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Long-term Weight Loss Maintenance, Sex Steroid Hormones and Sex Hormone Binding Globulin

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Abstract

Objective: We tested the effects of weight loss on serum estradiol, estrone, testosterone and sex hormone binding globulin (SHBG) in overweight/obese women 18-months after completing a year-long, 4-arm, randomized-controlled dietary weight loss and/or exercise trial.

Methods: From 2005–2008, 439 overweight/obese, postmenopausal women (BMI>25 kg/m²), 50–75 years, were randomized to a year-long intervention: diet (reduced calorie, 10% weight-loss, N=118), exercise (225 min/week moderate-to-vigorous activity, N=117), combined diet+exercise (N=117), or control (N=87). At 12-months, 399 women provided blood; of these, 156 returned at 30 months and gave a blood sample. Hormones and SHBG were measured by immunoassay. Changes were compared using generalized estimating equations, adjusting for confounders.

Results: At 30-months, participants randomized to the diet+exercise intervention had statistically significant increases in SHBG levels vs. controls (P=0.001). There was no statistically significant change in SHBG in the exercise or diet intervention arms. Hormone levels did not vary by intervention arm from baseline to 30-months. Participants who maintained weight loss at 30-months had statistically significantly greater decreases in free estradiol and free testosterone (P_{trend}=0.02, P_{trend}=0.04, respectively) and increases in SHBG (P_{trend}<0.0001) vs. those who did not have sustained weight loss. Levels of other analytes did not vary by weight loss at 30 months.

Conclusions: Sustained weight loss results in reductions in free estradiol and testosterone and increases in SHBG 18-months post-intervention.

Keywords

Sex steroid hormones; weight loss maintenance; SHBG

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INTRODUCTION

Increased body mass index (BMI) and adiposity are strongly associated with risk of postmenopausal breast cancer: a population-based report estimated that the global population attributable fraction of postmenopausal breast cancer attributable to high BMI was 34% (32%–36%), with the highest—48%—observed in North America.¹ In postmenopausal women, adipose tissue is the primary site of endogenous estrogen production via aromatization of androgens, and circulating levels of sex steroid hormones are strongly and positively associated with BMI.² This may partially explain the link between excess body fatness and increased risk of postmenopausal breast cancer:^{3, 4} higher levels of circulating estrogens are associated with risk of developing postmenopausal breast cancer,^{5, 6} with odds ratios in the highest vs. lowest quintile between 1.46 and 2.66.² We previously reported that weight loss, with or without exercise, produced large and statistically significant reductions in circulating levels of sex steroid hormones after 12 months. The Nutrition and Exercise in Women (NEW) study (<http://www.Clinicaltrials.gov> identifier NCT00470119), a 12-month randomized controlled trial (RCT), randomized 439 overweight/obese, postmenopausal women into one of four groups: a reduced-calorie weight loss diet and/or moderate- to vigorous-intensity aerobic exercise vs. control. Weight loss was associated with statistically significant reductions in circulating serum estrone, estradiol, free estradiol, and free testosterone and increases in SHBG, with greatest degrees of weight loss associated with larger reductions in estrogens.⁷ Here, we investigate whether long-term weight loss maintenance, 18 months after completion of the study (i.e., 30-months post-randomization) translate into long-term reductions in circulating levels of sex steroid hormones. To our knowledge, this is the first study to examine the long-term effects of weight loss interventions on circulating levels of sex steroid hormones in postmenopausal women.

METHODS

Setting and Participants.

This study is ancillary to the NEW (<http://www.clinicaltrials.gov> NCT00470119) study, carried out in the Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA USA, and performed with the approval of the FHCRC Institutional Review Board, in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. Written informed consent was obtained from each participant. The parent trial is described in detail elsewhere.⁸ Briefly, 439 postmenopausal, healthy overweight (BMI ≥ 25 kg/m²), sedentary women, aged 50–75 years, were recruited through media and mass mailings. Specific exclusion criteria included use of estrogen, progesterone, or testosterone hormones of any form in the three months prior to enrollment; history of breast cancer or other serious medical conditions; >100 min/week of moderate physical activity; diagnosed diabetes or other serious medical condition(s); consumption of >2 alcoholic drinks/day; current smoking; participation in another structured weight loss program; and contraindication to participation (e.g. abnormal exercise tolerance test, inability to attend sessions). In addition to reporting no menstrual cycles for ≥ 1 year, women aged 50–59 years who had undergone a hysterectomy were required to have a follicle-stimulating hormone

(FSH) level of > 23.0 IU/L. Eligible women were enrolled in the study from 2005–2008; 12-month follow-up for all participants was completed in 2009.

Interventions.

Eligible participants were randomly assigned to a (i) reduced-calorie dietary modification intervention (N=118); (ii) moderate-to-vigorous intensity aerobic exercise intervention (N=117); (iii) combined diet and exercise intervention (N=117); or (iv) control (no intervention; N=87). Permuted block randomization was used to achieve a proportionally smaller control arm, stratified according to BMI ($<30 \text{ kg/m}^2$) and race/ethnicity. Investigators and laboratory staff were blinded to randomization arm. The reduced-calorie weight loss diet intervention was a modification of the dietary component of the Diabetes Prevention Program⁹ and the Look AHEAD¹⁰ lifestyle intervention programs, with a goal of total daily energy intake of 1200–2000 kcal/day based on baseline weight; <30% daily energy intake from fat; and a 10% reduction in body weight by 6 months with maintenance thereafter to 12 months. The exercise intervention goal was 45 minutes of moderate-to-vigorous intensity aerobic exercise, 5 days per week (225 minutes/week). Participants attended 3 supervised sessions/week at our study facility and for remaining sessions exercised at home. The program progressed to the maintenance target of 70–85% maximal heart rate for 45 minutes by week 7. Activities with 4 metabolic equivalents (METs),¹¹ such as brisk walking, were counted towards the prescribed exercise target. Women randomized to the diet+exercise intervention received both the reduced-calorie weight loss diet and the aerobic exercise interventions. Women randomized to the control group were requested not to change their diet or exercise habits. *Food 'journaling', weekly weigh-ins, and session attendance were tracked to promote adherence to the diet intervention. Physical activity logs were reviewed weekly by study staff to monitor adherence. Participants not meeting exercise targets were contacted by staff to discuss barriers and approaches to increase activity. Dietitians and exercise physiologists met regularly with clinical health psychologists experienced in lifestyle behavior change to discuss participant progress and refine behavior modification goals. Women randomized to the control arm received no contact with study staff until their 12- and 30-month follow-up appointments.*⁸

Participant Follow-up at 12- and 30-months post-randomization.

399 (91.1%) participants completed physical exams and provided a blood sample at the 12-month time-point. For the current analysis, we excluded 17 women who had circulating baseline hormone concentrations that were outside the acceptable ranges for postmenopausal women. These additional exclusion criteria were: estrogen use during the trial (n=1); serum estradiol $>42 \text{ pg/mL}$ (n=13), testosterone $>100 \text{ ng/dL}$ (n=1), or SHBG $>180 \text{ nmol/L}$ (n=1). In addition, one participant was excluded due to a missing baseline blood sample. Of the 439 NEW study participants, 421 were eligible for inclusion in the current analysis, of whom 383 participants had attended the 12-month interview. For this extended follow up ancillary study, women were identified from study records as they approached their 30-month anniversary of randomization. Seventy-five women were >30 months post-randomization at the time recruitment began, and were thus ineligible for inclusion. To assess interest, women were contacted by study staff, and invited to be part of the follow-up study. Of 307 eligible women (i.e., those were still within the 30-month timeframe, and with hormone levels in

normal ranges) contacted from the parent trial, 204 (66.5%) women agreed to provide questionnaire-based follow-up data and of these, 151 (49.2%) provided fasting blood samples (N=44 diet (43.1%); N=42 (38.9%) diet+exercise; N=42 (42%) exercise; N=23 (31.5%), control).

Blood Specimen Collection and Processing.

Fasting (12 hours) venous blood samples (50 mL) were collected during clinic visits at baseline (pre-randomization), 12-months, when the original study was completed and at 30-months post-randomization. Participants refrained from alcohol (48 hours), vigorous exercise, or NSAID use (24 hours) prior to blood collection. Blood was processed within one hour, and stored at -70°C . Laboratory assays were performed at the Reproductive Endocrine Research Laboratory (University of Southern California). Estrone, total estradiol, total testosterone were quantified by radioimmunoassay after organic solvent extraction and Celite column partition chromatography.^{12, 13} SHBG was quantified via chemiluminescent immunometric assay using the Immulite Analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). Free estradiol and free testosterone were calculated using measured values for total estradiol, total testosterone, SHBG, and an assumed constant for albumin.^{14–16} Duplicate pooled blood samples were included for quality assurance purposes and to assess inter and intra-assay coefficient of variation (CV). Laboratory personnel were blinded to sample identity. To correct for assay drift over time and to incorporate these data into the baseline and 12-month data, we included a series of baseline and 12-month samples that matched samples that had been previously assayed in each batch of 30-month samples.⁷ We compared the results from these samples with previously measured results, and used mean of the difference to normalize the newly assayed 30-month data. The inter-assay coefficients of variation ranged from 8–13% for the steroid hormone assays and 5–7% for the SHBG assay. The sensitivities of the estrone, estradiol and testosterone assays are 4 pg/mL, 2 pg/mL, and 1.5 ng/dL, respectively.

Covariates.

All study measures were obtained at baseline, 12- and 30-month time-points by trained personnel blinded to participants' randomization status. Height and weight were measured and body mass index (BMI, kg/m^2) calculated. Fat mass and % body fat were measured by Dual-energy X-ray absorptiometry whole-body scanner (GE Lunar, Madison, WI). Cardiorespiratory fitness (VO_2max) was assessed using a maximal graded treadmill test according to a modified branching protocol.¹⁷ Validated questionnaires collected information on demographics, medical history, dietary intake, supplement use and physical activity patterns.⁸

Statistical Analyses.

The Kolmogorov-Smirnov test was used for the baseline comparisons between participants vs. non-participants since the assumption of homogeneous variances between the two samples may not hold for all variables. Descriptive data are presented as geometric means (95% confidence intervals (CI)). *We compared average changes in outcomes from baseline to 30-months by randomization arm, using the generalized estimating equations (GEE) modification of linear regression to account for the correlation within individuals over time.*

Main analyses were performed according to the intention-to-treat principle. For secondary, pre-planned analyses, we combined data for all women regardless of randomization group. Participants were divided into four categories based on change from baseline to 30 months: 1) gained weight/remained weight stable; 2) lost >0 to <5% of baseline weight; 3) lost 5- <10% of baseline weight; and 4) lost 10% of baseline weight. We then compared changes in levels of analytes from baseline to 30-months stratified by these four weight loss categories. *All models were adjusted for age, baseline BMI (<30kg/m², >30kg/m²) and race/ethnicity. P_{trend} values were obtained from a GEE model for the comparison of the changes from baseline to 30 months in the biomarkers between higher versus lowest (Loss<0%) levels of 30 months weight change percentage adjusted for race/ethnicity and age and BMI at baseline.* All statistical tests were two-sided. After Bonferroni correction for 4 comparisons, $p<0.01$ was considered statistically significant. Statistical analyses were performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC).

RESULTS

Extended follow-up study participants.

At 30 months, 151 (49%) of 308 women who had completed the 12-month assessment, who had not passed their 30-month post randomization anniversary at time of recruitment, and who had hormone levels within normal ranges for post-menopausal ranges, provided a blood sample and completed questionnaires. At baseline, extended follow-up study participants were on average 58.5 years, and were predominantly non-Hispanic Whites (87.4%; Table 1). Compared to non-participants, women who agreed to take part in the extended follow-up study had lower baseline BMIs (*31.4 vs. 30.0 kg/m²; P=0.007*), and lower lean mass (*40.9 vs. 39.1 kg P<0.001*). Baseline levels of estrone, estradiol and SHBG did not differ between extended follow-up study participants vs. non-participants, but circulating levels of testosterone, free estradiol and free testosterone were statistically significantly lower among extended follow-up study participants vs. non-participants (*27.4 vs 24.8 ng/dl P=0.01; 0.36 vs. 0.33 pg/mL P=0.004; and 5.9 vs. 5.2 pg/mL P=0.003* respectively).

Changes in circulating levels of sex steroid hormones at 30-month time-point (the extended follow up study).

At 30-months the mean reduction in BMI was -2.6% in the control arm, -6.6% (P=0.05) in the diet arm, -2.0% (P=0.53) in the exercise arm, and -7.9% (P=0.005) in the diet+exercise arm. Participants randomized to the diet+exercise intervention arms had statistically significant increases in SHBG levels compared to controls (controls 47.9%; diet+exercise +80.5%, P=0.001). *There were no statistically significant changes in levels of SHBG in either the diet and exercise arms compared to controls* (diet +67.7%, P=0.05; exercise +53.7%, P=0.52). There were no statistically significant changes from baseline to 30 months in levels of total estradiol estrone, total testosterone, free testosterone, or free estradiol in any intervention arm compared to control (data not shown).

Weight loss effects.

We combined all participants' data regardless of intervention arm and stratified by categories of weight loss. Participants who maintained any degree of baseline weight loss at 30-months

had statistically significant greater increases in SHBG compared to those who lost no weight/gained weight (Table 2). Circulating levels of SHBG increased in tandem with increasing degree of weight loss: no weight loss (0%), +31.7%; 0–5% weight loss, +58.2%; 5–10% weight loss, +72.7%; and 10% weight loss, +107.9% ($P_{\text{trend}} < 0.0001$). Statistically significant reductions were observed in circulating levels of free testosterone (0% weight loss +1.4%; 0–5% –7.7%; 5–10%, –7.5%; and 10%, –18.0% $P_{\text{trend}} = 0.04$) and free estradiol (0% weight loss, –46.7%; 0–5%, –54.2%; 5–10%, –51.2%; 10%, –61.5% $P_{\text{trend}} = 0.02$) across all three weight loss categories compared to those who lost no weight/gained weight. Levels of estrone, estradiol or testosterone did not vary by degree of weight loss at 30 months.

DISCUSSION

At 30-months, participants randomized to either diet or diet+exercise arms only had statistically significant increases in levels of SHBG between baseline and 30-months, compared to participants randomized to the control arm. In contrast, there were no statistically significant changes among levels of estrone, estradiol, testosterone, free estradiol, or free testosterone, in participants randomized to any of the intervention arms, compared to participants who had been randomized to the control arm. In the parent study, weight loss appeared to account for most of the reductions in sex steroid hormones and increases in SHBG, with increasing weight loss associated with statistically significant linear changes at 12 months in all analytes.⁷ Because of the relatively small numbers for the 30-month analysis, we were unable to stratify individual randomization arms by weight loss categories. However, when we combined all participants and stratified by four weight loss categories, participants who maintained any degree of weight loss compared to baseline weight, had statistically significant reductions in circulating levels of free estradiol and free testosterone, with greater effects seen with higher degrees of weight loss. Weight loss maintenance was also associated with statistically significant increases in circulating levels of SHBG, again, with greater amounts of weight loss associated with greater increases in SHBG. Unlike the 12-month results, there were no statistically significant reductions in levels of total estradiol, estrone, or testosterone with any level of weight loss maintenance compared to those who did not lose weight/gained weight, and reductions in circulating levels of free estradiol and free testosterone at 30 months are likely to be in part attributable to increases in SHBG due to weight loss. Weight loss maintenance after cessation of weight loss/prevention activities, and associations with disease risk-factor reduction have been studied in the context of type 2 diabetes. The Diabetes Prevention Study reported that, despite a pattern of weight regain, participants successfully maintained some weight loss for a number of years after completion of the intervention, and risk of developing diabetes was reduced for several years post-intervention.¹⁸ This and other diabetes prevention studies found sustained and long-term benefits including a reduction in the cumulative incidence of diabetes, and lower fasting glucose levels, even after intervention activities ceased.^{18–20}

Beneficial changes in circulating sex steroid hormones with weight loss have been observed in shorter-term randomized clinical trials of exercise or dietary weight loss, and are principally attributed to reductions in adiposity,^{21–26} including the 12-month results in our study.⁷ An ancillary study nested within the Diabetes Prevention Program (DPP) reported

that reductions in visceral and subcutaneous adipose tissue in overweight glucose intolerant participants, were independently associated with significant increases in SHBG and estrone in postmenopausal women.²⁷ However, to our knowledge, no studies have examined the effects of long-term weight loss maintenance on circulating levels of sex steroid hormones after completion of a weight loss or exercise intervention. Here, postmenopausal overweight women who completed a 12-month reduced calorie weight loss program, (with or without exercise) 18 months prior to recruitment to the ancillary study, and who successfully maintained weight loss, demonstrated sustained decreases in free estradiol and free testosterone. These effects may have implications for modifying risk for postmenopausal breast cancer. Cumulative exposure of the breast epithelium to estrogens — either endogenous or exogenous — is an accepted risk factor for breast cancer, with higher exposures in postmenopausal women associated with increased risk. This increase in postmenopausal breast cancer risk, associated with increasing BMI has been attributed to an associated increase in estrogens, particularly bioavailable estradiol.^{28, 29} Weight loss maintenance in the present study was also associated with increased circulating levels of SHBG, which regulates the bioavailable fraction of circulating estradiol. While several studies reported that elevated SHBG levels are associated with decreased risk of postmenopausal breast cancer^{2, 5, 30}, one found no such association.³¹ However SHBG may also have direct effects on cells, including inhibition of progesterone receptor expression,³² increased apoptosis,³³ and regulation of cell growth.³⁴ As the extended follow-up ancillary study participants were not part of any formal weight-maintenance program post-intervention, the sustained beneficial changes in SHBG, free estradiol and free testosterone suggests that weight loss intervention effects may have long-term benefits for cancer risk reduction.

Limitations of our study include the relatively small number of women that returned for the 30-month follow-up visit, which limits the power to investigate effects in subgroups of data. However, the retention rate at 30 months is broadly similar those observed in other studies that assessed weight maintenance study participants more than 12 months after study interventions were completed— 40.3%³⁵; 57.1%²⁰; 48.4%³⁶; and 39.6%³⁷. Another concern is bias among women who returned for the 30-month assessment. Baseline differences between extended follow-up ancillary study participants vs. non-participants demonstrated statistically significant differences in baseline BMI and waist circumference, and women who maintained more weight loss at 30 months might have been more likely to return for the assessment. Strengths of our study include the well-characterized study population, a focus on postmenopausal women, a group at high risk for obesity-related breast cancer, and excellent participant adherence to interventions.

CONCLUSIONS

Maintenance of breast cancer risk-factor reduction via favorable changes in circulating sex steroid hormones in postmenopausal women who had completed a weight-loss intervention 18-months prior, indicate longer-term benefits of behavioral weight-loss strategies in overweight postmenopausal women. The findings should be investigated further in other randomized controlled trials of weight-loss with extended follow-up.

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Table 1.

Baseline covariates by extended follow-up study participation vs. non-participation

Characteristics	Extended follow-up study: Non-participants		Extended follow-up study: Participants		P ^a
	N=270		N=151		
	Mean	SD	Mean	SD	
Age (years)	57.9	4.7	58.5	5.5	0.48
VO ₂ max, ml/kg/min	23.0	3.9	22.6	4.2	0.92
Pedometer steps/day ^c	5625	2356	5853 N=149	2175	0.21
Daily calorie intake, kcal ^c	1890 N=261	643.9	1969 N=149	610.2	0.32
Weight (kg)	85.3	12.3	80.7	10.0	0.007
BMI ^c , kg/m ²	31.4	4.0	30.0	3.7	0.007
Body fat (%)	48.0	4.4	47.6	4.2	0.56
Lean mass (kg)	40.9	5.2	39.1	4.1	0.005
Alcohol (g/day) ^c	7.0 N=261	9.9	7.87 N=149	12.1	0.20
Total Estradiol (pg/mL)	13.0	6.0	11.9	5.3	0.08
Estrone (pg/mL)	37.9	15.1	35.9	16.3	0.13
Total Testosterone (ng/dL)	27.4	13.0	24.8	12.7	0.01
SHBG ^c (nmol/L)	38.9	18.1	40.1	16.7	0.35
Free Testosterone (pg/mL)	5.9	2.9	5.2	2.5	0.003
Free Estradiol (pg/mL)	0.36	0.17	0.33	0.16	0.004
Categorical variables	N	%	N	%	P ^b
Non-Hispanic white	226	83.7	132	87.4	0.31
Married/living with a partner	167	62.1	100	66.2	0.37
College degree	172	63.7	103	68.2	0.51
Never smoker	159	58.9	92	60.9	0.33

^aP-value from a non-parametric Kolmogorov-Smirnov two-sample test for difference between study participants vs. non-participants.^bP-value from a Chi-square test for a difference in proportions between study participants vs. non-participants.^cQuestionnaire data not completed by all participants (pedometer data, alcohol or food frequency questionnaires). Superscripts indicate completed available data.^dBMI Body Mass Index; SHBG Sex Hormone Binding Globulin

Table 2.

Change from baseline to 30-months in the log-transformed (normalized) circulating levels of sex hormones by category of weight loss (all participants combined).

Biomarker	Weight change	Baseline			30 Months			Change	P value ^a
		N	Geometric mean	95% CI	N	Geometric mean	95% CI	(%)	
Total Estradiol	Loss 0%	33	11.2	9.8, 12.8	31	7.3	6.1, 8.6	-4.0 (-35.4)	Ref.
	Loss 0-5%	51	11.1	9.7, 12.6	46	6.5	5.7, 7.4	-4.6 (-41.3)	0.28
	Loss 5-10%	35	10.6	9.1, 12.2	33	6.8	5.8, 7.9	-3.8 (-35.8)	0.89
	Loss 10%	31	10.6	9.0, 12.5	30	5.8	4.9, 6.8	-4.8 (-45.4)	0.15
	P-trend								0.28
Estrone	Loss 0%	33	34.1	29.8, 39.2	31	37.6	33.2, 42.5	3.4 (10.0)	Ref.
	Loss 0-5%	51	33.7	29.8, 38.1	46	37.3	32.7, 42.5	3.6 (10.6)	0.97
	Loss 5-10%	35	31.6	27.4, 36.4	33	33.8	29.9, 38.2	2.2 (6.9)	0.70
	Loss 10%	31	30.5	26.0, 35.8	30	33.3	29.2, 37.9	2.8 (8.9)	0.79
	P-trend								0.72
Total Testosterone	Loss 0%	33	24.3	20.8, 28.2	31	26.6	22.5, 31.5	2.4 (9.7)	Ref.
	Loss 0-5%	51	22.5	19.9, 25.1	46	24.3	21.2, 27.9	2.0 (8.9)	0.89
	Loss 5-10%	35	20.5	18.4, 22.9	33	23.4	19.3, 28.3	2.8 (13.7)	0.76
	Loss 10%	31	22.8	19.2, 27.0	30	25.5	21.0, 30.9	2.7 (11.2)	0.95
	P-trend								0.84
SHBG ^b	Loss 0%	33	42.0	36.0, 48.9	33	55.3	47.4, 64.4	13.3 (31.6)	Ref.
	Loss 0-5%	51	35.0	30.9, 39.7	51	55.4	48.4, 63.3	20.4 (58.2)	<0.001
	Loss 5-10%	35	35.9	32.10, 40.2	34	62.0	54.4, 70.8	26.1 (72.7)	<0.001
	Loss 10%	31	35.3	30.5, 41.1	31	73.5	64.9, 83.1	38.1 (107.9)	<0.001
	P-trend								<0.001
Free Testosterone	Loss 0%	33	4.8	4.2, 5.5	31	4.9	4.1, 5.8	0.1 (1.4)	Ref.
	Loss 0-5%	51	4.8	4.3, 5.4	46	4.5	3.8, 5.2	-0.4 (-7.7)	0.29
	Loss 5-10%	35	4.4	3.9, 5.0	33	4.1	3.4, 5.0	-0.3 (-7.5)	0.29
	Loss 10%	31	4.9	4.1, 5.9	30	4.0	3.3, 4.9	-0.9 (-18.0)	0.03
	P-trend								0.04
Free Estradiol	Loss 0%	33	0.28	0.24, 0.33	31	0.15	0.13, 0.18	-0.13 (-46.7)	Ref.
	Loss 0-5%	51	0.30	0.26, 0.34	46	0.14	0.12, 0.16	-0.16 (-54.2)	0.09
	Loss 5-10%	35	0.28	0.24, 0.33	33	0.14	0.12, 0.16	-0.15 (-51.2)	0.36
	Loss 10%	31	0.29	0.24, 0.34	30	0.11	0.09, 0.13	-0.18 (-61.5)	0.005
	P-trend								0.02

^aP-value obtained from a generalized estimating equation (GEE) model for the comparison of the changes from baseline to 30 months in the biomarkers between higher versus lowest (Loss 0%) levels of 30 months weight change percentage adjusted for race/ethnicity and age and BMI at baseline.

^bBMI Body Mass Index; SHBG Sex Hormone Binding Globulin