time points A to C (baseline to follow up)
Values are mean ± SEM. * Differs from WL-M group, P<0.05

A subset of subjects had blood drawn at all three time points (WL-M, n= 10; WL-R, n=8). Over the 2 year period, there was a trend ($p<0.09$) for cortisol concentrations to increase by 49.5 ± 46.1% in the WL-M group compared to the WL-R group (1.6 ± 36%) and for osteocalcin concentrations to decrease by -48.1 ± 12.8% in the WL-M group compared with -10.6 ± 61.9% decrease in the WL-R group ($p< 0.08$) (Table 6).

Table 6. Percent Change of Hormone and Bone Marker Measurements from Baseline to Final

	WL-M (n=10)	*WL-R (n=8)*	*G-WL (n= 9)*	*L-WL (n=9)*
Hormones				
Estradiol	-0.49± 22.2	-7.4 ± 30.1	0.7 ± 24.7	6.5 ± 27.3
Estrone	56.2 ± 52.8	63.2 ± 36.8	59.2 ± 48.0	58.2 ± 47.5
25(OH)D	41.5 ± 44.1	29.1 ± 56.2	27.3 ± 50.0	46.9 ± 48.0
PTH	69.3 ± 111.0	29.9± 114.3	38.4 ± 122.3	35.0 ± 112.2
Cortisol	49.5 ± 46.1	1.6 ± 36.0‡	45.4 ± 44.7	6.4 ± 44.7
Bone Markers				
Pyd/creatinine	-49.7 ± 31.2	-33.0 ± 38.4	-36.3 ± 40.2	-49.7 ± 26.6
Dpd/creatinine	-31.2 ± 58.0	-30.52 ± 3.7	-15.9 ± 56.0	-52.3 ± 23.8
Serum NTX	87.8 ± 63.2	27.5 ± 100.9	90.6 ± 63.4	32.6 ± 95.4
OC	-48.1 ± 2.8	-10.6 ± 2.0†	-37.2 ± 55.1	-31.9 ± 34.9

Data (mean ± SD)
‡Trend towards difference from WL-M group ($p < 0.1$) † Trend towards difference from WL-M group ($p <0.08$)
WL-M(Weight Loss Maintenance) WL-R (Weight Loss Regain), G-WL (Greater Weight Loss), L-WL (Less Weight Loss)

Less Weight Loss vs. Greater Weight Loss during the first 6 months

At baseline, there were no significant differences in age, body weight, or BMI between the L-WL and G-WL groups. During the weight loss protocol, the G-WL group lost 12.2 ± 2.2% total body weight during the intervention while the L-WL group lost 6.35 ± 2.2%. Overall, the G-WL group lost 10.0 ± 4.7 % of total body weight while the L-WL group lost 4.0 ± 4.7 % during the entire study period (A to C), resulting in significant differences in body weight ($p<0.001$), fat mass ($p<0.001$), and a trend towards a difference in

lean mass ($p<0.1$) at time point C. At baseline, when all subjects did three day food diaries, there was a significant difference in Ca intake with the G-WL group consuming 1025 ± 426 mg/day while the L-WL group had a mean intake of 827 ± 215 mg/day. However, reported calcium intake was not significantly different at the completion of the weight loss protocol (time point B; G-WL 1288 ± 409; L-WL 1204 ± 425 mg), although individuals within each groups were assigned to different levels of supplementation (G-WL group: 12 NL-Ca, 7 Hi-Ca; L-WL group: 12 NL-Ca, 9 Hi-Ca; Table 2) and at time point C (G-WL 1254 ± 497, L-WL 1196 ± 583 mg, respectively).

The G-WL group had greater losses at the 1/3 radius in BMD ($p < 0.05$) and BMC ($p < 0.02$), and in total body BMC ($p < 0.02$), and a trend at the FN BMD ($p < 0.08$) during weight loss (time point A to B). Over the entire study period (time point B to C), there was greater bone loss in the G-WL group at the 1/3 radius BMD ($p <0.01$) and BMC ($p=0.001$), total body BMD ($p < 0.01$), and trochanter ($p<0.05$) with a trend towards loss at the spine BMD ($p = 0.06$; Table 5). Of the subset of women who had blood drawn at each time point (G-WL n=9; L-WL n= 9), biochemical analysis revealed that there were no significant differences between the groups in any of the biochemical markers of bone turnover or in any of the hormones tested (Table 6).

Correlations

Correlations were used to determine if relationships existed between the changes in BMD and the changes in soft tissue (lean body mass, total fat, leg fat, and trunk fat) as well as the interactions between the changes in BMD and the changes in hormones or markers of bone turnover. Over the entire study period, there was a positive correlation between the percent change in body weight with the percent change in trochanter ($r=0.440$, $p<0.01$), 1/3

radius (r=0.047, p<0.01), and total body BMD (r=0.367, p<0.02). The percent change in total fat mass was correlated with 1/3 radius BMD (r=0.41, p<0.02) and total body BMD (r=0.519, p<0.001). The change in trunk fat was positively correlated with total body BMD changes (r=0.463, p<0.01) and leg fat correlated with trochanter BMD (r=0.422, p<0.01), 1/3 radius BMD (r=0.63, p<0.001), and total body BMD (r=0.429, p<0.01). In addition, there was a positive relationship between lean mass change and trochanter BMD change (r=0.39, p<0.02).

Table 7. Correlations of the relationship between the percent change in various bone sites and weight (wt), lean body mass (LBM), fat mass (FM), truck fat, Leg fat, estradiol, estrone, vitamin D3, parathyroid hormone (PTH), and cortisol from baseline to final.

		FN	**Troch**	**UD Radius**	**1/3 Radius**	**SPINE**	**Total Body**
Weight	*r*	NS	0.440	NS	0.470	NS	0.367
	p-value		<0.01		<0.05		<0.02
LBM	*r*	NS	0.390	NS	NS	NS	NS
	p-value		<0.02				
FM	*r*	NS	NS	NS	0.410	NS	0.518
	p-value				<0.02		<0.001
Trunk Fat	*r*	NS	NS	NS	NS	NS	0.463
	p-value						<0.01
Leg Fat	*r*	NS	0.422	NS	0.630	NS	0.429
	p-value		<0.01		<0.001		<0.01
Estradiol	*r*	NS	NS	NS	NS	NS	NS
	p-value						
Estrone	*r*	NS	NS	NS	NS	NS	NS
	p-value						
25(OH)D	*r*	NS	NS	NS	NS	NS	NS
	p-value						
PTH	*r*	NS	NS	NS	NS	NS	NS
	p-value						
Cortisol	*r*	NS	NS	-0.550	NS	NS	0.693
	p-value			<0.08			p<0.02

Non-significant (NS; p>0.1)
LBM (Lean Body Mass), FM (fat Mass), 25(OH)D (25-hydroxy Vitamin D), PTH (parathyroid hormone)

Hormonal data correlations over the entire study period showed a negative correlation between cortisol changes and total body BMD changes (r=-0.693, p<0.02) and a trend towards significance at the ultradistal radius (r=-0.55, p=0.08; Table 7). During period B to

C, estradiol and 25OHD showed a positive correlation with the UD radius BMD ($r>0.52$; $p< 0.05$) and trend with total body BMD ($r= 0.045$; $p> 0.08$).

Annualized Bone Loss

Annualized bone mineral density loss was determined for all four groups at each site by dividing the percent change in BMD by the number of months during the entire study period (time point A to C) to determine the monthly loss and then multiplying by 12 months. There was a greater ($P <0.05$) annual total body BMD loss in the WL-M (-1.0 ± 1.8%) compared with the WL-R group (-0.2 ± 1.6%), and a trend towards greater annual troch BMD loss ($p<0.06$) in the G-WL group (-4.1 ± 3.7%) compared with the L-WL group (-1.6 ± 4.0%).

Table 8. Percent Annual Change of Bone Mineral Density compared to a Reference Range of Weight Stable Postmenopausal Women.

	WL-M (n= 22)	*WL-R (n=18)*	*G-WL (n=19)*	*L-WL (n=21)*	*Literature Range : Weight Stable Women*
FN	-1.5 ± 2.8	-1.4 ± 2.0	-1.5 ± 2.4	-1.4 ± 2.5	-0.91 to -0.36 [1-8]
Trochanter	-3.7 ± 3.6	-1.7 ± 4.3	-4.1 ± 3.7	-1.6 ± 4.0††	-0.45 to 0.41 [2,5,6]
UD Radius	-1.0 ± 3.1	-0.6 ± 2.1	-1.4 ± 2.8	-0.3 ± 2.6	- 0.77 to -0.14 [1, 8]
Spine	-1.0 ± 3.5	-2.1 ± 4.6	-2.7 ± 3.2	-0.4 ± 4.4††	-1.32 [2]
Total Body	-1.0 ± 1.8	-0.2 ± 1.6‡	-1.1 ± 1.0	-0.1 ± 0.8†	-0.88 [2]

Data (mean ± SD)
‡Differs from WL-M group ($p < 0.02$)
†Differs from G-WL group ($P < 0.001$) ††Trend towards difference from G-WL group ($P<0.07$)
BMD (bone mineral density), UD (ultradistal), BMC (bone mineral content)
WL-M(Weight Loss Maintenance) WL-R (Weight Loss Regain), G-WL (Greater Weight Loss), L-WL (Less Weight Loss)
Literature Range References: [1]Makovey, 2008; [2]Park, 2007; [3]Sirola, 2003; [4] Nguyen, 1998; [5]Guthrie, 1998, [6]Young 1996; [7]Pouilles, 1995; [8]Harris, 1992

The annual bone loss in this study is compared to data found in the literature for populations of similar sex, age, and menopausal status at specific sites (Table 8, Figure 4), showing an increased rate in bone loss at the femoral neck and trochanter in all groups compared to weight stable women. The rates of change at the ultra-distal radius appear greater than weight stable women in the WL-M and G-WL group, but both the WL-R and L-

WL groups appear similar to weight stable women. At the spine, the rates of loss are greater in the WL-M and the L-WL groups are lower than weight stable women while the WL-R and G-WL groups are higher. The rates of loss of total body BMD in all groups are similar to weight stable women.

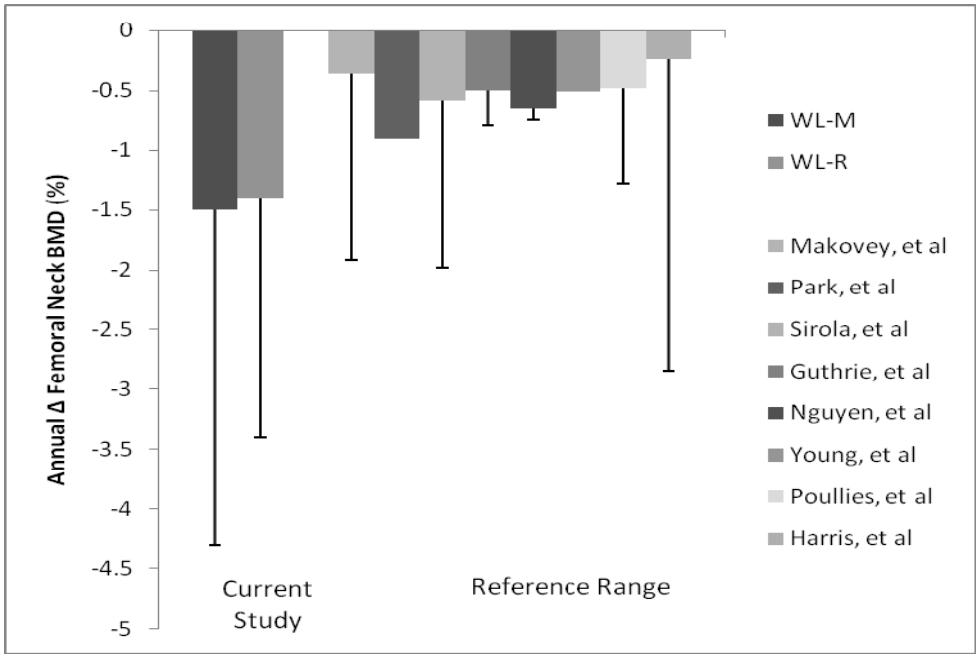


Figure 4. Annual Percent Change of Bone Mineral Density) compared to the Reference Range Weight Stable Postmenopausal Women at the Femoral Neck.
Data (Mean – SD)
WL-M (Weight Loss Maintenance) WL-R (Weight Loss Regain), G-WL (Greater Weight Loss), L-WL (Less Weight Loss)
*no SD reported

DISCUSSION

The results of the present study are twofold: overweight and obese women who maintain their weight loss over approximately one and a half years have greater bone loss at the troch, 1/3 radius, and total body compared to those who regain their weight; women who lose approximately 10% of initial body weight or greater will have greater bone mineral density losses both immediately after weight loss and after another year and a half of weight maintenance. Previous studies from this laboratory have shown bone loss at some sites after

six months of weight loss (Ricci, 2001; Reidt 2005). This study was designed to determine whether these losses remain a year and a half after the completion of weight loss. The rationale for examining the data in two separate and subtly different analyses was to explore the longer-term implications of weight reduction on bone in both subjects who were able to maintain the weight they lost, independent of quantity, for 16 months after the intervention ended as well as those who lost at least approximately ten percent of their weight during the intervention, which is thought to be enough to reduce risk of many chronic diseases. Few previous studies have addressed the influence of both weight loss and weight regain on bone and the results of these studies have been contradictory (Avenell, 1994; Compston, 1992; Fogelholm 2001). To our knowledge, there are no studies of obese/overweight postmenopausal women comparing a group that maintained their weight loss with a group that regained the weight, thereby examining the reversibility of the bone loss with weight loss. Furthermore, few studies have examined a group ~1 1/2 years after the initial period of weight reduction. These data indicate that the effect of weight loss on bone remains a significant factor, despite decreases in rate of weight loss and maintaining Ca intake, over time, but weight regain partially attenuates these losses at some sites.

The maintenance of weight loss seen in this cohort during the post-intervention period show that 55% of eligible subjects maintained the weight they lost during the first portion of the study. The literature differs on the prevalence of weight loss maintenance of 10 % weight loss over one year ranging from 20-49% (Wing, 2005; McGuire, 1999). There are several possible reasons for this higher than average success rate. It may be that those subjects who responded well to the structure and time-intensity of this particular weight loss protocol were better able to continue with the diet principles on their own, as evidenced by

the higher initial weight loss in the WL-M group than the WL-R group. Another possibility is that there may have been a self-selection bias towards those who remained successful (i.e. those who regained the weight did not respond to requests for follow-ups).

Although groups in this study were determined based on body weight outcomes, there is a relatively even distribution of both levels of calcium supplementation in all weight loss groups. The differences in calcium supplementation could be seen as a confounding variable with regard to changes in bone. However, it is important to note that the final measurements were done an average of 16 ± 7 months after this supplementation was stopped, and thereafter, the recommended calcium intake for all subjects was 1200 mg/day or greater. Estimated intake showed that each group was compliant with this suggestion with similar intakes (WL-M,1286 ± 521 mg; WL-R 1148 ± 550 mg; G-WL,1254 ± 497, L-WL 1196 ± 583 mg, respectively). Additionally, with the exception of lean body mass, there were no differences in bone or body composition parameters (from time point A to C) between the normal and high calcium groups. While there is research to suggest that calcium attenuates fat accumulation (Zemel, 2005), no differences were seen between supplementation groups with regard to changes in fat mass. There is little, if any, research to support an effect of calcium supplementation on changes in lean body mass.

The hip showed a number of changes throughout the study period in both the WL-M and WL-G groups. Despite the significant difference in the percent weight lost during the intervention (period A to B) between the groups, there was no significant difference in the loss of trochanter BMD. Yet, during the entire study period (A to C), the WL-M group had significantly greater losses of BMD at the trochanter than the WL-G group, showing that these changes were not based solely on initial weight loss, but also by the subsequent

changes in weight during the follow-up period. The WL-M group had losses in trochanter BMD (4%) compared to baseline after 10% weight loss, and further losses after a year and a half weight maintenance (7% trochanter loss). In contrast, the WL-R group who lost 8% of their body weight at the end of the 6 month intervention showed a significant loss of BMD at the trochanter (3% loss), but after weight regain during the follow-up period, there was no difference from baseline. This demonstrates that the trochanter is particularly sensitive to weight changes, both positive and negative, possibly due to the high trabecular content, as hypothesized by Riedt, et al (2005). This study highlights that these losses continue at a greater rate than weight stable women, even after the weight stabilizes. Hence, reduced obese women who maintain their lost weight may ultimately show greater risk for hip fracture, especially considering that both groups had significantly less femoral neck BMD 2 years after the start of the weight loss intervention.

The decreases at the 1/3 radius seen during this study may be the result of slower response to acute weight changes at this site, continuing well after the rate of weight loss decreases. There were differences in the rate of change at the 1/3 radius after weight loss, indicating that the greater rate of bone loss in the WL-M group at the forearm is based more on the greater overall weight loss in that group compared to the WL-R group. By the end of the study, the BMD at the 1/3 radius was significantly less than baseline in the WL-M group and only a trend towards significance in the WL-G group, so it is difficult to determine whether this site recovers with weight regain. Epidemiological studies, however, have shown an increased risk of forearm fracture with increased weight cycles in older men (Sogaard, 2008) and weight loss as a negative predictor of BMD at the forearm in postmenopausal women, although weight gain had no effect (Forsmo, 2006).

The total body BMD was lost at a greater rate in the WL-M group and showed a significant loss after 2 years, similar to findings after 6 months of weight loss (Ricci, 2001), which may be reflective of losses at the specific sites, as discussed above. Interestingly, regaining weight (WL-R group) showed no significant loss of total body BMD after 2 years, suggesting that there was recovery with weight regain, similar to the findings of Compston (1992).

In subjects who lost greater than 10% of their body weight during the intervention, there was a significantly greater losses in BMD at the trochanter, 1/3 radius, and total body BMD as well as 1/3 radius BMC one and a half years after weight loss. Additionally, there was a trend towards greater loss at the spine loss in the G-WL group, similar to Riedt, 2005. Since there were no differences between the WL-M and WL-R groups, it can be concluded that this was a function of greater overall weight loss, possibly related to the high trabecular content of this site. While this is of concern due to higher risk of spinal fractures, it differs from the bone loss at the trochanter in that the losses are not significantly different in the time period after weight loss between the groups, suggesting that spinal bone loss stabilizes after the completion of weight loss.

There was a trend towards decreases in osteocalcin, a marker of bone formation, and increases in cortisol, which may inhibit bone formation, in the WL-M group compared with the WL-R group. These data support the hypothesis that there is a continued alteration in bone formation well after weight stabilizes. There were no other differences in biochemical data between groups based on weight maintenance or total weight loss. One might expect to see differences between the groups in estradiol levels, given that one potential mechanism for the negative effect of weight loss on bone is a decrease in estrogen production from adipose

tissue, since fat mass differed significantly between groups. However, the lack of differences between groups may be more indicative of the small sample size, since only a subset of subjects had blood drawn at all three time points. Furthermore, determination of calcium and vitamin D intake from time points B to C were based entirely on subject's self reports of supplement use and a three-day food diary. Variations in some bone-regulating hormone concentrations may be attributable to a smaller degree of compliance with these supplements during the follow-up period.

The relationship between weight, soft tissue, and bone is not well understood. In this study, total fat mass, leg fat, and trunk fat were used to determine if there are any differences in the relationship between different regions of fat and bone. Leg fat is considered subcutaneous fat while trunk fat contains both visceral and subcutaneous adipose tissue. Given that the subcutaneous fat in both regions may be expected to similar biochemical activities, any differences in relationships found between bone sites and these distinct regions may be explained by the visceral fat content in the trunk (Kuwahata, 2008). There was a positive correlation between change in body weight with the change in trochanter, 1/3 radius, and total body BMD, which is well defined in the literature (Finkelstein, 2007, Zhai 2008, Sirola J, 2003), although the reason for this relationship is debated. The positive relationship between the change in leg fat and changes in trochanter, as well as the association between increased lean body mass and trochanter BMD support the idea that weight provides mechanical loading to strengthen bone. Yet, the change in total fat mass was positively associated with the changes in non-weight bearing radius and total body BMD, but not the weight-bearing trochanter BMD, suggesting that the mechanical loading of fat mass is not the sole reason for this relationship. Sherk et al found similar results in a

postmenopausal population, concluding that lower body strength had a greater influence on hip BMD than fat mass (2009). Additionally, changes in leg fat had a positive association with 1/3 radius, supporting the hypothesis that there is also a biochemical regulatory pathway between bone and adipose. Most likely, there is complex mechanism involving numerous inter-related central and adipocyte- produced hormones involved in bone changes during weight change (Reid, 2008; Zhao 2007), although whether this has an overall positive or negative effect remains debatable. The current study demonstrates an apparently positive relationship between fat mass and bone, although it should be noted that there were no associations seen between trunk fat and regional bone sites. This may indicate that the positive effects of fat on bone excludes visceral fat. Although only a few hormones were measured in the present study, there was a negative correlation between the change in serum cortisol concentrations, which may be derived from visceral fat (Mattson, 2007), and total body BMD. Further studies using magnetic resonance imaging or computed tomography will be needed to elucidate the exact nature of regional adiposity and the biochemical link with bone.

When the bone change is annualized, comparisons can be made to weight stable subjects of similar menopausal status from previous studies to demonstrate the overall impact of weight loss over time. While there were significantly greater losses in the total body in both the WL-M and G-WL groups compared to the WL-R and L-WL group respectively, each of the groups in this analysis had a greater rate of loss than that reflected in the literature at the trochanter. Both the G-WL and WL-M groups have higher rates of annual bone loss at most sites (except the spine in the WL-M group) when compared to the literature, thereby

confirming the hypothesis that both weight bearing and non-weight bearing sites are affected by weight loss over time.

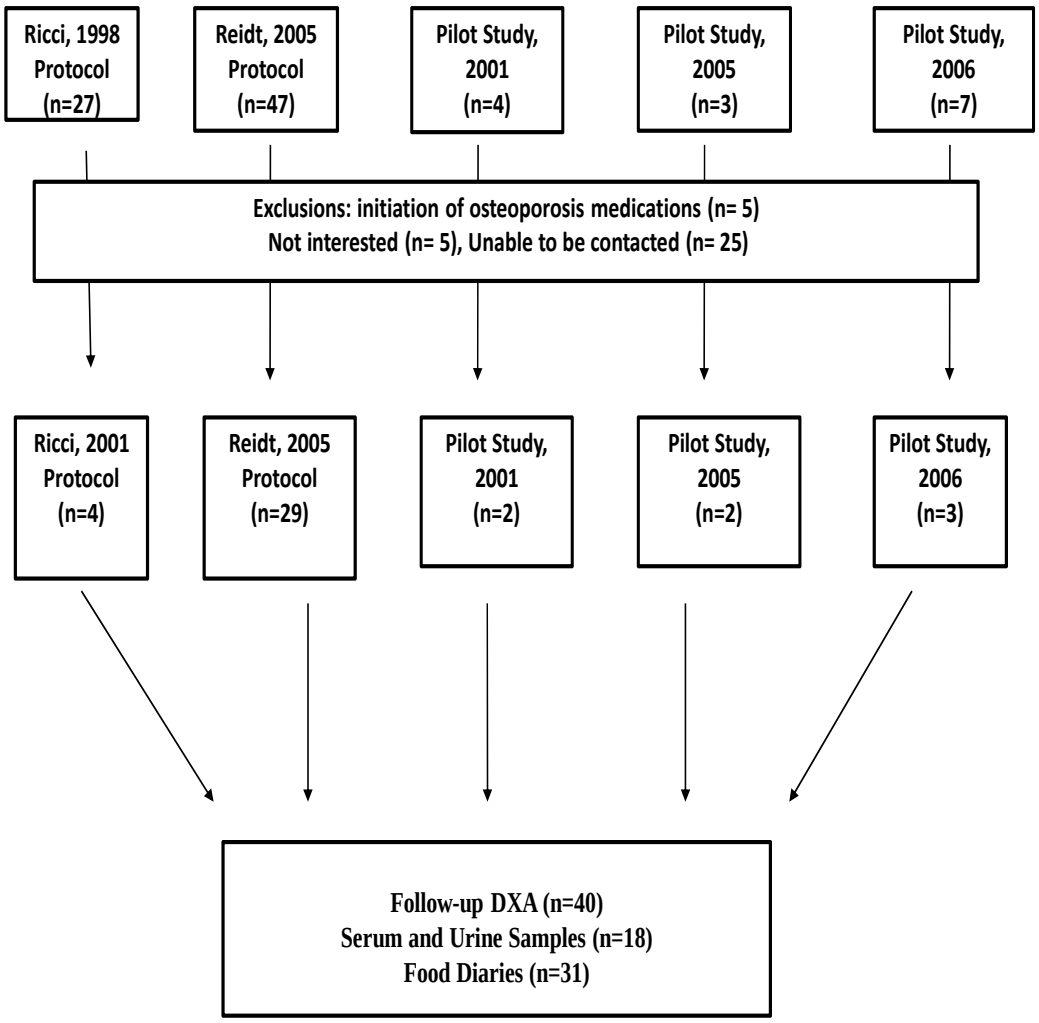
It is interesting to note that while bone loss was less extreme in the WL-M than WL-R group, the WL-R group still appeared to have greater mean rates of loss than seen in weight stable women of similar age and menopausal status in the literature. Although weight re-gain appears to have a positive effect on bone compared to weight maintenance, the rate of loss remains higher than in weight stable postmenopausal women reported in the literature at the femoral neck, trochanter, and spine (Figure 5; Makovey; 2008, Park, 2007; Sirola, 2003; Uusi-Rasi, 2001; Guthrie, 1998; Nguyen, 1998; Young, 1996; Pouilles, 1995; Harris, 1992). These findings are consistent with Avenell, et al who found that the spine did not recover with weight gain in postmenopausal women, yet they contradict that study's findings that femoral neck BMD recovered (1994). The rate of change in total body BMD during weight regain seemed less than the weight stable individuals, however, similar to Compston, et al who observed that total body BMD decreased with weight loss but recovered with weight gain in a small group of pre- and post-menopausal women (1992). Although the current study observed only one weight cycle, in can be hypothesized that multiple episodes of weight loss and regain during the menopausal period may indeed increase fracture risk at some sites, given that bone losses are not completely recovered by weight regain.

Dietary recalls were closely monitored during the weight loss protocol in order to determine calcium intake. Upon completion of the first phase of the study, all subjects were encouraged to continue to use calcium supplements to maintain the recommended daily allowance of 1200 mg per day. It was observed that many, but not all, of the subjects reported taking supplements at the final time point. It should be noted that there was a

greater bone loss in the WL-M group, even with ~1200 mg Ca intake, as seen previously (Riedt, 2005). Although 3 day food diaries and compliance of supplement intake were analyzed throughout the six month weight loss protocol, at time point C the Ca intake was estimated by a single 3 day food diary and self reports of supplementation without other more intensive monitoring to measure compliance. Yet, these results suggest that it may be important to exceed the current dietary recommended intake of calcium of 1.2 g per day (or perhaps other bone-active nutrients such as vitamin D) not only during but after weight loss.

This study shows that weight loss induced bone loss continued at the trochanter and femoral neck one and a half years after weight stabilized in obese/overweight postmenopausal women who successfully maintained their moderate weight loss, with women with greater weight loss having greater bone loss. Weight regain resulted in recovery of trochanter and total body BMD. It should be noted that even after weight loss, the WL-M group had an average BMI of 24.9 ± 2.4 kg/m^2, just within the normal weight range (NIH, 1998), and thus, a clinician might not consider this individual at risk of osteoporosis based on their relatively high body weight, although their rate of bone loss may be higher than women of similar weight who have been weight stable. Similarly, women who regained their weight would be still be considered overweight, and may be urged to attempt further weight loss, creating an environment of weight cycling. Current recommendations appropriately encourage weight loss in overweight individuals to reduce the risks of many important health conditions, but the long-term consequence of weight loss-induced bone loss must be considered. Future studies will be needed to determine the biochemical mechanisms of this relationship and possible dietary and/or pharmaceutical therapies to ameliorate these losses both during and after weight loss in the postmenopausal population.

Appendix A. Recruitment Flow Chart



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