Sex Differences in Bone Loss With Weight Loss in the Pounds Lost Trial: Evaluation of Mechanisms

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Sex Differences in Bone Loss with Weight Loss in the Pounds Lost Trial: Evaluation of Mechanisms

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Abstract

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Purpose: Obesity is an increasingly prevalent health concern, and according to the CDC, over 2/3 of Americans are overweight or obese. While weight loss has been shown to prevent the medical complications of obesity, it is important to understand potential risks of weight loss in overweight patients. Loss of bone mass is a known complication of weight loss and increases patients' risk of osteoporosis and fracture(s). Minimizing bone loss should thus be a goal of weight loss treatment. In the POUNDS LOST trial, 811 obese and overweight individuals were randomized to 1 of 4 diets varying in macronutrient content and followed for two years. While participants lost similar amounts of weight across the four diets, females lost BMD at the spine (LS) and femoral neck (FN) while males gained BMD at the LS. The purpose of this study was to understand mechanisms by which females lost BMD with weight loss and males did not.

Methods: In 231 POUNDS LOST participants we analyzed biomarkers of bone turnover (osteocalcin, C-telopeptide), sclerostin, adipokines (leptin, adiponectin), and calcium homeostasis (calcium, 25-OH Vitamin D, PTH) at baseline and after 2 years of weight loss. Multivariate analyses correlated the 2-year changes in these biomarkers with changes in LS BMD and with visceral adipose tissue and evaluated whether there were sex differences in these interactions.

Results: Males lost more weight than females, though the weight change was not different between sexes once adjusted for baseline weight. Change in C-telopeptide was associated with a change in BMD in pre- and post-menopausal females; change in C-telopeptide explained 42 percent of the change in BMD in pre-menopausal females and 9 percent of the change in BMD in post-menopausal females; there was not an association between change in C-telopeptide and change in BMD in males. Males had significantly lower levels of sclerostin at month 24 than pre-and post-menopausal females (1100 pg/mL in males, 1131 pg/mL pre-menopausal females, 1269 post-menopausal females), but sclerostin was not correlated with degree of weight lost or change in BMD. There were no sex differences in the relationship between sclerostin and BMD. There were sex differences in the change in leptin with weight loss (males change -.62 ln ng/mL; pre-menopausal females +0.17 ln ng/mL; post-menopausal females change +0.04 ln ng/mL). There were also sex differences in the relationship of change in leptin to change in BMD. In

males, decreases in leptin were associated with an increase in BMD; decrease in leptin accounted for 3.6 percent of the increase in BMD. This relationship was not seen in pre-menopausal females or post-menopausal females. All groups had low vitamin D levels (<30ng/mL)at baseline. There was a relationship between change in vitamin D and change in BMD; however, there were no sex differences in change in vitamin D and change in BMD. There were sex differences in the change in parathyroid hormone with weight loss (males decreased -0.1 pg/mL; pre-menopausal females increased 27.2 pg/mL; post-menopausal females increased 6.3 pg/mL). Change in parathyroid hormone was not associated with a change in BMD, and there were not sex differences in the relationship between change in parathyroid hormone and change in BMD.

Conclusions: Sex differences in bone loss with weight loss may be explained by increased bone resorption as indicated by the biomarker c-telopeptide. The sex differences are seen in both pre- and post-menopausal females, though they are more significant in premenopausal females. Lower leptin levels may have a protective effect on bone in males with weight loss. Vitamin D levels may be low in obese and overweight individuals, and changes to vitamin D are associated with change in BMD in both males and females. Ongoing studies are examining in this cohort whether there are sex differences in the relationships between changes in body composition (fat, BMD and lean tissue) and adipokines and measures of calcium homeostasis.

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Glossary of Terms

BMD: Bone Mineral Density

BMI: Body Mass Index

Ca: Calcium

CTX: C-Telopeptide

FN: Femoral neck

HRT: Hormone Replacement Therapy

LS: lumbar spine

PTH: Parathyroid Hormone

Section 1: Introduction

Obesity is an increasingly prevalent health concern, and current Center of Disease Control (CDC) estimates report that over one-third of Americans are obese and another one-third are overweight (CDCPrevention, 2012). Medical consequences of excess weight are some of the leading causes of death in the United States including heart disease, Type 2 Diabetes, stroke, depression, and some kinds of cancer. The most effective non-surgical treatment of obesity is weight loss through diet and lifestyle intervention. There are many known benefits to weight loss for overweight patients, including a significant reduction in the risk of cardiovascular disease and depression as well as overall mortality (Pourier et al 2006; Dixon et al 2003); however, an understanding of the potential risks of weight reduction is essential for treating patients interested in losing weight.

Bone loss is a known risk of weight loss. Several studies have shown that weight reduction, from diet or bariatric surgery, leads to reduced bone mass at sites of common fractures and increased bone turnover (Brozozowska et al 2013; Hinton 2012; Shapses 2001; Villareal 2006). Bone loss such as this is concerning because it increases a patient's risk of osteoporosis and fractures that can lead to significant morbidity and mortality. This reduction in bone mass may be the result of reduced mechanical stress on bone that occurs with weight loss, though the precise biological mechanisms of the relationship between reduced weight bearing and bone loss remains unclear (Villareal et al 2012, Reid IR 2002). This study aimed to evaluate some of the mechanisms of bone loss with weight loss.

In the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial, 811 overweight women and men were randomized to four diets differing in macronutrient content. After two years, there was no effect of diet macronutrient content on amount of weight lost, and participants in all groups lost an average of 4 kilograms (6.9% of total body weight) (Sacks et al., 2009). Approximately half of the participants were randomly selected to complete body composition assessments at the beginning of the study and after six months and two years of follow-up (de Souza et al., 2012). Measurements of body fat and lean mass were assessed using dual-energy X-ray absorptiometry (DXA), and abdominal and hepatic fat were measured using computed tomography (CT). Participants lost on average more fat mass than lean mass throughout the trial, but there were no differences in changes in body composition among diet groups.

There were, however, differences in body composition by sex with this diet and weight loss intervention. Compared to women, men lost more fat mass than lean mass after two years of study participation. Women lost more visceral fat than did men relative to total-body fat loss. During the two-year dietary intervention, women, moreover, showed a decrease in bone mineral density (BMD) in the spine and femoral neck, while men showed a gain in BMD at the spine and no change at the femoral neck.

(de Souza et al., 2012; Tirosh et al., 2015). These sex differences in weight-loss induced bone-loss have, to our knowledge, never been described in a sample size of this magnitude.

The goal of this ancillary study is to understand the mediators of these changes in bone health in women but not men with weight loss. Even without weight loss, women are more likely to suffer from osteoporosis than men; current studies estimate that in adults aged 50 years and older, 40% of women and 13% of men will develop an osteoporotic fracture in their remaining lifetime (Hannan et al., 2000). Understanding the factors that may put women at an additional risk of bone loss and fractures with weight loss may be important for optimization of bone health and fracture reduction in women who embark on medically important weight loss diets.

In Pounds Lost participants who completed bone density and body composition measures, we measured biomarkers related to bone turnover, calcium homeostasis, and adipokines at baseline and after 2 years of the dietary intervention. To determine whether bone turnover was increased with weight loss in women compared with men, we measured serum levels of osteocalcin, a biomarker of bone formation, and c-telopeptide (CTX), a biomarker of bone resorption. We additionally measured the change in sclerostin, an inhibitor of bone formation known to increase with weight loss, in women and men before and after weight loss. To investigate whether adipokines, hormones released from adipose tissue, change with weight loss and if changes in signaling from the adipokines, leptin and adiponectin are associated with decreases in BMD in women but not men, we measured serum levels of leptin and adiponectin. Finally, to assess whether alterations in calcium homeostasis contribute to reductions in BMD in women but not men, we measured serum levels of calcium, vitamin D, and parathyroid hormone at baseline and after two years of follow-up. Changes in these measured biomarkers were correlated with changes in BMD. To further assess the relationship between these biomarkers and changes in body composition, we correlated changes in these markers with changes in body fat, visceral adipose tissue and lean tissue.

To our knowledge, an investigation of the sex differences in biomarkers contributing to bone loss with weight loss has never been published. The original Pounds Lost body composition analyses are the first of that sample size to show such a robust difference in bone loss with weight loss. This study is the first to investigate the mechanisms underlying those sex differences. Since women are at high risk of bone loss and osteoporosis, understanding of these mechanisms may inform treatment and prevention of bone loss in females attempting to lose weight.

Section 2: Student Role:

My contribution to this study was to understand why females, but not males, lost BMD with weight loss. This project was carried out in conjunction with my mentor, Dr. Meryl LeBoff, Chief of the Calcium and Bone Section in the Endocrinology, Diabetes and Hypertension Division at Brigham and

Women's Hospital. To begin this project, I requested the POUNDS LOST serum samples collected from the participants during the study from the research facility in Louisiana where they had been stored. When the samples arrived, I sorted them by participant and date so they could be analyzed. I coordinated with the directors of the Harvard Catalyst Core Clinical Laboratory to arrange for analysis of these specimens.

I then determined which analyses I wanted to run on these samples. I did a literature review of the studies that had been done on bone loss and weight loss. My literature yielded three general mechanisms for bone loss that had been studied: increased bone turnover, contribution of hormones released from fat, and changes to vitamin D and calcium homeostasis. I designed specific aims based on my comprehensive review of the available literature. First, to evaluate the role of biomarkers of bone turnover in bone loss with weight loss, I chose to analyze c-telopeptide, a marker of bone resorption, and osetocalcin, a marker of bone formation at baseline and after two years of dietary weight loss. To evaluate the role of adipocytes in bone loss with weight loss, I chose to analyze two hormones released from fat cells: leptin and adiponectin, at baseline and after two years of dietary weight loss. To evaluate the role of vitamin D homeostasis in bone loss with weight loss, I chose to analyze vitamin D, parathyroid hormone, and calcium at baseline and after two years of dietary weight loss. Additionally, I read about the hormone sclerostin, which is released from the osteocyte and has a catabolic effect on bone. There is now a sclerostin antibody, Romosozumab, in Phase II trials of pharmaceutical development that has been shown to increase bone mineral density at the spine and hip in postmenopausal women. Based on this reading, I hypothesized whether sclerostin might play a role in bone loss in the spine and hip with weight loss in our participants. I applied for and received the 2014 Harvard Catalyst Clinical Research Center Batch Sample Analysis and Assay Validation Award to run the sclerostin analyses.

Dr. LeBoff and I coordinated with Dr. Russ DeSouza, the clinical epidemiologist who had originally completed the body composition analyses in the POUNDS LOST trial, to complete the statistical analyses of the data. I shared with him the specific aims, and together we created a model to run the multivariate analyses to investigate whether there were changes in the biomarkers with weight loss and whether those changes correlated with changes in bone mineral density and whether if there were differences in those correlations by sex.

I submitted an abstract and presented preliminary results of this data at the annual meeting of the *Obesity Society and American Society for Metabolic and Bariatric Surgery* as a poster. In addition, I presented additional data from this project at the Connors-BRI Center for Women's Health and *Gender Biology Workshop on Sex Differences*. I also completed the writing of the project and will be submitting a first-author publication later this year.

Section 3: Methods

Participants and Study Design

The POUNDS LOST protocol was a randomized controlled trial that examined the effects of four energy-restricted diets on body weight and composition in overweight and obese participants. The four diets consisted of either low (20% of energy) or high (40% of energy) fat, average (15%) or high (25%) protein, and 25%, 35%, 45%, 55%, or 65% carbohydrate. The study was conducted at two sites: the Harvard School of Public Health and Brigham and Women's Hospital in Boston, MA, and the PBRC at Louisiana State University in Baton Rouge, LA, with project staff support from the National Heart, Lung, and Blood Institute. The study methods, participant inclusion and exclusion criteria, and protocols for diet and exercise have been described in detail elsewhere (Sacks et al., 2009). Participants in the original protocol were aged 30-70 with a BMI (in kg/m^2) between 25 and 40. Major exclusion criteria included a diagnosis of diabetes or unstable cardiovascular disease, use of medications that affect body weight, and lack of motivation as assessed by a standardized questionnaire and survey. 811 participants met these criteria and were enrolled in the trial. All subjects gave written informed consent before participating in the protocol. The study was approved by the human subjects committee at both institutions and by a data safety monitoring board approved by the National Heart, Lung, and Blood Institute.

A randomly selected group of these participants (n=424, 242 women and 182 men) completed DXA scans for fat mass, lean mass, and bone mineral density at baseline and after six months and two years of follow up. The details of this ancillary protocol are described previously (de Souza et al., 2012). Briefly, body composition was measured by DXA on a Hologic QDR 4500A bone densitometer (Hologic Inc, Bedford, MA) at BWH and PBRC after an overnight fast with the subject in the supine position while wearing a hospital gown. Fat mass and lean mass were calculated from percent body fat and body weight. Fat and lean mass index was calculated by dividing fat and lean mass respectively by body mass index. Bone mineral density was measured at the femoral neck and lumbar spine.

Of the participants who completed DXA measurements, approximately 40% (n=165, 91 women and 74 men) completed CT scans for total fat, abdominal fat, subcutaneous fat, and visceral fat at baseline and 24 months after study enrollment. These measurements were completed using either a General Electric High-Light computed tomographic (CT) scanner (General Electric) at BWH or a GE LightSpeed volume CT scanner (General Electric) at PBRC. Digital files from BWH were sent to PBRC and reanalyzed by a single reader and stored on the PBRC clinical database.

424 participants (242 women and 182 men) distributed evenly across the four diet intervention groups, completed DXA measurements at baseline. A sub group of 165 participants (91 women and 74 men) completed CT scans at baseline. 236 of these participants provided DXA measurements at the two-year follow-up time point, and 89 participants provided CT measurements at that timepoint. Of the 236

who completed DXA, 231 provided fasting blood samples at both time points. These 231 individuals with fasting blood samples and body composition measurements at baseline and 2 years were included in the analyses for this study. One participant was found to have elevated parathyroid hormone, low calcium, and high vitamin D, consistent with hyperparathyroidism, and was excluded from the study. One participant was found to have a baseline C-telopeptide level out of reference range [>3 standard deviations (SDs)] that could not be explained physiologically and this participant was excluded from analyses. With these two excluded participants, there were a total of 229 participants with baseline and month 24 levels of c-telopeptide, osteocalcin, adiponectin, leptin, calcium, and vitamin D. Due to an insufficient amount of serum, there were 229 PTH levels at baseline but only 119 PTH levels available at month 24, and sclerostin was only run at month 24, with 226 total samples.

Lab Measurements

Fasting blood samples were collected at both sites at baseline and 24 months of the diet interventions. Serum was analyzed for osteocalcin, CTX, sclerostin, calcium, PTH, 25-hydroxyvitamin D, leptin, and adiponectin at the MGH Clinical Laboratory Research Core in Boston, MA. All baseline and 24 month serum samples were measured in the same assay to reduce inter-assay variability.

Statistical Analyses

Baseline and end of study characteristics are summarized using means (standard deviation) for continuous variables; and as counts (proportions) for nominal variables; differences between sexes (i.e. men, premenopausal, and postmenopausal women) were assessed using analysis of variance (F-tests), and Fisher's exact tests as appropriate. Changes from baseline are expressed as means +- SEMs. The significances of between-diet differences in changes from baseline to year 2 in continuous outcome measures (e.g. bone mineral density, bone mineral content, and fat and lean tissues) were assessed using an analysis of covariance, with change from baseline as the outcome, and diet assignment, baseline weight, age, weight change, and baseline biomarker values as covariates. The association of changes in biomarkers from baseline to m24 with changes in bone mineral density, were assessed using multivariate regression analyses, which included formal tests of interaction to assess gender-specific associations. All analyses were performed with SAS software (version 9.4; SAS Institute, Cary, NC). The pre-specified level of significance for all tests was set to $P<0.05$.

Section 4: Results

Table 1 Participant baseline characteristics

Variable	Males	Pre-M Females	Post-M Females	P-diff
N	104/42-46[a]	29/0[a]	96/52-65[a]	
Age, y	53.1 (9.4)[a]	48.1 (6.1)[b]	57.2 (5.7)[c]	**<0.0001**
Height, cm	176.6 (5.8)[a]	164.5 (7.3)[b]	163.0 (5.8)[b]	**<0.0001**
Weight, kg	103.6 (12.4)[a]	89.8 (13.9)[b]	83.4 (12.7)[c]	**<0.0001**
BMI, kg/m2	33.2 (3.4)[a]	33.1 (4.0)[a,b]	31.4 (4.2)[b]	**<0.0001**
HRT [n (%)]	-	2 (6.9)	37 (38.5)	**<0.0001**
Adiponectin (ln)* [native units]	8.67 (0.48) [6504 (3089)]	8.67 (0.56) [6842 (4500)]	8.77 (0.53) [7360 (3940)]	0.35
Calcium (serum)	9.83 (0.42)	9.80 (0.40)	9.96 (0.40)	
CTX (square root)* [native units]	0.54 (0.11)[a,b] [0.30 (0.12)]	0.49 (0.11)[a] [0.25 (0.11)]	0.55 (0.13)[b] [0.31 (0.14)]	0.0502
Leptin (ln)* [native units]	1.57 (0.55)[a] [5.51 (3.0)]	2.38 (0.41)[b] [11.7 (4.8)]	2.17 (0.53)[b] [9.9 (5.0)]	**<0.0001**
Osteocalcin (ln)* [native units]	3.53 (0.32)[a,b] [35.9 (10.9)]	3.37 (0.36)[a] [30.8 (10.4)]	3.59 (0.34)[b] [38.5 (13.2)]	**0.007**
PTH	44.9 (14.0)	41.6 (11.7)	42.6 (11.7)	0.32
Sclerostin (only available at 24 months)	-	-	-	
Vitamin D (ng/ml, mean)	23.7 (7.3)	20.8 (7.8)	22.3 (7.0)	0.12
<20, n (% of gender category)	39 (37.5)	15 (51.7)	39 (40.6)	0.74
20-29.9	49 (47.1)	10 (34.5)	43 (44.8)	
30+	16 (15.4)	4 (13.8)	14 (14.6)	
Baecke Activity Factor	1.59 (0.10)	1.56 (0.09)	1.57 (0.11)	0.19

Table 2 biomarkers before and after weight loss for all participants:

Variable	N	Mean	Std Dev	Min	Max
ln_adiponectin_0	229	8.71	8.51	7.27	10.02
ln_adiponectin_24	229	8.81	.049	7.50	9.96
calcium_0	229	9.88	0.41	8.50	11.10
calcium_24	229	10.08	049	8/80	11.60
sq_ctx_0	229	0.53	0.12	0.22	0.87
sq_ctx_24	229	0.56	0.12	0.24	0.96
ln_leptin_0	229	1.92	0.62	0.10	3.35
ln_leptin_24	229	1.67	0.79	-0.69	3.28
ln_osteocalcin_0	229	3.54	0.34	2.34	4.50
ln_osteocalcin_24	229	3.53	0.35	2.53	4.38
pth_0	229	43.50	12.82	15.00	82.00
pth_24	229	48.01	14.74	23.00	111.00
sclerostin_0	0				
sclerostin_24	226	1181.98	372.16	290.00	3019.00
vitd_0	229	22.72	7.25	6.70	52.4
vitd_24	229	25.52	8.67	8.20	58.00

Table 3: Changes in Biomarkers

Parameter	Men (n=104)	Pre-M women (n=29)	Post-M women (n=96)	P
Weight change (kg)[1]	-8.0 (0.8)[a]	-5.4 (1.5)[ab]	-4.8 (0.8)[b]	**0.013**
Weight change (kg)[2]	-6.8 (0.9)	-6.4 (1.5)	-5.8 (0.9)	0.74
CTX change[3*] (ng/mL)	0.025 (0.010)	0.039 (0.017)	0.014 (0.010)	0.465
Osteocalcin change (*ln* ng/mL)[4**]	-0.027 (0.035)	0.102 (0.062)	-0.013 (0.037)	0.187
Sclerostin 24 (pg/mL)[5]	1100 (42)[a]	1131 (70)[ab]	1269 (40)[b]	**0.026**
B-coefficient of weight change on sclerostin [2*]	-3.0 (4.2) [P=0.48]	3.5 (11.4) [P=0.77]	-0.7 (5.9) [P=0.91]	
Leptin change (*ln* ng/mL)[7**]	-0.62 (0.06)	0.17 (0.09)[a]	0.04 (0.06)[a]	**<0.0001 ; r^2=0.53**
Adiponectin change (*ln* ng/mL)[8**]	0.182 (0.052)	0.082 (0.091)	0.023 (0.055)	0.16
Vitamin D Change[9]	2.8 (0.6)	2.5 (1.0)	2.7 (0.6)	0.97
PTH change (pg/mL)[10**]	-0.1 (1.9)	7.2 (3.1)	6.3 (1.7)	**0.047**
Ca change (mg/dL)[4**]	0.21 (0.04)	0.21 (0.07)	0.19 (0.04)	0.97
Total BMD change[1]	0.022 (0.003)[a]	-0.010 (0.006)[a]	-0.009 (0.004)	**<0.0001 ; r^2=0.18**
Total BMD change[6]	0.023 (0.003)[a]	-0.009 (0.007)[a]	-0.010 (0.004)	**<0.0001 ; r^2=0.18**

[1]Adjusted for diet assignment
[2]Adjusted for diet assignment, baseline weight, age
[3]Adjusted for diet assignment, baseline weight, age, weight change, baseline CTX
[4]Adjusted for diet assignment, baseline weight, age, weight change, baseline osteocalcin
[5]Adjusted for diet assignment, baseline weight, age, weight change
[6]Adjusted for diet assignment, baseline BMD, age
[7]Adjusted for diet assignment, baseline weight, age, weight change, baseline leptin
[8]Adjusted for diet assignment, baseline weight, age, weight change, baseline adiponectin
[9]Adjusted for diet assignment, baseline weight, age, weight change, baseline vitamin D
[10]Adjusted for diet assignment, baseline weight, age, weight change, baseline PTH
[11]Adjusted for diet assignment, baseline weight, age, weight change, baseline Ca

Interactions of Sex with Change in Biomarkers and Change in Bone Mineral Density

1) Change in BMD = sex + CTX (change) + CTX*sex

Change in BMD by Sex **P<0.0001**

Change in BMD by Change in CTX **P<0.0001**

Interaction of Sex*CTX on Change in BMD **P<0.0071**

Given this interaction, we evaluated the relationship between change in BMD and change in CTX by sex and adjusted for baseline CTX and weight change for each sex group:

Males: change in BMD = -0.0101 **– 0.01137***change in CTX + **0.0446***baseline CTX (**Non-significant**) – 0.0011*change in weight (Non-significant)

Pre-M Females: change in BMD = 0.047 **-0.2422***change in CTX -0.0932*baseline CTX (**P<0.0001**) – 0.00007*change in weight

Post-M Females: change in BMD = 0.0192 **– 0.0981***change in CTX (**P=0.0031**) -0.0380*baseline CTX + **0.00125***change in weight **(P<0.021)**

Figure 1: Relationship between Change in CTX and Change in BMD for Males

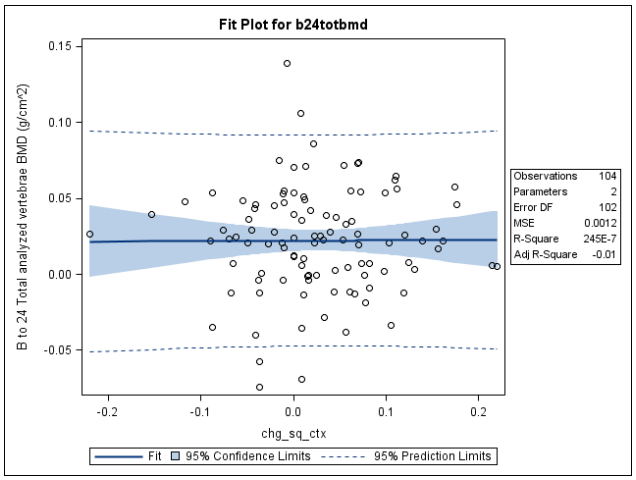


Figure 2: Relationship between Change in CTX and Change in BMD in Pre-Menopausal Females

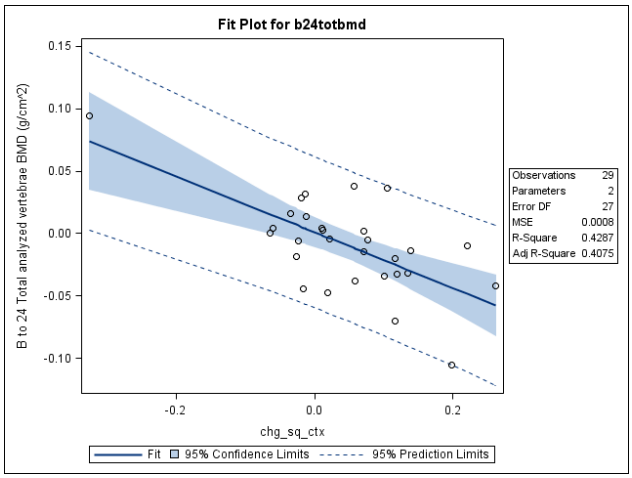
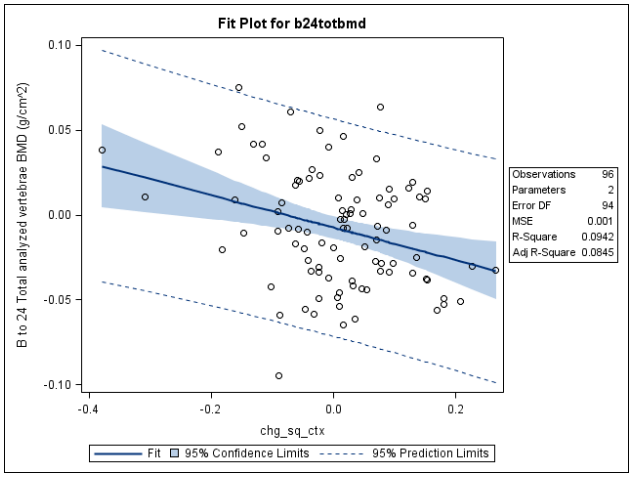


Figure 3: Relationship between Change in CTX and Change in BMD in Post-Menopausal Females



1) Change in BMD = sex + osteocalcin (change) +osteocalcin*sex

Change in BMD by **Sex** **P<0.0001**

Change in BMD by Change in osteocalcin **P<0.0151**

Interaction of Sex*osteocalcin on Change in BMD P<0.1223

2) Change in BMD = sex + sclerostin (24) +sclerostin*sex

Change in BMD by Sex **P<0.0001**

Change in BMD by month 24 Sclerostin P<0.22

Interaction of Sex*month 24sclerostin on change in BMD P<0.56

3) Change in BMD = sex + leptin (change) + leptin*sex

Change in BMD by Sex **P<0.0001**

Change in BMD by change in leptin P<0.348

Interaction of Sex*change in leptin on change in BMD **P<0.0085**

Given this interaction, we evaluated the relationship between change in BMD and change in leptin by sex and adjusted for baseline leptin and weight change for each sex group:

Males: change in BMD = 0.024**- 0.0041***change in leptin (**P=0.54**) -0.0068*baseline leptin (P=0.30) - 0.00083*change in weight (P=0.07)

Pre-Menopausal Females: change in BMD = -0.0537**- 0.0157***change in leptin (**P=0.31**) + 0.01809*baseline leptin (P=0.34) + 0.0001*wt change (P=0.92)

Post-Menopausal Females: change in BMD = 0.0178**+ 0.0025***change in leptin (**P=0.71**) - 0.0087*baseline leptin (P=0.19) + 0.0015*wt change (P=0.022)

Figure 4: Relationship between Change in Leptin and Change in BMD in Males

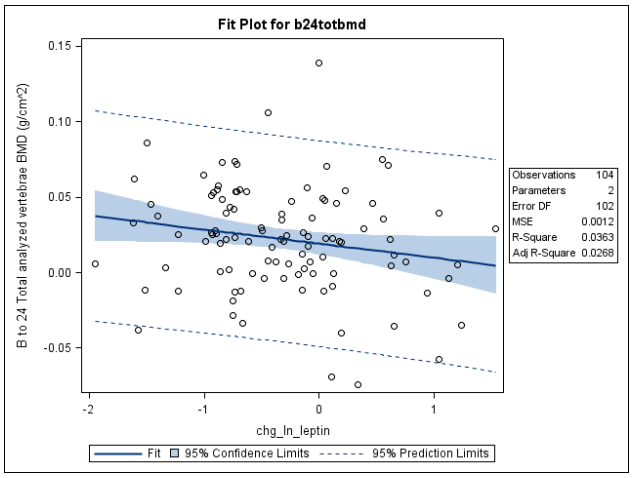


Figure 5: Relationship between Change in Leptin and Change in BMD in Pre-Menopausal Females:

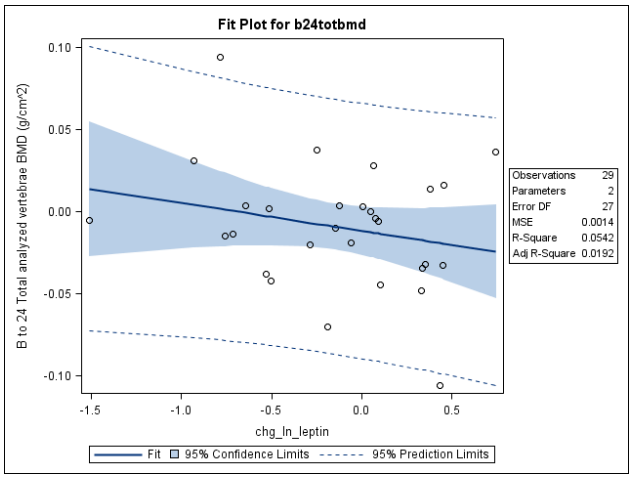
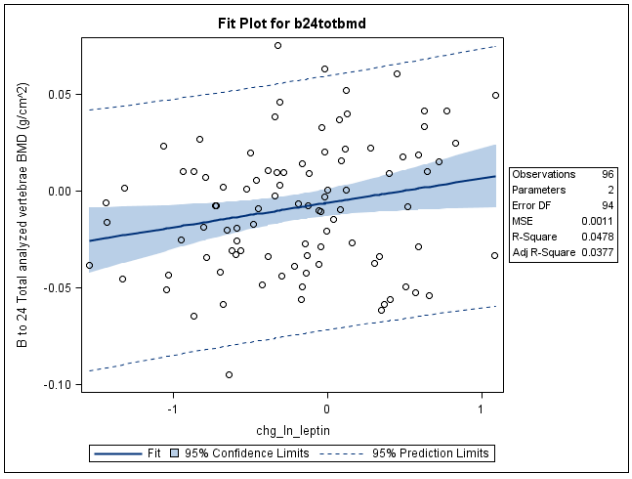


Figure 6: Relationship between Change in Leptin and Change in BMD in Post-Menopausal Females



4) **Change in BMD = sex + adiponectin (change) + adiponectin*sex**
   Change in BMD by sex **P<0.0001**
   Change in BMD by change in adiponectin P<0.33
   Interaction of Sex*change in adiponectin on change in BMD P<0.95

5) **Change in BMD=sex+ vitD (change) + vitD*sex**
   Change in BMD by Sex **P<0.0001**
   Change in BMD by change in Vitamin D **P<0.08**
   Interaction of Sex*change in vitamin D on change in BMD P<0.68
6) **Change in BMD = sex + PTH(change) + PTH*sex**
   Change in BMD by Sex **P<0.0001**
   Change in BMD by change in PTH P<0.84
   Interaction of Sex*change in PTH on change in BMD P<0.91
7) **Change in BMD = sex + calcium (change) + calcium*sex**
   Change in BMD by Sex **P<0.0001**
   Change in BMD by change in Ca P<0.42
   Interaction of Sex*change in Ca on change in BMD P<0.82

Section 5: Discussion, Limitations, Conclusions, and Suggestions

Participant Characteristics:

In this study, males started at a significantly higher BMI than females. Males also lost significantly more weight than females, but when adjusted for baseline weight, the changes in weight between females and males were similar, suggesting that though males lost more weight, they did not lose more weight relative to their starting weight. Males gained BMD at the spine while both pre and post-menopausal women lost BMD at the spine, consistent with the original body composition analyses completed in this study population (Tirosh et al 2015). This BMD loss remained significant when adjusted for diet assignment, baseline weight, age, weight change, and baseline BMD.

Bone turnover

We evaluated the biomarkers of bone turnover c-telopeptide (CTX) and osteocalcin. CTX is cleaved by osteoclasts during bone resorption and is thus used as a specific marker as bone resorption. Bisphosphonate treatment for osteoporosis works by reducing bone turnover and has been associated with a decline in CTX (Rosen et al 2000). Osteocalcin is a protein secreted by the osteoblast and is important for building bone; higher serum osteocalcin levels have been correlated to increases in bone mineral density during osteoporosis treatment with teriparatide. Additionally, higher osteocalcin levels have been observed in patients in the months following a fracture, suggesting is role in bone formation and repair. More recent research has demonstrated that osteocalcin stimulates the pancreatic beta cells to proliferate and secrete insulin and stimulates adipocytes to enhance adiponectin secretion and reduce insulin resistance, suggesting a general anabolic role for this peptide (Chapurlat et al 2016).

In this study, there were no sex differences in the change in CTX with weight loss. There was, however, a correlation between change in CTX and change in BMD, and there was an interaction of sex on that correlation, suggesting that there were sex differences in the relationship between change in CTX and change in bone mineral density. For males, there was not a significant association between change in bone mineral density and change in CTX (as shown in Figure 1). In both pre-menopausal and post-menopausal females, there was an association between change in BMD and change in CTX: as females lost more BMD, there was a greater increase in CTX (as shown in Figures 2 and 3). Changes to CTX explained 42% of the loss in BMD in the pre-menopausal females (as shown in Figure 2) and 9% of the loss in BMD in the post-menopausal females (as shown in Figure 3). This suggests that increases in bone resorption are correlated with bone loss with weight loss in females.

In this study, there were no sex differences in change in osteocalcin with weight loss. There was an association between change in osteocalcin and change in bone mineral density, but there was not a significant interaction of sex on this association, suggesting that sex differences in bone loss with weight loss are not explained by differences in changes to osteocalcin.

Other studies have shown that bone turnover increases with weight loss, though none to our knowledge have demonstrated sex differences in CTX (Shapses 2001; Shapses 2012). This is clinically important because increases in bone turnover have been associated with increased rates of bone loss and elevated fracture risk in postmenopausal women (Garnero and Hausherr 1996; Garnero and Sournay-Rendu 1996; Garnero 2010; Shapses 2001). Treatment aimed at reducing bone turnover, such as with bisphosphonates, may be helpful for protecting bone health in women who want to lose weight.

Sclerostin

Sclerostin is a Wnt antagonist that is secreted by the osteocyte, leading to inhibition of osteoblast maturation and proliferation and reduced bone formation (Villareal et al 2012). In animal studies, sclerostin has been shown to increase with skeletal unloading and decrease with loading mechanical stimulation of bone (Robling et al 2008). Villareal et al demonstrated that sclerostin significantly increased with weight loss and was inversely related to lean body mass change, suggesting that sclerostin may mediate the relationship between reduced mechanical stress and bone loss in obese patients who lose weight (Villareal et al 2012). The rise in sclerostin and the fall in bone mineral density was prevented in patients who received weight-bearing exercise therapy in addition to dietary intervention with weight loss. This further implicates sclerostin as a mediator of reduced mechanical stress-induced bone loss in patients undergoing weight loss. Sclerostin monoclonal antibodies such as blosozumab are currently in clinical trials as treatment for osteoporosis (Recker et al 2014; Ishtiaq et al 2014; Lewiecki EM 2011).

In our study, there were sex differences in sclerostin at month 24 with weight loss; when adjusted for diet assignment, baseline weight, age, and weight change, postmenopausal females had higher levels of sclerostin than pre-menopausal females and than males. There was no correlation of weight change with month 24 sclerostin, suggesting that the magnitude of weight change did not correlate with levels of sclerostin at 24 months. Furthermore, there was no correlation of change in bone mineral density with sclerostin at 24 months, and there was no interaction of sex and sclerostin on the change in BMD. These results suggest that in our study, while there were sex differences in sclerostin after weight loss, sclerostin did not correlate with amount of weight lost or amount of change in bone mineral density. Additionally, there were not sex differences in the correlation between sclerostin and bone mineral density change with weight loss. This suggests that sclerostin at month 24 does not explain the sex differences in bone loss with weight loss in this study.

Adipokines

Leptin is a hormone released from adipose tissue that acts through the hypothalamus to reduce food intake (Upadhyay 2015). Prior studies have demonstrated that leptin levels are higher in obese patients than non-obese individuals and that leptin decreases with weight loss (Poonpet 2014, Fathy 2014, Miller 2014). Leptin has been shown to increase bone mineral density through both direct and indirect

pathways. Leptin may peripherally increase bone mass by activating the osteoblast through its receptor on osteoblast cells as well as through the activation of fibroblast growth factor 23 (FGF-23). Centrally, leptin acts on the leptin receptor in the hypothalamus to activate osteoblast formation (Upadhyay 2015, Wee 2014). Leptin has been associated with increased total bone mineral mass and bone mineral density as well as increased lumbar spine and femoral neck bone mineral density (Jurimae 2008).

In this study, there were sex differences in change in leptin with weight loss (as shown in Table 3). While there was not a correlation between change in leptin and change in bone mineral density, there was an interaction of sex and change in leptin on the change in bone mineral density. In males, there was a significant decrease in leptin as BMD increased (as shown in Figure 4). This relationship was not seen in pre- and post-menopausal women (as shown in Figures 5 and 6).

Adiponectin is also released from adipose tissue and is inversely correlated with body fat percentage (Ukkola 2002). Weight loss has been associated with an increase in adiponectin (King 2014, Poonpet 2014, Shapses 2006). Prior studies have also demonstrated that women have higher adiponectin levels than men (Filkova 2009). It has been posited that adiponectin suppresses the number of osteoclasts and may activate osteoblastogensis (Shapses 2006). This would suggest that an increase in adiponectin with weight loss would lead to less reduction in bone mineral density.

In this study, there were not sex differences in the change in adiponectin with weight loss. Furthermore, there was not a correlation between change in adiponectin with change in bone mineral density, and there was no interaction of sex on the association between change in adiponectin and change in bone mineral density. This data suggests that, in this cohort, sex differences in bone loss with weight loss are not explained by changes in adiponectin.

Calcium Homeostasis

In this sample, both women and men showed low 25-hydroxyvitamin D levels (≤ 30 ng/ml) at baseline. This is consistent with previous studies that have shown vitamin D deficiency in obese participants, most likely because vitamin D is sequestered in adipose tissue (Hultin 2010) and/or obese individuals have a reduction in vitamin D binding protein (VDBP). Vitamin D increased in both women and men with weight loss. If obese individuals have a lower VDBP, it remains to be clarified whether they have a similar or lower free vitamin D level compared with normal weight individuals (Powe et al 2011). Thus it is possible that overweight and obese patients may be deficient in vitamin D and may need supplementation to ensure adequate levels.

In this study, there were no sex differences in the change in vitamin D with weight loss. There were also not sex differences in the relationship between change in vitamin D and change in bone mineral density. In both sexes, there was a trend toward an association between change in vitamin D and change

in bone mineral density, consistent with the known biological activity of vitamin D as important for calcium absorption and bone formation.

Parathyroid hormone (PTH) is released by the parathyroid gland and plays an important role in regulating serum calcium. It enhances calcium release from bone by stimulating osteoblasts, which in turn stimulate the production of osteoclast precursors that break down bone and release calcium. It also increases the reabsorption of calcium in the renal distal tubules and renal collecting ducts and inhibits the reabsorption of phosphate in the kidney. Finally, PTH is responsible for the conversion of 25-hydroxy vitamin D into 1,25-dihydroxy vitamin D (calcitriol), the active form of vitamin D that is important for stimulating calcium uptake from the intestine (Blaine et al 2015). PTH is thus an important contributor to bone resorption. In this study, there were sex differences in the change in PTH with weight loss. Men lost PTH, while women gained PTH over the course of the study. However, those change in PTH were not correlated with changes in BMD. Furthermore, there was no interaction of sex on the relationship between change in PTH and change in BMD, suggesting that there were no sex differences in the relationship between change in PTH and change in BMD with weight loss. Thus, in this study, change in PTH does not explain the change in BMD with weight loss.

Finally, there were not sex differences in change in calcium with weight loss in this cohort. There was also no association between change in serum calcium and change in BMD throughout the study, and there was no interaction of sex on the association between change in serum calcium and change in BMD. This suggests that, in this cohort, changes to serum calcium did not explain the observed changes in BMD with weight loss that occurred in females but not males.

Limitations

Due to limited amount of serum available for analyses, we were not able to measure sclerostin at baseline, so we have data only for sclerostin after two years of weight loss. We accounted for this limitation by measuring the B-coefficient of weight change on sclerostin to evaluate if there was a relationship between the amount of weight lost and the month 24 sclerostin level. There was no relationship between month 24 sclerostin and amount of weight lost or amount of bone change. In a future study, it would be beneficial to evaluate change in sclerostin as related to change in BMD to determine if there is a relationship between changing sclerostin levels and change in BMD and if there are sex differences in that change. Furthermore, due to limitations in the amount of serum, we were not able to measure all of the month 24 PTH levels. We did have sufficient baseline and month 24 PTH levels to measure a change in PTH and to correlate that change with BMD.

In this cohort, the average age of pre-menopausal females was 48.1 years, which is close to the average age of menopause (51 years old). However, the perimenopausal period may last four to eight years, and it is possible that, while these females were classified as pre-menopausal because they had not

had one year of cessation of menses, they had begun to enter the perimenopausal period (North American Menopause Society). As such, it is possible that some of the similarities between the groups classified as pre-menopausal females and post-menopausal females exist because those in the pre-menopausal group had entered perimenopause and have begun to experience hormonal changes of menopause that may affect their bone health and biomarkers. It would be useful in future analyses to look at younger pre-menopausal females to evaluate if there are differences between early pre-menopausal and late pre-menopausal females in terms of BMD and biomarkers of bone health.

Future Directions

This study examined the relationship between biomarkers of bone turnover, adipokines, and calcium homeostasis and changes in BMD at the spine with weight loss. The next step in our analyses is to evaluate changes in BMD at the total hip and femoral neck as in the original POUNDS LOST study, females lost more bone mineral density than males at those sites (Tirosh et al 2015). Additionally, it is possible that these biomarkers may lead to bone loss indirectly through changes in body composition. For example, changes in leptin may reflect a change in visceral adipose tissue that changes the mechanical stress on bone, thus leading to greater bone loss. In the original analysis of body composition in this study population, changes in lean mass and fat mass were correlated with changes in bone mineral density, and there were sex differences in these correlations (Tirosh et al 2015). The next direction for analysis of this data is to test the hypothesis that changes in the studied biomarkers may lead to change in BMD indirectly through changes in body composition by measuring the relationship between changes in biomarkers, changes in adipose and lean mass tissue, and changes in BMD and evaluating sex differences in these relationships.

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