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Weight-loss-associated changes in bone mineral density and bone turnover after partial weight regain with or without aerobic exercise in obese women

Pamela S. Hinton, Ph.D.¹, R. Scott Rector, Ph.D.^{1,2,4}, Melissa A. Linden, M.S.¹, Shana O. Warner, Ph.D.¹, Kevin C. Dellsperger, M.D.^{2,3}, Anand Chockalingam, M.D.^{2,4}, Adam T. Whaley-Connell, D.O.^{2,4}, Ying Liu, M.S.¹, and Tom R. Thomas, Ph.D.¹

¹Department of Nutrition and Exercise Physiology

²Department of Internal Medicine

³Department of Medical Pharmacology and Physiology

⁴Harry S. Truman Veterans Affairs Medical Center

Abstract

Background/Objectives—Moderate, long-term weight loss results in loss of bone mass in overweight or obese premenopausal women. However, whether these changes persist during weight maintenance or regain remains to be determined.

Subjects/Methods—Overweight or obese (BMI: 25.8–42.5 kg/m²) women (n=40) with at least two risk factors for the metabolic syndrome participated in this 12-mo study that examined the effects of prescribed weight loss and regain, with or without exercise, on bone turnover and on bone mineral density (BMD) in a subset of participants (n=24). During the first 6 mo, participants lost ~10% of their initial body weight via energy restriction and supervised aerobic exercise. Following weight loss, participants were randomly assigned to either an exercise or a no-exercise treatment for the regain (+50% of weight lost) phase. A one-way (time) repeated measures ANOVA tested the effects of weight loss on BMD and bone turnover, and a two-way RM ANOVA (time, exercise) was used to examine the effects of exercise during weight regain.

Results—Hip (p=0.007) and lumbar spine (p=0.05) BMD decreased with weight loss, and remained reduced after weight regain with or without exercise. Likewise, the weight-loss-associated increases in osteocalcin (p<0.001) and C-terminal peptide of type I collagen (p<0.001) persisted following weight regain, independent of exercise.

Conclusions—the results of the present study, which is the first to examine changes in bone mass and turnover during carefully controlled weight regain, suggest that weight-loss-induced

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Address all correspondence to: Pamela S. Hinton, 106 McKee, Columbia, MO 65211, Telephone: (573) 882-4137, FAX: (573) 884-4885, hintonp@missouri.edu.

Disclosures

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perturbations in bone mass and turnover persist after partial weight regain, regardless of whether regular, weight-bearing aerobic exercise was continued.

Keywords

weight loss; weight regain; bone mineral density; bone turnover

Introduction

Weight loss improves metabolic fitness and reduces morbidity and mortality associated with overweight and obesity (Fernandez 2007). However, results of weight-loss intervention studies suggest that weight reduction might adversely affect long-term bone health. Even moderate weight reduction (~10%) in overweight or obese individuals both decreases bone mineral density (BMD) (Avenell et al. 1994; Pritchard et al. 1996; Ricci et al. 1998; Van Loan et al. 1998; Fogelholm et al. 2001; Jensen et al. 2001; Ricci et al. 2001; Riedt et al. 2005; Villareal et al. 2006; Villareal et al. 2008) and accelerates bone turnover, as assessed by serum biomarkers of bone formation and resorption (Ricci et al. 1998; Salamone et al. 1999; Ricci et al. 2001; Villareal et al. 2006; Holecki et al. 2007; Hinton et al. 2009; Rector et al. 2009). The proposed mechanisms for these weight-loss-associated changes included reduced mechanical loading due to body weight reduction, decreased secretion of adipose-derived hormones, and increased secretion of cortisol and parathyroid hormone, which stimulate osteoclast activity and bone resorption (Shapses and Riedt 2006). Although these relatively short-term weight loss intervention studies, which are typically 3–6 months long, have demonstrated significant reductions in total body or regional BMD, others have reported increased BMD following weight reduction (Bosy-Westphal et al. 2011). In particular, there is evidence that diets high in protein, calcium and/or dairy might prevent or ameliorate the detrimental effects of weight loss on bone immediately post-weight reduction (Jensen et al. 2001; Shapses et al. 2001; Riedt et al. 2007; Sukumar et al. 2011) or after a period of weight maintenance (Bowen et al. 2004; Thorpe et al. 2008). Thus, in addition to the ambiguity regarding the effects of brief weight loss interventions on bone, the long-term consequences of weight loss on bone mass, bone turnover, and subsequent fracture remain relatively unknown. However, results from the few studies that included a post-weight loss follow-up suggest that alterations in BMD and bone turnover may persist during weight maintenance (Avenell et al. 1994; Fogelholm et al. 2001; Jensen et al. 2001; Villareal et al. 2008; Hinton et al. 2009).

Consistent with these findings, observational data indicate that both weight loss and repeated cycles of weight loss and regain increase fracture risk in both men and women (Langlois et al. 1998; Meyer et al. 1998; Langlois et al. 2001; Bacon et al. 2004). Moreover, chronic dieting is associated with reduced bone mineral content (BMC) (Bacon et al. 2004) and repeated cycles of weight loss and regain increase the risk of bone fracture (Meyer et al. 1998). As approximately one-half of adult women are on a weight-reduction diet (Bish et al. 2005), and many women who successfully lose weight undergo partial weight regain (Franz et al. 2007), weight loss may negatively affect long-term bone health in many women.

Conversely, weight-bearing exercise that produces adequate mechanical strain increases bone strength due to improvements in bone mineral density (Nelson et al. 1991; Vainionpaa et al. 2005) and bone structure (Robling et al. 2006). Results of both cross-sectional and intervention studies support the beneficial effects of weight-bearing physical activity on bone health in weight-stable premenopausal women (Borer 2005). However, whether weight-bearing exercise facilitates recovery of bone mass following weight reduction remains to be determined.

Thus, the purpose of the present study was two-fold: to evaluate the effects of partial weight regain on total body and regional bone mineral density and on bone turnover markers in overweight or obese women following moderate weight loss; and to determine if weight-bearing exercise affects BMD and/or serum markers of bone turnover during partial weight regain. We hypothesized that partial weight regain would not restore BMD or bone turnover markers to pre-weight reduction values. We also hypothesized that regular weight-bearing exercise would result in greater increases in BMD during weight regain compared with no exercise in association with increased bone formation and reduced bone resorption, as assessed by serum markers.

Materials and Methods

Study participants

Sedentary, overweight or obese (BMI: $>25.0 \text{ kg/m}^2$) women (non-pregnant and non-lactating) aged 19–50 y were eligible to participate in this study. Sedentary was defined as no more than one systematic exercise session over 30-min duration per week over the previous 4 mo. All but two of the participants, who had undergone hysterectomies, had regular menstrual cycles; 13 women were taking hormonal contraceptives throughout the duration of the study. Subjects with any diagnosed cardiovascular disease, diabetes, or disease symptom according to the American College of Sports Medicine (ACSM 2009) that would limit exercise were excluded. Exclusion criteria also included smoking or any medications or supplements that affect weight loss or bone metabolism. Inclusion and exclusion criteria, including habitual physical activity level, were assessed via questionnaire and verbal questioning during participant screening. The Health Sciences Institutional Review Board at the University of Missouri-Columbia approved this study. All procedures were in accordance with both the Helsinki Declaration of 1975 as revised in 1983 and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised November 13, 2001. Prior to enrollment, participants provided written informed consent.

Experimental Design

This study was part of a larger randomized, clinical trial that evaluated the effects of moderate weight loss and subsequent partial weight regain with or without exercise on body weight, body composition, and parameters of the metabolic syndrome (Thomas et al. 2010). All participants (n=40) were prescribed a reduced-energy diet designed to result in a 10% reduction in initial body weight during the 4- to 6-month weight-loss phase of the study. After completion of the weight-loss phase of the study, participants were randomized to either the exercise (EX, n=22) or no exercise (NOEX, n=18) intervention during 4 to 6

months of prescribed regain of 50% of the weight lost during the first phase of the study. The weight-loss and weight-regain interventions, which have been described in detail previously (Thomas et al. 2010), are summarized in brief below.

Intervention

Weight-Loss—During the initial, 4- to 6-month weight-loss phase, all participants lost 10% of their baseline body weight through a combination of supervised aerobic exercise (treadmill walking) 5 d/wk at approximately 55–60% $\text{VO}_{2\text{peak}}$ (65–75% HR max) for 45 min per session (>200 min/wk, 2000–2500 kcal/wk) and moderate energy restriction (reduced by ~600 kcal/d).

Weight-regain—Following weight loss, each participant was randomized to either the EX or NOEX group for controlled partial weight regain (i.e., regained 50% of the weight loss) during the second 4- to 6-month phase of the study. Participants randomized to the NOEX group discontinued regular aerobic exercise and increased their daily energy intake, while participants randomized to the EX group continued supervised, regular exercise and achieved positive energy balance by increasing energy intake. In particular, participants were instructed by the study nutritionist to increase their energy intake by increasing portion size and by consuming energy- and-nutrient-dense snacks, i.e., dried fruit and nuts, which were provided to them.

Treatment compliance—During both the weight-loss and weight-regain phases of the study, body weight was monitored weekly to ensure adequate progress towards the target weight-loss or weight-gain goal. Dietary intake was monitored via 7-day written food records obtained at baseline, during weight loss and weight regain, and participants were provided weekly dietary counseling by the study nutritionist. Subsequently, the food records were analyzed for nutrient content using Food Processor 8.0 computer program (esha, Salem, OR). Exercise training was performed in the Exercise Physiology Lab and was monitored by study personnel. In addition, changes in maximal oxygen consumption were used to verify exercise compliance during weight loss and regain.

Outcome Measures

Anthropometrics and bone measurements—Height was assessed at baseline using a wall tape calibrated to 0.1 cm. Body weight was measured weekly on a Toledo scale (Mettler-Toledo Inc., Columbus, OH) for the duration of the study and was used to calculate body mass index (BMI). Skinfold thickness was measured at three sites (triceps, suprailiac, and mid thigh) with skinfold calipers. Three measurements were taken at each site and the mean of the two closest measurements was used to calculate percent body fat according to the Jackson-Pollock equation (Jackson 1985). Dual-energy X-ray absorptiometry (DXA, Hologic QDR 4500, Waltham, MA), which became available mid-study, was used to measure bone area, BMC, and areal BMD in a subset of the participants (weight loss: n=24; weight regain: EX, n=14; NOEX, n=10). This subset of participants did not differ from the rest of the study population in age, initial body weight, BMI, percent body fat, maximal oxygen consumption, nor in percent weight lost or gained (data not shown). Scans of the total body, lumbar spine (L1–L4), and total hip were performed. Areal BMD (g/cm^2) was

calculated from bone area (cm²) and BMC (g) by the software supplied with the DXA scanner (version 12.4). All DXA scans for each participant were performed and analyzed by one investigator. CVs for BMC and BMD were <1%.

Serum markers of bone formation and resorption—Changes in the bone turnover markers following weight loss and partial regain were assessed in all participants (n=40). Blood collection was preceded by 48 hours of dietary control and no exercise in the early morning following an overnight, 12-hr fast to reduce the variation associated with circadian rhythms and feeding. Likewise, for each participant, blood samples were collected during the same phase of the menstrual cycle, to minimize intra-individual variation associated with menstrual-cycle phase. Menstrual cycle regularity was not altered by weight loss. A portion of each blood sample was dispensed into a 10-ml Serum Separator Tube™ (Becton-Dickinson, Franklin Lakes, NJ USA) followed by centrifugation for 15 min at 2000g. The separated serum was transferred to cryogenic vials and stored at -70 °C for later analysis.

Serum markers of bone formation and resorption are used clinically as indirect measures of bone remodeling (Seibel 2005). Osteocalcin (OC) is secreted by mature osteoblasts, while bone-specific alkaline phosphatase (BAP) is secreted by newly differentiated osteoblasts (Aubin et al. 1995). C-terminal telopeptide of type I collagen (CTX) is released when bone collagen is broken down during bone resorption. The concentrations of OC, BAP, and CTX in serum were assessed in duplicate using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Osteocalcin and Metra® BAP, Quidel, San Diego CA; Serum CrossLaps®, Immunodiagnostic Systems, Fountain Hills, AZ). We measured CTX in serum rather than urine to minimize intra-individual variation (Christgau et al. 2000). The anti-OC antibody used in the ELISA recognizes only intact OC; therefore, because the antibody does not bind OC fragments, which are released during bone resorption, OC measured using this ELISA results only from de novo synthesis. All assays were performed in the same run to eliminate inter-assay variability. Coefficients of variation for the OC, BAP and CTX assays were <10%.

Statistics

To examine the effects of weight loss, a repeated measures 1-factor analysis of variance (RMANOVA) was used to test for a significant main effect of time (baseline vs. post weight loss). The main effects and interaction of partial weight regain and exercise during weight regain were examined using a 2-factor (time and exercise treatment) RMANOVA. In the case of a significant interaction, follow-up paired t-tests were used to test differences from post-weight loss to post-weight regain within each group. Pearson's correlation was used to examine bivariate relationships between absolute and percent changes in the bone turnover markers during weight loss and partial regain. The normality assumption was verified using the Shapiro-Wilk test. Data are means ± standard error of the mean (SEM). P-values less than 0.05 were considered statistically significant.

Results

Weight-loss

Sedentary, overweight or obese (BMI= 33.1 ± 0.6 kg/m²) women aged 39 ± 1 y completed this ~12-month randomized clinical trial that examined the effects of exercise during partial weight regain on serum markers of bone turnover (n=40), and on BMD in a subset of participants (n=24). Anthropometrics and maximal oxygen consumption measured at baseline are shown for the entire study population in Table 1; there were no differences in these measures between participants who underwent DXA scanning and the rest of the study population. During the initial 4- to 6-month weight-loss phase of the study, total energy intake was reduced by ~600 kcal/d compared with baseline energy intake (Table 2). Mean calcium and vitamin D intakes at baseline were less than the adequate intake (AI) (1000 mg Ca/d and 5 µg vitamin D/d, (Food and Nutrition Board 1997); calcium intake was reduced after weight loss (Table 2).

After completion of the weight-loss phase of the study, the mean weight loss was $9.5 \pm 0.2\%$ (-8.5 ± 0.3 kg) and ranged from -6.6 to -13.2% (-12.5 to -5.6 kg) of initial body weight; there were significant reductions in both fat mass and lean body mass (Table 1). Cardiorespiratory fitness was significantly improved after 6 months of regular aerobic exercise, as evidenced by improvements in both absolute and relative VO_{2peak} (Table 1).

BMD of the total hip ($-1.6 \pm 0.5\%$) and lumbar spine ($-1.1 \pm 0.6\%$) were significantly reduced following weight loss (Figure 1). Weight loss had no effect on bone area of the total body (Baseline= 1987 ± 33 cm²; Post-weight loss= 1970 ± 34 cm²), lumbar spine (Baseline= 57.1 ± 1.7 cm²; Post-weight loss= 57.3 ± 1.4 cm²) and total hip (Baseline= 31.1 ± 0.7 cm²; Post-weight loss= 31.2 ± 0.7 cm²). There were no differences in weight-loss-associated changes in total hip or lumbar spine BMD between participants who were subsequently randomized to the EX and NOEX groups during weight regain (Total hip BMD: EX= $-1.8 \pm 0.6\%$ vs. NOEX= $-2.2 \pm 1.0\%$; Lumbar spine BMD: EX= $-1.2 \pm 0.6\%$ vs. NOEX= $-1.1 \pm 1.0\%$).

Osteocalcin ($50 \pm 10\%$) and CTX ($28 \pm 4\%$) increased significantly with weight loss, while BAP ($1 \pm 6\%$) remained unchanged (Figure 2). The changes in OC and CTX during weight loss were not significantly correlated (data not shown). There were no differences in weight-loss-associated changes in OC or CTX between participants who were subsequently randomized to the EX and NOEX groups during weight regain (OC: EX= $47 \pm 13\%$ vs. NOEX= $44 \pm 14\%$; CTX: EX= 27 ± 6 vs. NOEX= $29 \pm 8\%$).

Weight-regain

During the partial weight-regain phase of the study, energy intake was significantly increased by ~500 kcal/d. Calcium intake also increased significantly, while the increase in mean vitamin D intake was not statistically significant; there were no differences in nutrient intakes between the EX and NOEX groups (Table 2). On average, participants regained $55.2 \pm 1.9\%$ (absolute weight regained: mean= 4.6 ± 0.2 kg; range= 2.7 to 6.9 kg) of the weight lost during the first phase of the study with significant increases in both lean and fat mass in both the EX and NOEX groups (Table 1). The NOEX group exhibited a significant

reduction in VO_{2peak} , while the EX group maintained VO_{2peak} during weight regain (Table 1).

There was a significant effect of exercise during weight regain on total body BMD, i.e., a significant time-by-exercise interaction in the 2-way RMANOVA ($p=0.012$), such that individuals in the EX group experienced a significant reduction in total body BMD during the weight-regain phase (Post-weight loss: $1.169 \pm 0.028 \text{ g/cm}^2$; Post-weight regain: $1.156 \pm 0.028 \text{ g/cm}^2$, $p=0.04$), while total body BMD of the NOEX group remained unchanged (Figure 1). By contrast, exercise during weight regain had no effect on BMD of the hip or lumbar spine, with BMD at these sites remaining at their reduced post-weight-loss values in both the NOEX and EX groups (Figure 1). Weight regain had no effect on bone area of the total body (Post-weight loss = $1979 \pm 34 \text{ cm}^2$; Post-weight regain = $1967 \pm 35 \text{ cm}^2$), lumbar spine (Post-weight loss = $57.5 \pm 1.5 \text{ cm}^2$; Post-weight regain = $57.5 \pm 1.5 \text{ cm}^2$) or total hip (Post-weight loss = $31.3 \pm 0.8 \text{ cm}^2$; Post-weight regain = $31.1 \pm 0.7 \text{ cm}^2$).

Likewise, weight regain had no effect on serum markers of bone turnover. OC and CTX remained elevated relative to baseline following weight regain ($44 \pm 12\%$ and $23 \pm 7\%$, respectively) and did not differ from post-weight loss (Figure 2). Exercise during weight regain had no effect on the bone turnover markers, although the interaction between exercise and time for CTX approached statistical significance ($p=0.06$).

Discussion

In the present study, we observed a statistically significant reduction in lumbar spine and hip BMD after moderate weight loss in overweight or obese, middle-aged women with a concurrent increase in serum markers of bone formation and breakdown. This study, which is the first to examine changes in bone mass and turnover during carefully controlled weight regain, found that weight-loss-induced perturbations in bone mass and turnover persisted after partial weight regain, regardless of whether regular, weight-bearing aerobic exercise was continued. However, it is important to note that continuation of aerobic exercise countered the detrimental effects of partial weight regain on many markers of the metabolic syndrome, as previously reported (Thomas et al. 2010).

There is considerable evidence from relatively short-term weight loss intervention studies, which are typically 3–6 months long, that weight reduction, through energy restriction alone or combined with exercise, results in loss of total body or regional BMD (Avenell et al. 1994; Pritchard et al. 1996; Ricci et al. 1998; Van Loan et al. 1998; Fogelholm et al. 2001; Jensen et al. 2001; Ricci et al. 2001; Riedt et al. 2005; Villareal et al. 2006; Villareal et al. 2008) and accelerated bone turnover (Ricci et al. 1998; Salamone et al. 1999; Ricci et al. 2001; Villareal et al. 2006; Holecki et al. 2007). By contrast, Bosy-Westphal et al. (2011) reported significant increases in leg, lumbar spine and total body BMD following weight loss in overweight or obese men and women, some of which were fully or partially reversed with subsequent weight regain. However, results from other studies that included a post-weight loss follow-up suggest that alterations in BMD and bone turnover may persist during weight maintenance (Avenell et al. 1994; Fogelholm et al. 2001; Jensen et al. 2001; Villareal et al. 2008; Hinton et al. 2009). The present study extends these findings to partial weight

regain following moderate weight loss in overweight or obese women, regardless of activity status. The persistent reduction in BMD and sustained elevation in bone turnover markers following a cycle weight loss and partial regain have clinical relevance, as both are independent predictors of fracture risk (Garnero and Delmas 2004).

Because of the reported benefits of weight-bearing physical activity on maintenance of bone mass and strength in weight-stable premenopausal women (Eickhoff et al. 1993; Basse and Ramsdale 1994; Borer 2005), we examined the possibility that continuation of regular weight-bearing aerobic exercise would affect BMD and bone turnover during weight regain. While the ability of exercise to “protect” bone against the deleterious effects of weight loss has been examined previously with mixed results (Andersen et al. 1997; Salamone et al. 1999; Nakata et al. 2008; Rector et al. 2009), the present study is the first to examine the use of weight-bearing exercise as a means to enhance “recovery” of bone mass and turnover following weight loss. We hypothesized that exercise during partial weight regain would increase BMD and alter the bone turnover markers in favor of bone formation, i.e., greater reductions in CTX and greater increases in OC and BAP. However, we found that exercise did not enhance recovery of BMD during weight regain, and, in fact, was associated with a significant decrease in total body BMD. Likewise, exercise did not affect serum markers of bone turnover (Figures 1 and 2). There are several biologically plausible reasons that exercise during weight regain appeared to have no effect on BMD or bone turnover markers.

According to Frost’s mechanostat theory (Frost 1997), for an activity to be osteogenic, the strain of the mechanical load must exceed a certain strain intensity or threshold. The participants in the present study were previously untrained and, for many, the target exercise intensity (60% of VO_2 peak) was achieved by fast treadmill walking on an incline. Although walking is a weight-bearing activity, it produces ground reaction forces only equal to, or slightly greater than, body weight (Nilsson and Thorstensson 1989). Therefore, a plausible explanation for the negative findings in the present study is that the exercise stimulus was insufficient to induce bone formation. These findings are also consistent with those of previous interventions that also reported no effect of walking on BMD in women during weight maintenance (Nelson et al. 1991; Wu et al. 2006) or weight loss (Rector et al. 2009). Thus, it appears that low-impact aerobic exercise may be ineffective in increasing bone mass and normalizing bone turnover markers during partial weight regain in premenopausal women. Moreover, the absence of an increase in BMD during weight regain with or without exercise suggests that recovery of the bone lost during weight reduction is difficult to achieve.

It is important to recognize that calcium and vitamin D status might moderate the effects of weight loss or regain on bone turnover and BMD. In the present study, mean intakes of both calcium and vitamin D during weight loss were less than the Adequate Intake, and, although, calcium intake increased during weight regain, 73% of participants consumed less than 1000 mg Ca/d. Sufficient dietary calcium and vitamin D are especially important under conditions of reduced calcium absorption (Borer 2005), such as occurs with weight loss (Cifuentes et al. 2004). High dietary calcium (1.5–1.8 g Ca/d) preserves bone mass and turnover during weight loss in premenopausal women (Ricci et al. 1998; Jensen et al. 2001; Shapses et al. 2001; Riedt et al. 2007; Wagner et al. 2007), possibly by preventing increased secretion of

the pro-resorptive parathyroid hormone (Riedt et al. 2007). Thus, low dietary calcium and vitamin D might have contributed to the decrease in BMD observed following weight reduction in the present study.

Although there is evidence that high dietary calcium can mitigate some of the deleterious effects of weight loss on bone, the skeletal effects of dietary calcium post-weight loss are unclear. We previously reported no differences between a recommended- and low-dairy weight-maintenance diet, containing ~600 vs. ~1300 mg Ca/d, on total body BMD following weight reduction in obese women (Hinton et al. 2010). By contrast, Thorpe et al. (2008) reported that a high-protein diet (1000 mg Ca/d) positively affected total body BMD during weight stabilization compared with a high-carbohydrate diet (700 mg Ca/d) in overweight men and women (Thorpe et al. 2008). To the best of our knowledge, no studies have examined the skeletal effects of dietary calcium during weight regain.

Consistent with the results of the present study, we have repeatedly observed that CTX and OC, but not BAP, increase with weight reduction (Hinton et al. 2009; Rector et al. 2009). It is unclear if the discrepant response between BAP and OC is due to their differential expression during osteoblast maturation (Aubin et al. 1995), or to a possible glucoregulatory endocrine function of OC. Recent evidence in genetically altered mice indicates that osteoblasts secrete OC, which improves insulin sensitivity in liver, adipose, and skeletal muscle (Lee et al. 2007). In obese humans, OC increases with weight loss concurrent with improved insulin sensitivity (Fernandez-Real et al. 2009). Therefore, the increase in OC observed in the present study may reflect an osteoblast endocrine function that is unrelated to bone formation. These possibilities are intriguing and warrant future investigation.

The present study has several strengths. First, both the prescribed weight loss and prescribed weight regain were carefully controlled through regular monitoring of body weight and energy intake. Similarly, all exercise training sessions were monitored by study personnel. A second strength of the present study is that we measured changes in bone area during weight loss and regain. In adults, BMD and BMC should change in parallel, as bone area does not change appreciably; thus, discordant BMD and BMC results are biologically implausible and are probably artifacts of DXA. This issue is particularly problematic for evaluation of changes in BMD associated with alteration in body mass or composition (Tothill et al. 1999; Tothill 2005). For example, Bosy-Westphal et al. (2011) demonstrated that weight-loss-associated changes in arm, leg, lumbar spine and pelvis BMD were negatively correlated with changes in bone area, but not BMC. Thus, because bone area did not change with weight loss or partial regain, we verified that the alterations in BMD were likely not the result of artifactual changes in bone area.

Regarding study limitations, it is important to note that the sample size affected the statistical power for the outcome variables, indicating an increased likelihood of Type II errors, i.e., failure to reject the null hypothesis that exercise had no effect, when in fact it is false. Another limitation, which is not unique to our study, is the necessity of using indirect measures, i.e., serum markers, of bone formation and bone breakdown that reflect the entire skeletal response and are not site-specific. Finally, we did not control calcium and vitamin D intake during weight loss and regain, which might be viewed as a strength or a limitation.

Because we did not control calcium and vitamin D intake, we cannot assess the independent effects of changes in energy balance. However, because calcium and vitamin D intakes are often reduced during energy-restriction (Truby et al. 2008) and the prevalence of inadequacy is high among dieting individuals (Ashley et al. 2007), our study design more accurately reflects dieting in the “real world.” Finally, future studies that aim to examine the protective and restorative effects of exercise should test exercise interventions that are specifically designed to have maximal osteogenic effect, i.e., <100 loading cycles per session of high-impact, dynamic, multi-directional activity (Turner and Robling 2005).

In summary, the results of the present study, which is the first to examine changes in bone mass and turnover during carefully controlled weight regain, suggest that weight-loss-induced perturbations in bone mass and turnover persisted after partial weight regain, regardless of whether regular, weight-bearing aerobic exercise was continued. The persistent reduction in BMD and sustained elevation in bone turnover markers following weight loss have clinical relevance, as both are independent predictors of fracture risk.

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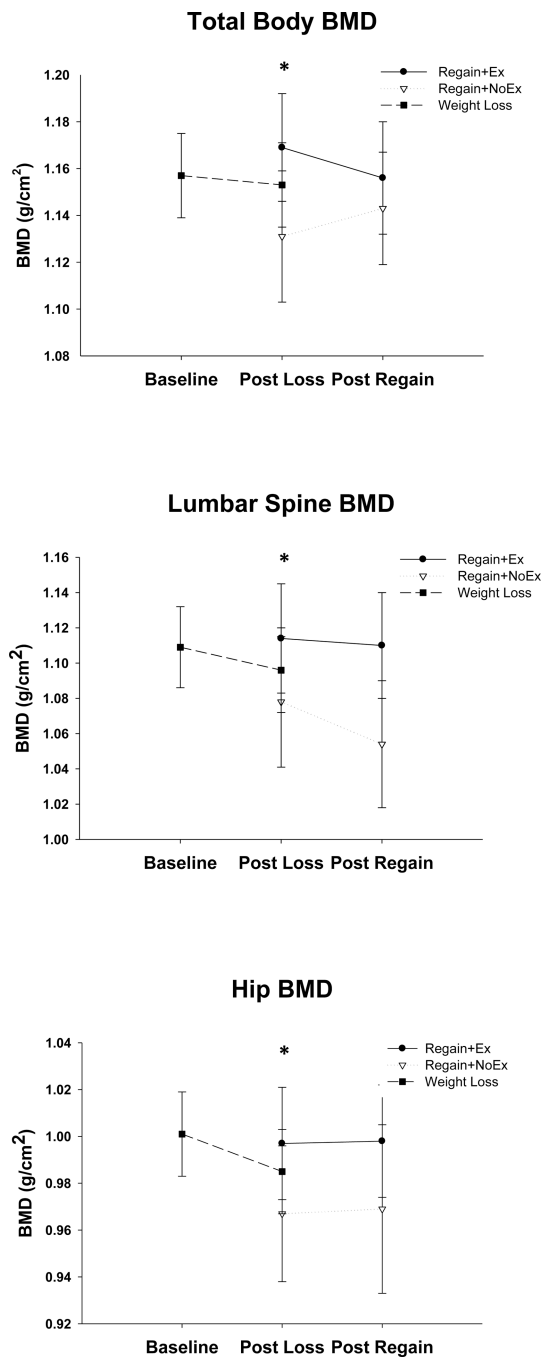


Figure 1. Changes in BMD (means \pm SEM) with weight loss (n=24) and weight regain with (n=14) or without exercise (n=10). *, Post-Loss mean is significantly different from Baseline, i.e., main effect for Weight Loss (time), <0.05. †, Post-Regain is significantly different from Post-Loss within the NOEX and EX groups, p<0.05; see Results for details of significant main effects and interactions.

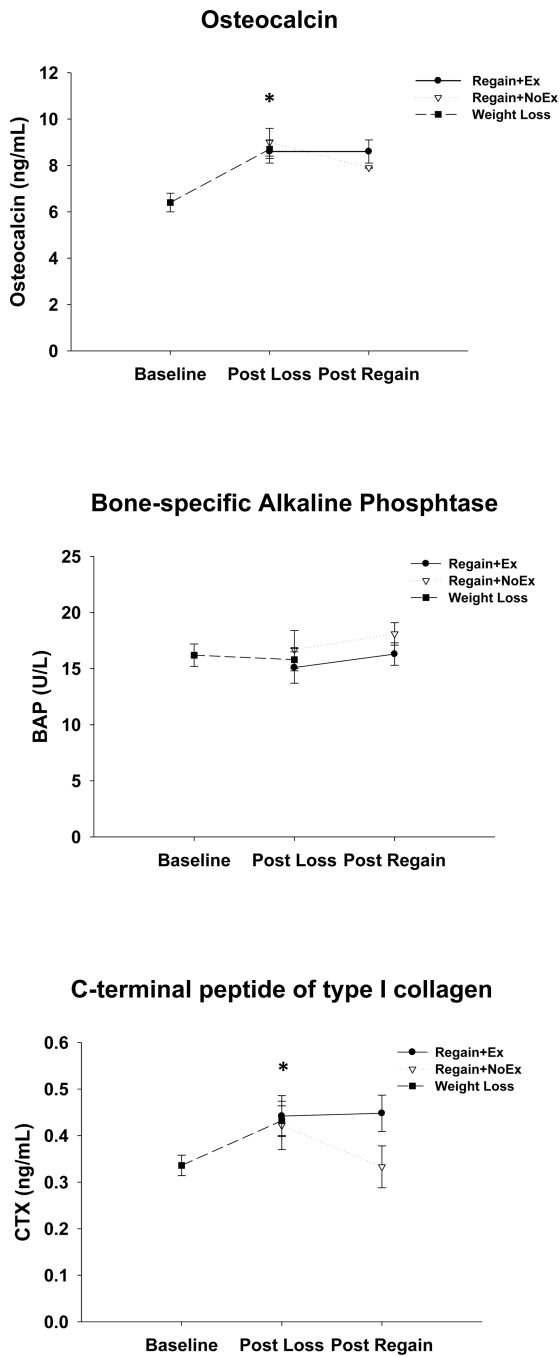


Figure 2.

Changes in serum bone turnover markers (means \pm SEM) with weight loss (n=40) and weight regain with (n=22) or without exercise (n=18). *, Post-Loss mean is significantly different from Baseline, i.e., main effect for weight loss (time), $p < 0.05$. †, Post-Regain is significantly different from Post-Loss within the NOEX and EX groups, $p < 0.05$; see Results for details of significant main effects and interactions.

Anthropometrics and peak oxygen consumption at baseline, post-weight loss and post-weight regain with and without exercise.

Table 1

	Weight Loss (n=40)		Weight Regain-NOEX (n=18)		Weight Regain-EX (n=22)	
	Baseline	Post-Loss	Post-Loss	Post-Regain	Post-Loss	Post-Regain
Body weight (kg)	89.7 ± 2.2	81.2 ± 2.0*	82.4 ± 2.9	87.6 ± 3.1 [†]	79.1 ± 2.6	83.3 ± 2.7 [†]
BMI (kg/m ²)	33.0 ± 0.7	29.8 ± 0.6*	30.6 ± 0.9	32.6 ± 1.0 [†]	29.0 ± 0.8	30.6 ± 0.8 [†]
Body fat (%)	38.9 ± 0.7	34.4 ± 0.7*	34.5 ± 1.1	37.1 ± 1.1 [†]	33.7 ± 1.0	35.9 ± 1.0 [†]
Fat mass (kg)	35.2 ± 1.3	28.3 ± 1.2*	29.2 ± 1.6	33.6 ± 1.8 [†]	25.0 ± 1.4	28.2 ± 1.7 [†]
LBM (kg)	54.5 ± 1.1	52.9 ± 1.0*	54.6 ± 1.5	55.6 ± 1.4 [†]	51.0 ± 1.3	51.8 ± 1.3 [†]
VO _{2peak} (L/min)	2.16 ± 0.05	2.35 ± 0.06*	2.50 ± 0.08	2.34 ± 0.06 [†]	2.23 ± 0.07	2.29 ± 0.06
VO _{2peak} (mL/kg/min)	24.3 ± 0.5	29.2 ± 0.6*	29.9 ± 1.0	26.4 ± 0.9 [†]	29.7 ± 0.9	29.0 ± 0.8

Data are means ± SEM. BMI, body mass index; LBM, lean body mass. Body fat, fat mass, and LBM were derived from skinfold thicknesses as described in the Methods.

* Post-Loss mean is significantly different from Baseline, i.e., main effect for weight loss (time), p<0.05.

[†] Post-Regain is significantly different from Post-Loss within the NOEX and EX groups, p<0.05; see Results for details of significant main effects and interactions.

Table 2

Nutrient intakes at baseline, post-weight loss and post-weight regain with and without exercise.

	Weight Loss (n=40)		Weight Regain-NOEX (n=18)		Weight Regain-EX (n=22)	
	Baseline	Post-Loss	Post-Loss	Post-Regain	Post-Loss	Post-Regain
Energy (kcal)	2221 ± 75	1591 ± 54*	1565 ± 92	2146 ± 84 [†]	1640 ± 84	2224 ± 110 [†]
Fat (g/d)	89 ± 4	58 ± 3*	58 ± 4	86 ± 6 [†]	58 ± 4	81 ± 5 [†]
Fat (% kcal)	36.1 ± 0.9	32.9 ± 0.9*	34.1 ± 1.5	36.3 ± 1.3	31.4 ± 1.4	32.9 ± 1.2
CHO (g/d)	271 ± 11	198 ± 8*	194 ± 14	260 ± 16 [†]	209 ± 12	287 ± 15 [†]
CHO (% kcal)	48.9 ± 1.0	49.6 ± 1.0	48.5 ± 1.7	48.3 ± 1.6	51.5 ± 1.6	51.8 ± 1.5
PRO (g/d)	81 ± 3	67 ± 2*	67 ± 4	84 ± 6 [†]	69 ± 3	87 ± 5 [†]
PRO (% kcal)	14.8 ± 0.4	17.5 ± 0.5*	17.6 ± 0.8	15.7 ± 0.5 [†]	16.8 ± 0.7	15.4 ± 0.5 [†]
Calcium (mg/d)	835 ± 56	693 ± 34*	711 ± 56	797 ± 176 [†]	713 ± 51	1108 ± 160 [†]
Vitamin D (µg/d)	1.5 ± 0.2	1.3 ± 0.2	1.6 ± 0.4	2.2 ± 0.4	1.1 ± 0.3	1.6 ± 0.4

Data are means ± SEM.

* Post-Loss mean is significantly different from Baseline, i.e., main effect for weight loss (time), $p < 0.05$.

[†] Post-Regain is significantly different from Post-Loss within the NOEX and EX groups, $p < 0.05$; see Results for details of significant main effects and interactions.