Weight regain and breast cancer-related biomarkers following an exercise intervention in postmenopausal women

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Abstract:

Background: Epidemiologic studies have reported associations between weight fluctuations and postmenopausal breast cancer risk, however, the biological markers involved in this association are unknown. This study aimed to explore the associations between breast cancer-related biomarkers and weight regain following exercise-induced weight loss. Methods: From the 400 participants included in the Breast Cancer and Exercise Trial in Alberta, a total of 214 lost weight during the intervention and had follow-up blood samples, body composition and covariate measurements. Outcomes were measured at baseline, 12-months (end of the study) and 24-months (follow-up). Results: During follow-up, weight regain was 1.80 kg (95% CI: -0.40, 3.90), and was significantly associated with increases in estradiol (treatment effect ratio (TER) = 1.03, 95% CI: 1.01, 1.04), estrone (TER = 1.02, 95% CI: 1.01, 1.03), free estradiol (TER = 1.04, 95% CI: 1.02, 1.05), the homeostatic model assessment for insulin resistance (TER = 1.03, 95% CI: 1.02, 1.05), insulin (TER = 1.03, 95% CI: 1.01, 1.04), and decreases in sex hormone binding globulin (SHBG) (TER= 0.98, 95% CI: 0.97, 0.99) levels. Non-statistically significant associations were found for glucose and CRP. Furthermore, a statistically significant linear trend of increasing levels for all biomarkers, and decreasing SHBG, across weight regain categories was found. Conclusions: These results suggest that weight regain following exercise-induced weight loss is associated with breast cancer-related biomarker changes. Impact: These findings provide evidence to support the importance of developing effective strategies to prevent weight regain and consequently, decrease postmenopausal breast cancer risk via changes in adiposity-related biomarkers.

Keywords: Body composition | breast cancer prevention | weight fluctuations | physical activity

Article:

INTRODUCTION

Breast cancer is one of the most commonly diagnosed cancers in women worldwide (1). Obesity is associated with postmenopausal breast cancer risk (2,3) since increases in body fat are related with a 1.5 to 2.0-fold increased risk (4). Multiple interrelated biologic pathways have been proposed to explain the associations between obesity and postmenopausal breast cancer risk (5), including increased circulating levels of sex steroid hormones coupled with lower levels of sex hormone-binding globulin (SHBG), as well as insulin resistance and inflammation (6).

Lifestyle and pharmacological interventions with the aim of promoting weight loss and decreasing breast cancer-related biomarkers have been assessed in clinical trials for the primary prevention of postmenopausal breast cancer (7-11). Sustained weight loss is associated with a reduction in breast cancer risk for women over 50 years (12). Unfortunately, the data available suggest that approximately 80% of participants who achieve weight loss will experience approximately 50% weight regain within one year following the end of the intervention (13-16). Regular physical activity participation and reduced energy and fat intake are important for long-term weight maintenance, influencing the relationship between postintervention weight regain and adverse health outcomes (17-19). The Women's Health Initiative Observational Study reported associations between weight gain and weight cycling (intentional weight loss and subsequent weight regain) with an increase in postmenopausal breast and endometrial cancer risk (20). However, the potential associations between changes in biologic markers associated with breast cancer risk and weight regain have yet to be fully explored. Given the associations between weight fluctuations and breast cancer risk, there is a need to clarify the biological markers involved in this relationship (20).

The Breast Cancer and Exercise Trial in Alberta (BETA) was designed to assess the effects of 12-month aerobic exercise interventions differing in prescribed exercise volume (150 versus 300 minutes/week of aerobic exercise) on biomarkers of postmenopausal breast cancer risk with 24-month follow-up measurements (21,22). During the follow-up period, no exercise intervention was prescribed. We have previously reported that 72% of participants regained weight during the follow-up period and some of the leading behavioral predictors of this weight regain were reductions in moderate-vigorous intensity physical activity time, increases in energy intake, fat intake, and sedentary time, as well as a delay in sleep timing midpoint (19). The present secondary analysis aims to complement these initial analyses by exploring the associations between breast cancer-related biomarkers (estradiol, free estradiol, estrone, SHBG, insulin, glucose, HOMA-IR and CRP) and weight regain following exercise-induced weight loss in postmenopausal women from BETA. We hypothesized that weight regain during 12-month follow-up would be associated with negative changes in these biomarkers.

MATERIALS AND METHODS

Study design and participants

The methods and study design for BETA are described in more detail elsewhere (23). This study was approved by the Alberta Cancer Research Ethics Committee, the Conjoint Health Research Ethics Board of the University of Calgary and the Health Research Ethics Board of the University of Alberta. BETA was a two-arm, two-center, 12-month randomized controlled trial (RCT) with 12-month follow-up assessments, conducted in Calgary and Edmonton, Alberta,

Canada. A total of 400 postmenopausal women were recruited and randomized to either 150 minutes/week (MODERATE volume) or 300 minutes/week (HIGH volume) of aerobic exercise. Women were postmenopausal, aged 50-74 years, had a body mass index (BMI) between 22-40 kg/m^2, were English speaking, did not have diabetes, recreationally inactive (<90 minutes/week of physical activity), non-smokers or excessive alcohol drinkers (no more than two alcoholic drinks/day), not taking hormone therapy, had normal levels of cholesterol, fasting blood glucose (<7mmol/L), thyroid-stimulating hormone and alanine aminotransferase, were not previously diagnosed with cancer, not planning to participate in any weight loss program or taking weight loss medications, and had received medical approval for participation in an exercise intervention. Written consent was obtained from each participant after a full explanation of the purpose and nature of all procedures used.

Intervention

The duration of the intervention was 12 months. Exercise prescription included moderate-to-vigorous intensity aerobic exercise at 65-75% of heart rate reserve on five days/week for either 30 minutes/session (MODERATE volume) or 60 minutes/session (HIGH volume). The exercise sessions were supervised on at least three days/week by certified exercise trainers during the entire 12-month intervention. Exercise prescription was gradually increased with a 12-week ramp-up period and adjusted during the intervention (23). Study participants were asked to maintain their usual diet during the intervention. During the 12-month follow-up period, participants did not receive an exercise prescription or regular follow-up/feedback from the certified exercise trainers. However, they were aware that a follow-up assessment of outcomes would be conducted.

Body composition measurements

Body composition measurements were assessed at baseline, 12-months (end of the intervention), and one year later (end of follow-up). Body weight was measured using a conventional balance beam scale with standardized methods. Measurements were taken in duplicate by research staff and if differences between the two measurements were noted, a third measure was taken, and the average was calculated. A whole-body dual X-ray absorptiometry (DXA) scan was used to measure fat-free mass (kg), fat mass (kg) and body fat percentage (%) to describe participants' body composition and to be used in sensitivity analyses. DXA scans were taken using a Hologic Discovery A DXA system in Calgary and a General Electric Lunar iDXA in Edmonton. Research staff calibrated DXA scanners every day before use, and staff members were blinded to the randomization group. All scans were done following a standardized procedure in Calgary and Edmonton.

Blood data and assays

The blood collection and processing protocol used in BETA have been published elsewhere (23). Briefly, blood samples were collected from all participants at baseline, 12 months and 24 months after at least a 10-hour fast. No exercise or alcohol intake was allowed for 24 hours before the blood sample was taken. Estrone and total estradiol levels were measured by radioimmunoassay after extraction by organic solvent and Celite column partition chromatography steps. The

sensitivity of the assays was 2 and 4 pg/ml, respectively. Inter-assay and intra-assay coefficients of variation were 11-16% and 8-10%, respectively. SHBG, insulin and high sensitivity CRP were measured by solid-phase, two-site chemiluminescent immunometric assays on the Immulite analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). The sensitivity of the assays was 1 nmol/L, 2 μIU/ml, and 0.2 mg/dL, respectively, and the inter-assay and intra-assay coefficients of variation were <10% for all outcomes. Glucose was quantified by a standard analytical procedure using the Vitros Chemistry System. Inter-assay and intraassay coefficients of variation were 2% and 1%, respectively. HOMA-IR was calculated as fasting glucose (nmol/L) x fasting insulin (μIU/mL)/22.5, and free estradiol levels were calculated using a validated formula with a constant for albumin concentrations of 43 g/L, total estradiol and SHBG concentrations (24). To monitor assay reliability, appropriate quality control samples were used. Participants' baseline and 12-month blood samples were analyzed in the same batch with an equal number of participants from each randomization group. All assays were repeated for the 24-month blood samples at a later time. Blind duplicates were included within and between batches to estimate coefficients of variation. All lab personnel were blinded to the intervention assignment.

Covariate measures

Baseline characteristics were measured with a Baseline Health Questionnaire, which included information on age, marital status (married/common-law versus not married or common-law), education (high school or less versus beyond high school), employment (full-time versus not employed or part-time work) and race/ethnicity (non-Hispanic White versus other). The use of nonsteroidal anti-inflammatory drugs and statins was also included in the model as possible confounders. Other covariates included in all analyses were the use study site (Calgary versus Edmonton), randomization group (150 minutes/week of aerobic exercise - MODERATE volume versus 300 minutes/week of aerobic exercise - HIGH volume) and weight loss during the exercise intervention (kg).

Energy intake was assessed using the Canadian Diet History Questionnaire-II and analyzed with the Diet*Calc Analysis Program (Version 1.4.3; National Cancer Institute Applied Research Program, Bethesda, MD, USA). Total physical activity time was assessed with the validated Past Year Total Physical Activity Questionnaire (25). Changes in total energy and fat intake (kcal), as well as changes in total physical activity time (MET-hours/week) during the follow-up period, were included as covariates in the models.

Statistical Analysis

For our primary analysis, linear regression models were used to estimate multivariable-adjusted beta coefficients (β) and 95% confidence intervals (95% CIs) between changes in breast cancer-related blood biomarkers (glucose, insulin, HOMA-IR, estradiol, free estradiol, estrone, SHBG and CRP) with changes in body weight during the follow-up period. Since the aim of these analyses was to explore associations between breast cancer-related biomarkers and weight regain following exercise-induced weight loss, only participants who had any degree of weight loss during the intervention were included in the present analysis. Participants from both randomization groups (HIGH and MODERATE) were combined for these analyses to maximize statistical power, however, these analyses did control for covariates related to the intervention

that could influence our results, including the randomization group (MODERATE versus HIGH), the study site (Edmonton versus Calgary) and the amount of exercise-induced weight loss during the intervention, given the strong association between weight loss and weight regain ($r = 1.00$, $p < 0.001$). Furthermore, analyses were adjusted by changes in total energy and fat intake (kcal), as well as changes in total physical activity, during the follow-up period. The model was also adjusted for the use of nonsteroidal anti-inflammatory drugs or statins, and other socio-demographic characteristics that could influence weight management (26),such as age at menopause, ethnicity, education level, employment and marital status. Sensitivity analyses were conducted including fat mass changes in the model instead of weight changes. Another sensitivity analysis was conducted including only women who lost ≥2 kg during the intervention period. Finally, we ran the model excluding women who were normal weight (BMI <25 kg/m^2) at baseline and, finally, adjusted the model for body mass index at baseline. Assumptions of normality and homogeneity were assessed by visual examination of quantile-quantile plots and histograms of the residuals and plots of the residuals versus the fitted values. Since all biomarker data were non-normally distributed, log-transformations were used in the analyses. Treatment effect ratios (TER) were estimated from these models using anti-log. A treatment effect ratio <1.0 indicated lower biomarker concentrations with increases in body weight during follow-up; a ratio >1.0 indicated higher biomarker concentrations with increases in body weight during follow-up; and a ratio equal to 1.0 indicated no change in biomarker concentrations with increases in body weight. The presence of influential observations was assessed using Cook's distance. Sensitivity analyses were conducted to determine if the results were robust to the removal of influential observations if present. After log-transformation, all results were robust to the removal of potentially influential points.

As a secondary aim, weight change during follow-up was categorized to explore mean differences in biomarker concentrations between participants who continued to lose weight or maintained weight loss during follow-up as the referent group with participants who regained between 0.1-2.5 kg (category 1), 2.6-4.9 kg (category 2) or ≥5 kg (category 3) of weight during follow-up. Trend analyses to explore the linear trend of biomarker levels across the weight regain categories were conducted. Treatment effect ratios were estimated from these models and represent the ratio of geometric mean biomarker levels during follow-up (reflecting changes from 12 months to 24 months) between the referent group with each category. A treatment effect ratio <1.0 indicated lower biomarker concentrations in the weight regain category versus the referent group during follow-up; a ratio >1.0 indicated higher biomarker concentrations in the weight regain categories versus the referent group during follow-up; and a ratio equal to 1.0 indicated no change in biomarker concentrations between weight categories.

Analyses were conducted using STATA (version 14.0, College Station, TX: StataCorp LLC). Statistical significance was set at $P < 0.05$.

RESULTS

The flow chart of all participants from BETA has been reported in previous studies (22,27). A total of 214 participants were included in the present analyses **(Figure 1)**. Participant characteristics for the complete sample and by weight regain categories are described in **Table 1**.

During the intervention, changes in weight for this sample were -3.60 kg (95% CI: -5.80, -1.80), and during follow-up, weight regain was 1.80 kg (95% CI: -0.40, 3.90). Furthermore, participants who regained more weight during follow-up had more weight loss during the intervention (**Table 1**). Results for changes in biomarkers, body composition and exercise adherence during the intervention period are described elsewhere (22,27). For this sample of participants who lost weight during the intervention, a decrease in estradiol, estrone, free estradiol, insulin, HOMA-IR, glucose and CRP, and an increase in SHBG, were observed after the 12-month exercise intervention, which is consistent with lower breast cancer risk (**Table 1**).

Weight regain during follow-up was significantly associated with increases in estradiol (TER = 1.03, 95% CI: 1.01, 1.04; P<0.001), estrone (TER = 1.02, 95% CI: 1.01, 1.03; P < 0.001), free estradiol (TER = 1.04, 95% CI: 1.02, 1.05; P < 0.001), and decreases in SHBG (TER= 0.98, 95% CI: 0.97, 0.99; P < 0.001) levels **(Figure 2)**. Furthermore, weight regain during follow-up was significantly associated with increases in HOMA-IR (TER = 1.03, 95% CI: 1.02, 1.05; P < 0.001) and insulin (TER = 1.03, 95% CI: 1.01, 1.04; P < 0.001). Non-statistically significant associations were found for glucose (TER = 1.00, 95% CI: 0.99, 1.01; P = 0.08) and CRP (TER = 1.02, 95% CI: 0.99, 1.05; P = 0.08) **(Figure 3)**. Similar results were found when fat mass change, instead of weight change, was used in the model **(Supplementary figure 1 and 2).** When restricting the analyses to women who lost ≥2 kg during the intervention period and had body composition, blood samples and covariate measurements at follow-up (n=155), results did not change to those noted for the primary analyses. Furthermore, only including women with a BMI >25 kg/m^2 at baseline (n=175) in the statistical model lead to the same results for all biomarkers except for CRP (TER = 1.04, 95% CI: 1.01, 1.06; P = 0.02). Lastly, our models were adjusted by BMI at baseline and results did not change to our main results.

Results from our categorized weight regain analyses revealed increases in all breast cancer-related biomarkers and decreases in SHBG in all weight regain groups compared with those who continued to lose or maintained weight loss during follow-up, with larger associations being noted in participants who regained ≥5 kg during follow-up **(Table 2)**. Furthermore, a statistically significant linear trend of increasing biomarker levels across weight regain categories was found for all biomarkers **(Table 2).**

DISCUSSION

In this secondary analysis of data collected from a previously conducted exercise intervention trial in postmenopausal women, we found strong associations between breast cancer-related biomarkers and weight regain during the follow-up period in participants who had exercise-induced weight loss. Specifically, during the 12-month follow-up, weight regain was significantly associated with increases in estradiol, estrone, free estradiol, insulin and HOMA-IR, as well as decreases in SHBG in postmenopausal women, with no associations noted for glucose and CRP. It is important to note that 71% of participants who experienced weight loss during the intervention regained weight during the follow-up period, and this weight regain was mostly attributed to fat mass gains. Furthermore, participants with a higher amount of weight loss during the intervention had larger decreases in biomarkers, however, during the follow-up period, these same participants experienced larger amounts of weight regain, in addition to larger increases in breast cancer-related biomarkers and decreases in SHBG. Lastly, our categorical analyses found

a linear trend of increasing biomarker levels across weight regain categories, with larger changes found in participants who regained ≥5 kg during follow-up.

There is evidence for an association between weight regain and postmenopausal breast cancer risk, however, the biological mechanisms involved in this relationship need to be further explored (20). Sex hormone concentrations are a plausible mechanism for this relationship (6). Adipose tissue is the main source of estrogen production during menopause via androgen aromatization (28). Increases in fat mass may contribute to circulating sex hormone upregulation, increasing estrogen production, and decreasing SHBG (29). This hormone profile has been associated with increased postmenopausal breast cancer risk (30,31). Our results found that postmenopausal women who experienced weight regain following exercise-induced weight loss had a 3% increase in estradiol, a 4% increase in free estradiol concentrations, a 2% increase in estrone and a 2% decrease in SHBG, for every 1 kg increase in body weight. Furthermore, those who regained ≥5 kg during follow-up had a 24% increase in estradiol, 15% in estrone, 32% in free estradiol and a 17% decrease in SHBG compared with those who lost or maintained weight. It is important to note that, in this study, weight regain was mostly attributed to increases in fat mass. Consistent with our results, a similar lifestyle intervention trial conducted in premenopausal women with obesity and metabolic syndrome found decreased SHBG with a controlled and supervised intentional weight regain phase after an intervention that combined diet and exercise (32). However, to our knowledge, there are no previous trials that have analysed associations between estrogen levels and weight regain. Most previous trials have focused on comparing different approaches to weight loss (e.g., diet, exercise or surgery), and how these approaches may restore sex hormone homeostasis in postmenopausal women with obesity directly or through adiposity reduction, and have found evidence that these interventions can reduce estrogens and increase SHBG in postmenopausal women (33,34). Future trials assessing sex hormone responses to weight regain in postmenopausal women are needed to corroborate our findings. It is a challenge to estimate the clinical impact of these changes in breast cancer risk since there are no established cut-off points identified to estimated breast cancer risk yet (35). However, existing data support the inclusion of circulating estradiol concentrations in prediction models of breast cancer risk as a future goal (36).

Insulin resistance has been directly and indirectly related to postmenopausal breast cancer risk (37,38). Insulin has been shown to regulate cancer cell growth and proliferation (39). Indirectly, insulin can inhibit SHBG synthesis in the liver (40), therefore, increasing estrogen bioavailability (41). We found that every 1 kg increase in body weight was significantly associated with a 3% increase in insulin and HOMA-IR, and no statistically significant associations were found for glucose. Furthermore, those who regained ≥5 kg during follow-up had a 34% increase in insulin, 5% increase in glucose, 41% increase in HOMA-IR compared with those who lost or maintained weight. These results are consistent with previous studies that found increases in insulin and HOMA-IR during weight regain after a weight loss program in postmenopausal women (42), as well as other populations, such as breast cancer survivors and pre-diabetic adults (43,44). Furthermore, another study that included participants with obesity or overweight in a 4-6 month weight loss program with diet and exercise, followed by a second phase where participants were randomly assigned into two groups, partial weight regain with exercise or partial weight regain without exercise (45). This study reported that those participants who regained weight without exercise had an increase in insulin and HOMA-IR, however, the decrease in these markers

induced by weight-loss was maintained in the exercise group regardless of the amount of weight regained (45). Hence, promoting exercise during follow-up may counteract some of the detrimental effects of possible weight regain following the end of a weight loss intervention. Future trials are needed to corroborate these findings and explore the use of different weight maintenance strategies to counteract the negative changes in biologic markers that may occur with weight regain.

Chronic low-grade inflammation is also a hypothesized mechanism related to postmenopausal breast cancer risk (46). Adipose tissue plays an important role in the regulation of inflammation (47) and can release pro-inflammatory cytokines (5). CRP is produced by the liver and is regulated by two cytokines (IL-6 and TNF-α) (48). Increased levels of CRP have been associated with several chronic diseases, however, findings are inconsistent in the literature for the association between CRP and breast cancer risk, with some, but not all (49), studies finding a positive association (50). In our results, we noted that every 1 kg increase in body weight was associated with a 2% increase in CRP concentrations, which was non-statistically significant. Moreover, those who regained ≥5 kg during follow-up had a 42% increase in CRP compared with those who lost/maintained weight. The Diet, Obesity and Genes (DIOGENES) Dietary study was a multi-centre, randomized dietary intervention examining the role of different diets on preventing weight regain (51). Results from this trial also found that CRP was associated with weight regain (51). Other studies have also reported an increase in other biomarkers for breast cancer risk, such as IL-6 and leptin, with weight regain (43,52). However, a five-year follow-up study reported that weight cycling after a weight loss program has little influence on systemic inflammation (53). Additional studies are needed to explore associations between CRP and weight regain, as well as other inflammatory markers related to breast cancer risk.

Strengths of BETA include a randomized controlled trial design with a 12-month supervised exercise intervention and a 12-month follow-up period. In addition, gold-standard methods for measuring anthropometry, excellent adherence to the intervention and a highly reputable laboratory for the assessment of the biomarkers all contributed to the success of the trial. A limitation of the study is that the laboratory assays were measured on two different occasions, the first batch of analyses included blood biomarkers for baseline and 12 months and the second included the follow-up assays, and this could potentially influence our results. Despite this limitation, the assays were conducted in a reputable laboratory following standardized protocols with established and excellent performance metrics (21). Furthermore, the use of self-reported measurements of dietary intake and physical activity have inherent limitations. BETA was not designed to be a weight loss trial or to assess the effectiveness of exercise volume on weight loss and subsequent weight maintenance. Finally, our sample included previously inactive postmenopausal women, mostly with overweight or obesity, and therefore our results may be not generalizable to other subgroups of women.

In conclusion, these results suggest that weight regain is associated with changes in several biomarkers related to breast cancer risk. Specifically, weight regain was associated with increases in estradiol, estrone, free estradiol, insulin, HOMA-IR and decreases in SHBG in postmenopausal women following exercise-induced weight loss. Our results also showed that even a small amount of weight regain was strongly associated with worsened estradiol, free estradiol, insulin, HOMA-IR and CRP levels. These findings have important clinical

implications. Clinical trials that focus on lifestyle interventions need to develop and assess the effectiveness of strategies to prevent negative changes in biomarkers associated with weight regain following successful weight loss. These findings also highlight the importance of weight regain and associated biomarker changes for postmenopausal breast cancer risk, which should be further explored in future randomized controlled trials targeting weight loss. Future studies should also analyse other circulating and tissue-specific markers of breast cancer risk and their association with weight regain after an exercise intervention.

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Table 1. Characteristics for participants who lost weight during the 12-month exercise interventions[a] from the Breast Cancer and Exercise Trial in Alberta (BETA), Alberta, Canada, 2010-2014.

Participant Characteristics	**All participants who had exercise-induced weight loss during the 12-month intervention[a] (n=214)**	**Participants who lost/maintained weight loss during follow-up (n=61)**	**Participants who re-gained 0.1-2.5 kg during follow-up (n=66)**	**Participants who re-gained 2.6-4.9 kg during follow-up (n=51)**	**Participants who re-gained ≥5 kg During follow-up (n=36)**
Baseline age (years)	59.1 [56.5, 63]	60.0 [56.6, 62.0]	59.4 [56.5, 64.0]	58.2 [56.7, 63.7]	58.6 [55.3, 63.1]
Age at menopause (years)	50 [48, 52]	51 [49, 52]	50 [47, 52]	50 [48, 53]	50 [48, 52]
Ethnicity					
White; N (%)	190 (88.8)	55 (90)	56 (85)	46 (90)	33 (92)
Other; N (%)	24 (11.2)	6 (10)	10 (15)	5 (10)	3 (8)
Employment at baseline					
Employed full time; N (%)	66 (31.0)	22 (36.0)	18 (27)	16 (31)	10 (28)
Not employed full time; N (%)	148 (69.0)	39 (64)	48 (73)	35 (69)	26 (72)
Education					
High school or less, N (%)	45 (21.0)	15 (25)	10 (15)	10 (20)	10 (28)
Beyond high school, N (%)	169 (79.0)	46 (75)	56 (85)	41 (80)	26 (72)
Married or common-law at baseline					
No, N (%)	59 (27.6)	22 (36)	20 (30)	8 (16)	9 (25)
Yes, N (%)	155 (72.4)	39 (64)	46 (70)	43 (84)	27 (75)
Study site					
Calgary, N (%)	166 (77.6)	47 (77)	46 (70)	43 (84)	30 (83)
Edmonton, N (%)	48 (22.4)	14 (23)	20 (30)	8 (16)	6 (17)
Intervention group					
High exercise volume	109 (51)	28 (46)	39 (59)	23 (45)	19 (53)
Moderate exercise volume	105 (49)	33 (54)	27 (41)	28 (55)	17 (47)
Use of nonsteroidal anti-inflammatory drugs (NSAID) at baseline					
User	13 (6)	4 (7)	5 (8)	1 (2)	3 (8)
Non-user	201 (94)	57 (93)	61 (92)	50 (98)	33 (92)
Use of statin drugs at baseline					
User	27 (13)	5 (8)	10 (15)	8 (16)	4 (11)
Non-user	187 (87)	56 (92)	56 (85)	43 (84)	32 (89)
Baseline anthropometrics and body composition					
Body weight (kg)	75 [67.2, 85.9]	73.9 [66, 88.3]	73.2 [66.3, 80.9]	74.2 [67.1, 82.1]	82.8 [74.2, 89.3]
Fat mass (kg)	29.1 [23.9, 36.1]	29.4 [23.7, 39.9]	27.8 [22.9, 32.8]	28.5 [23.9, 34.7]	34.1 [27.0, 39.2]

Fat-free mass (kg)	43.8 [39.9, 47.9]	43.8 [39.9, 47.2]	43.3 [38.3, 48.3]	42.8 [40.4, 47.9]	46.2 [40.7, 48.9]
12-month anthropometrics and body composition					
Body weight (kg)	69.8 [63.6, 8]	70.7 [64.5, 85.8]	69.2 [63.2, 78.1]	69.1 [63.2, 76.9]	73.4 [66.6, 81.9]
Fat mass (kg)	25.8 [21.1, 31.5]	26.2 [21.4, 36.2]	24.4 [19.7, 29.3]	24.9 [20.4, 30.2]	27.3 [22.3, 32.1]
Fat-free mass (kg)	43.4 [39.8, 47.0]	43.4 [39.7, 46.1]	43.3 [38.5, 46.5]	42.7 [39.8, 46.6]	44.4 [41.6, 48.4]
Follow-up anthropometrics and body composition					
Body weight (kg)	72.4 [65.3, 82.5]	68.4 [62.2, 80.6]	71 [64.1, 80.2]	72.3 [66.7, 80.5]	82.5 [74.2, 89.1]
Fat mass (kg)	27.5 [22.0, 33.8]	25.3 [20.6, 34.9]	26.1 [21.2, 31.4]	28.1 [23.6, 32.6]	34.1 [26.8, 37.7]
Fat-free (kg)	43.6 [39.8, 47.1]	42.6 [39.6, 45.6]	43.0 [38.3, 47.1]	43.3 [40.5, 46.9]	46.8 [42.4, 48.8]
Baseline biomarker concentrations					
Estradiol (pg/mL)	9.45 [7.29, 12.62]	9.91 [7.64, 12.61]	8.60 [6.55, 11.47]	8.88 [7.08, 11.59]	11.67 [9.09, 13.62]
Estrone (pg/mL)	37.91 [30.71, 46.27]	40.3 [29.7, 47.8]	35.2 [29.9, 44.7]	35.6 [27.4, 45.3]	39.1 [31.8, 48.9]
SHBG (nmol/L)	45 [34, 60.7]	45.5 [37.3, 58]	44.25 [32, 64.2]	46.1 [32.6, 61.5]	37.25 [30, 63.4]
Free estradiol (pg/mL)	0.21 [0.15, 0.31]	0.23 [0.17, 0.31]	0.19 [0.15, 0.30]	0.21 [0.15, 0.29]	0.28 [0.20, 0.35]
Insulin (μIU/mL)	8.73 [5.74, 12.90]	8.74 [5.77, 13.5]	8.37 [5.74, 13.2]	8.67 [4.61, 13.7]	9.60 [5.97, 12.2]
Glucose (mM)	91 [85, 96]	92 [85, 96]	90 [87, 96]	92 [85, 96]	90 [85, 97]
HOMA-IR	2.01 [1.23, 3.02]	2.11 [1.22, 3.03]	1.87 [1.23, 2.89]	1.95 [0.99, 3.24]	2.24 [1.45, 2.65]
CRP (mg/L)	1.82 [0.93, 3.88]	2.3 [0.96, 4]	1.97 [0.93, 3.88]	1.5 [0.88, 2.9]	1.78 [1.12, 4.38]
12-month biomarker concentrations					
Estradiol (pg/mL)	8.6 [6.7, 11.4]	10.36 [7.2, 12.2]	8.1 [6.3, 10.9]	8.8 [6.7, 10.6]	8.58 [7.1, 11.96]
Estrone (pg/mL)	35.1 [28.4, 45.0]	39.9 [31.05, 45.2]	32.9 [27.7, 44.8]	34.4 [26.4, 44.4]	36.3 [28.4, 45.01]
SHBG (nmol/L)	49.1 [38, 67.3]	46.9 [37.9, 60.4]	49.6 [37.7, 71]	54.6 [38.1, 67.5]	49.2 [39.3, 76.5]
Free estradiol (pg/mL)	0.20 [0.14, 0.28]	0.23 [0.15, 0.30]	0.18 [0.13, 0.26]	0.19 [0.14, 0.25]	0.19 [0.15, 0.30]
Insulin (μIU/mL)	6.92 [4.74, 9.93]	7.68 [5.65, 12.2]	6.95 [4.69, 11.6]	6.5 [3.92, 8.93]	6.41 [5.21, 8.30]
Glucose (mM)	88.0 [82.9, 95]	89 [83.9, 95]	89 [82.9, 98]	88 [83.9, 95]	85.9 [82.9, 91.5]
HOMA-IR	1.51 [0.99, 2.29]	1.68 [1.19, 2.60]	1.51 [0.90, 2.48]	1.33 [0.81, 2.13]	1.41 [0.97, 1.86]
CRP (mg/L)	1.38 [0.73, 2.75]	1.74 [0.82, 3.4]	1.37 [0.78, 2.89]	1.22 [0.70, 2.68]	1.21 [0.68, 2.3]
Follow-up biomarker concentrations					
Estradiol (pg/mL)	9.40 [7.3, 12.5]	9 [7.2, 12.1]	9.25 [7.1, 13]	9.6 [7.1, 12.3]	9.85 [7.59, 14.3]
Estrone (pg/mL)	34.15 [26.7, 42.5]	35 [25.7, 40.4]	31.65 [26.7, 42.5]	34 [26, 43.4]	35 [28.6, 43.4]
SHBG (nmol/L)	43.9 [32.7, 59.9]	45.8 [36.6, 61.8]	45.8 [35.2, 60.7]	43.2 [28.2, 59.9]	37.4 [31.8, 55.9]
Free estradiol (pg/mL)	0.23 [0.17, 0.33]	0.23 [0.16, 0.29]	0.22 [0.17, 0.34]	0.24 [0.18, 0.32]	0.27 [0.20, 0.36]
Insulin (μIU/mL)	7.43 [4.81, 11.1]	7.37 [4.32, 10.7]	7.13 [4.63, 12]	7.77 [4.49, 11.6]	8.35 [5.55. 10.15]
Glucose (mM)	83.9 [78.4, 89.2]	83.84 [78.38, 88.22]	83.77 [79.74, 89.06]	83.31 [77.5, 90.74]	84.59 [78.12, 89.59]
HOMA-IR	1.59 [0.99, 2.37]	1.55 [0.81, 2.17]	1.53 [1.07, 2.53]	1.62 [0.82, 2.54]	1.72 [1.19, 2.15]
CRP (mg/L)	1.74 [0.88, 3.72]	1.44 [0.85, 2.88]	2.15 [1.07, 4.09]	1.59 [0.85, 8.34]	1.72 [1.00, 3.82]

Total Physical Activity at Baseline (MET-h/week)	88.89 [60.64, 117.88]	81.79 [51.99, 131.13]	88.05 [62.27, 110.28]	98.11 [73.46, 131.77]	91.44 [60.99, 113.01]
Total Physical Activity at 12-months (MET-h/week)	114.40 [91.30, 141.92]	117.07 [91.46, 149.98]	114.15 [91.30, 128.44]	117.51 [98.59, 143.50]	107.15 [86.61, 138.89]
Total Physical Activity at Follow-up (MET-h/week)	96.75 [71.93, 127.02]	104.25 [71.35, 142.12]	97.06 [78.77, 135.32]	96.08 [71.94, 112.31]	87.76 [68.06, 114.47]
Total Energy Intake at Baseline (kcal)	1361 [1086, 1756]	1462 [1121, 1890]	1323 [963, 1707]	1322 [1103, 1838]	1360 [1101, 1645]
Total Energy Intake at 12-months (kcal)	1325 [1030, 1682]	1331 [1076, 1842]	1268 [944, 1745]	1393 [1130, 1680]	1299 [960, 1538]
Total Energy Intake at Follow-up (kcal)	1309 [1000, 1734]	1287 [948, 1699]	1267 [917, 1722]	1310 [1049, 1833]	1338 [1159, 1777]

Note: Values are reported as median [IQR] and N (%) for continuous and categorical variables, respectively.

[a] Participants who lost weight during the intervention and have complete DXA scans, anthropometry data, blood samples and covariate measurements.

Abbreviations: CRP, C-reactive protein; HOMA-IR, the homeostasis model assessment-estimated insulin resistance; Kcal, kilocalories; MET-h/week, metabolic equivalent of task hours per week; SHBG, sex hormone binding globulin.

Table 2. Change in biomarker levels from 12- to 24-months between participants who continued to lose weight/maintained weight loss (referent group) compared to participants who re-gained weight during follow-up from the Breast Cancer and Exercise Trial in Alberta (BETA), Alberta, Canada, 2010-2014.

Biomarker	N	12-month unadjusted geometric means (95% CI)[a]	24-month unadjusted geometric means (95% CI)[a]	% unadjusted change	Treatment effect ratio (95% CI) [b]	*P-value* [c]	*P-trend* [d]
Estradiol (pg/mL)							
Weight loss/maintenance	61	9.99 (8.92, 11.19)	9.51 (8.57, 10.56)	Referent	Referent		
Re-gained 0.1-2.5 kg	66	8.24 (7.43, 9.13)	9.56 (8.53, 10.71)	+23	1.23 (1.07 – 1.41)	*P = 0.003*	*P = 0.02*
Re-gained 2.6-4.9 kg	51	8.29 (7.54, 9.13)	9.54 (8.54, 10.65)	+23	1.23 (1.06 – 1.41)	*P = 0.007*	
Re-gained ≥5 kg	36	9.10 (7.71, 10.74)	10.71 (9.21, 12.46)	+24	1.24 (1.04 – 1.48)	*P = 0.02*	
Estrone (pg/mL)							
Weight loss/maintenance	61	38.55 (34.89, 42.59)	33.74 (30.73, 37.04)	Referent	Referent		
Re-gained 0.1-2.5 kg	66	35.91 (32.85, 39.26)	33.71 (30.90, 36.77)	+8	1.08 (0.99 – 1.18)	*P = 0.07*	*P = 0.01*
Re-gained 2.6-4.9 kg	51	33.70 (30.47, 37.28)	33.13 (30.29, 36.24)	+13	1.13 (1.03 – 1.23)	*P = 0.01*	
Re-gained ≥5 kg	36	37.84 (33.99, 42.13)	36.08 (31.47,41.36)	+15	1.15 (1.03 – 1.28)	*P = 0.01*	
SHBG (nmol/L)							
Weight loss/maintenance	61	49.23 (44.54, 54.41)	46.76 (42.13, 51.89)	Referent	Referent		
Re-gained 0.1-2.5 kg	66	50.26 (44.62, 56.63)	45.05 (40.19, 50.51)	-7	0.93 (0.86 – 1.01)	*P = 0.09*	*P < 0.001*
Re-gained 2.6-4.9 kg	51	50.56 (44.81, 57.05)	42.15 (36.93, 48.11)	-12	0.88 (0.81 – 0.96)	*P = 0.004*	
Re-gained ≥5 kg	36	52.30 (45.06, 60.71)	40.12 (34.78, 46.28)	-17	0.83 (0.75 – 0.92)	*P = 0.001*	
Free estradiol (pg/mL)							
Weight loss/maintenance	61	0.23 (0.20, 0.26)	0.23 (0.20, 0.26)	Referent	Referent		
Re-gained 0.1-2.5 kg	66	0.18 (0.16. 0.21)	0.23 (0.21, 0.27)	+27	1.27 (1.11 – 1.46)	*P = 0.001*	*P = 0.01*
Re-gained 2.6-4.9 kg	51	0.18 (0.16, 0.21)	0.24 (0.21, 0.27)	+28	1.28 (1.10 – 1.49)	*P = 0.002*	
Re-gained ≥5 kg	36	0.20 (0.16, 0.24)	0.27 (0.21, 0.29)	+32	1.32 (1.10 – 1.59)	*P = 0.003*	
Insulin (μIU/mL)							
Weight loss/maintenance	61	7.56 (6.52, 8.78)	6.88 (5.86, 8.07)	Referent	Referent		
Re-gained 0.1-2.5 kg	66	6.98 (5.98, 8.16)	7.34 (6.24, 8.64)	+18	1.18 (1.03 – 1.35)	*P = 0.02*	*P = 0.001*
Re-gained 2.6-4.9 kg	51	6.15 (5.22, 7.26)	7.29 (6.13, 8.65)	+30	1.30 (1.12 – 1.51)	*P = 0.001*	
Re-gained ≥5 kg	36	6.39 (5.54, 7.39)	7.64 (6.45, 9.04)	+34	1.34 (1.12 – 1.60)	*P = 0.002*	
Glucose (mM)							
Weight loss/maintenance	61	89.42 (87.46, 91.43)	83.17 (81.04, 85.35)	Referent	Referent		
Re-gained 0.1-2.5 kg	66	89.56 (87.10, 92.09)	84.17 (82.39, 85.98)	+2	1.02 (0.98 – 1.05)	*P = 0.48*	*P = 0.02*
Re-gained 2.6-4.9 kg	51	88.95 (86.52, 91.44)	85.91 (82.31, 89.66)	+3	1.03 (0.99 – 1.08)	*P = 0.09*	
Re-gained ≥5 kg	36	86.33 (84.09, 88.63)	84.34 (81.72, 87.06)	+5	1.05 (1.00 – 1.10)	*P = 0.03*	
HOMA-IR							
Weight loss/maintenance	61	1.67 (1.42, 1.96)	1.41 (1.19, 1.67)	Referent	Referent		
Re-gained 0.1-2.5 kg	66	1.54 (1.30, 1.83)	1.53 (1.28, 1.81)	+20	1.20 (1.03 – 1.39)	*P = 0.02*	*P < 0.001*
Re-gained 2.6-4.9 kg	51	1.35 (1.13, 1.61)	1.54 (1.27, 1.88)	+35	1.35 (1.15 – 1.58)	*P < 0.001*	
Re-gained ≥5 kg	36	1.36 (1.17, 1.58)	1.59 (1.33, 1.90)	+41	1.41 (1.17 – 1.70)	*P < 0.001*	
CRP (mg/L)							
Weight loss/maintenance	61	1.61 (1.28, 2.03)	1.60 (1.26, 2.04)	Referent	Referent		
Re-gained 0.1-2.5 kg	66	1.39 (1.08, 1.80)	1.94 (1.48, 2.56)	+37	1.37 (1.08 – 1.74)	*P = 0.01*	*P = 0.05*
Re-gained 2.6-4.9 kg	51	1.39 (1.08, 1.79)	1.88 (1.37, 2.57)	+29	1.29 (1.00 – 1.67)	*P = 0.05*	
Re-gained ≥5 kg	36	1.32 (0.98, 1.76)	1.87 (1.36, 2.55)	+42	1.42 (1.04 – 1.93)	*P = 0.03*	

Notes:

[a] Geometric means (lower 95% confidence limit to upper 95% confidence limit) unadjusted at 12 and 24-month.

[b] The treatment effect ratio (TER) was estimated from general linear models (24 month - 12 month) for each biomarker, estimating a parameter with anti-logarithm corresponding to the ratio of the adjusted geometric means among each weight re-gain category versus the referent group. A ratio greater than 1.0 indicates higher levels of biomarkers relative to the referent

group; a ratio less than 1.0 indicates a lower biomarker level compared to the referent group; and a ratio equal to 1.0 indicates no differences between comparison groups.

[c] *P*-values derived from the model correspond to the differences among each weight re-gain category with the referent group.

[d] *P*-trend for testing the linear association between biomarker levels across the weight regain categories.

∗These results are adjusted for the following covariates: age, years since menopause, marital status, employment, education, ethnicity, use of nonsteroidal anti-inflammatory drugs and statins, study site, randomization group, weight loss during the exercise intervention, changes in energy intake, fat intake and total physical activity during follow-up.

Abbreviations: CRP, C-reactive protein; HOMA-IR, the homeostasis model assessment-estimated insulin resistance; SHBG, sex hormone binding globulin.

FIGURE LEGENDS

Figure 1. Flow diagram of participants included in the present analysis from BETA, Alberta, Canada, 2010–2014.

Figure 2. Strength of the associations between changes in body weight during follow-up (24 months – 12 months) and changes in (A) estradiol; (B) estrone; (C) free estradiol; (D) SHBG, for 214 participants in BETA, Alberta, Canada, 2010-2014.

Note: The changes in body weight were calculated for each participant and plotted in the graphs, whereas changes in biomarkers during follow-up are presented by the line of best fit with 95% confidence intervals (CI), and were calculated as individual participant β values derived from the multivariable-adjusted linear regression model and transformed to represent the treatment effect ratio showing the ratio of geometric mean biomarker levels during follow-up (reflecting changes from 12 months to 24 months). A treatment effect ratio <1.0 indicated lower biomarker concentrations with changes in body weight during follow-up and a ratio >1.0 indicated higher biomarker concentrations. Multivariable-adjusted β and 95% confidence intervals (CI) were adjusted for the following covariates: age, age at menopause, employment, marital status, education, ethnicity, study site, randomization group, weight loss during the exercise intervention, use of nonsteroidal anti-inflammatory drugs and statins, changes in energy intake, fat intake and total physical activity during follow-up. **Abbreviations:** TER, treatment effect ratio; SHBG, sex hormone binding globulin; upper 95% CI, upper limit of 95% confidence interval; lower 95% CI, lower limit of 95% confidence interval.

Figure 3. Strength of the associations between changes in body weight during follow-up (24 months – 12 months) and changes in (A) insulin; (B) glucose; (C) HOMA-IR; (D) CRP, for 214 participants in BETA, Alberta, Canada, 2010-2014.

Note: The changes in body weight were calculated for each participant and plotted in the graphs, whereas changes in biomarkers during follow-up are presented by the line of best fit with 95% confidence intervals (CI), and were calculated as individual participant β values derived from the multivariable-adjusted linear regression model and transformed to represent the treatment effect ratio showing the ratio of geometric mean biomarker levels during follow-up (reflecting changes from 12 months to 24 months). A treatment effect ratio <1.0 indicated lower biomarker concentrations with changes in body weight during follow-up and a ratio >1.0 indicated higher biomarker concentrations. Multivariable-adjusted β and 95% confidence intervals (CI) were adjusted for the following covariates: age, age at menopause, employment, marital status, education, ethnicity, study site, randomization group, weight loss during the exercise intervention, use of nonsteroidal anti-inflammatory drugs and statins, changes in energy intake, fat intake and total physical activity during follow-up. **Abbreviations:** TER, treatment effect ratio; CRP, C-reactive protein; HOMA-IR, the homeostasis model assessment-estimated insulin resistance; upper 95% CI, upper limit of 95% confidence interval; lower 95% CI, lower limit of 95% confidence interval.

Figure 1

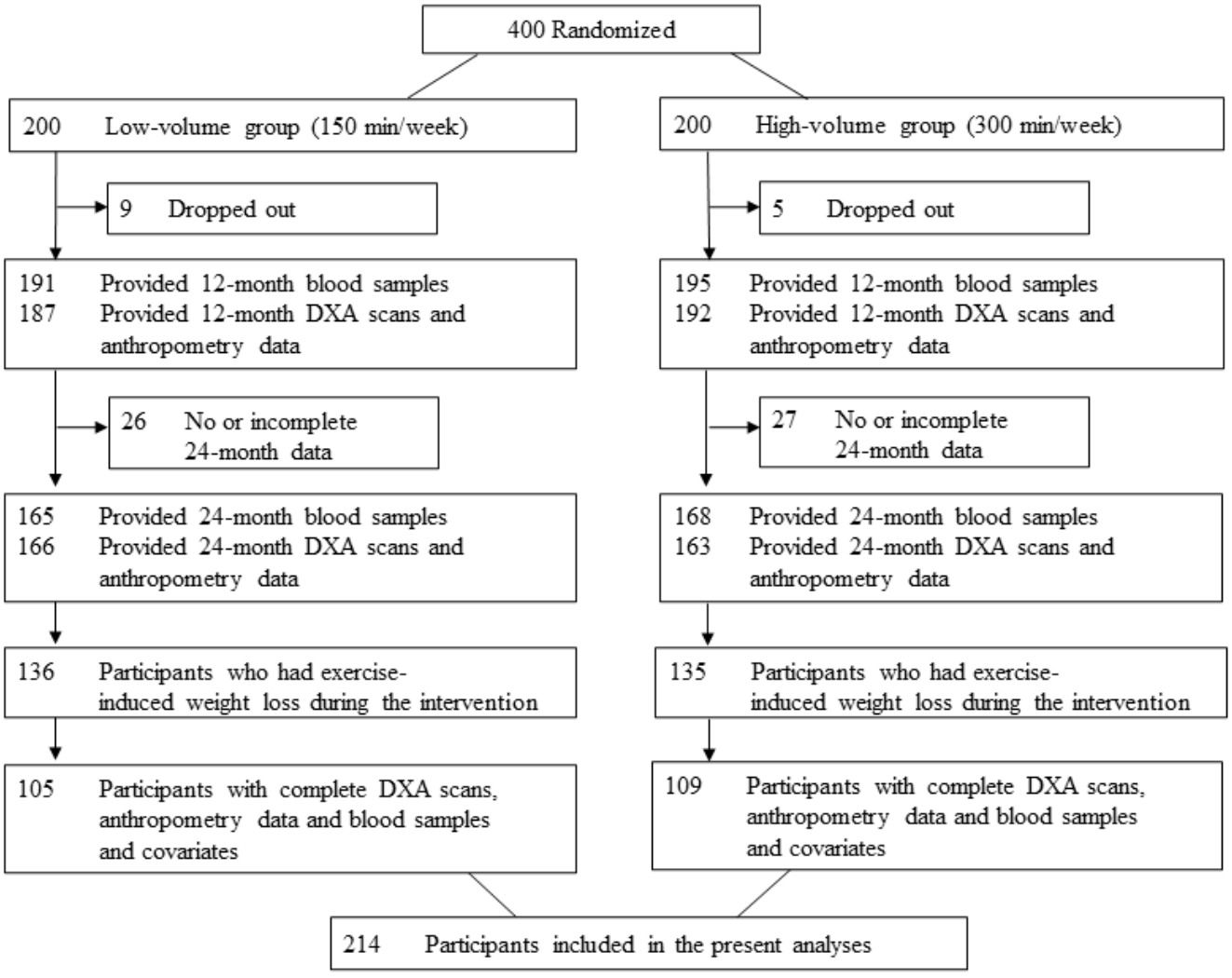

400 Randomized
200 Low-volume group (150 min/week)
200 High-volume group (300 min/week)
9 Dropped out
5 Dropped out
191 Provided 12-month blood samples
187 Provided 12-month DXA scans and anthropometry data
195 Provided 12-month blood samples
192 Provided 12-month DXA scans and anthropometry data
26 No or incomplete 24-month data
27 No or incomplete 24-month data
165 Provided 24-month blood samples
166 Provided 24-month DXA scans and anthropometry data
168 Provided 24-month blood samples
163 Provided 24-month DXA scans and anthropometry data
136 Participants who had exercise-induced weight loss during the intervention
135 Participants who had exercise-induced weight loss during the intervention
105 Participants with complete DXA scans, anthropometry data and blood samples and covariates
109 Participants with complete DXA scans, anthropometry data and blood samples and covariates
214 Participants included in the present analyses

Figure 2

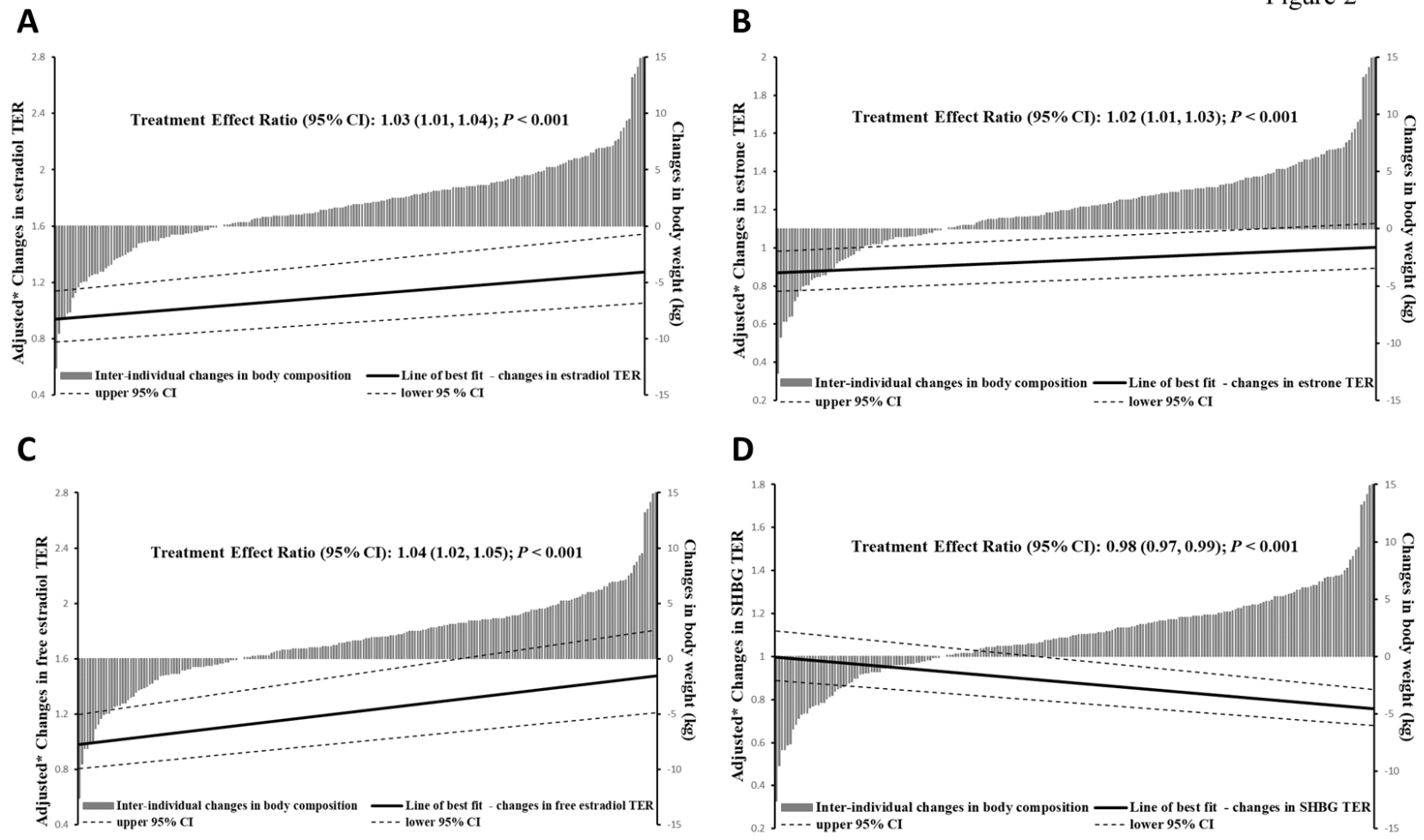
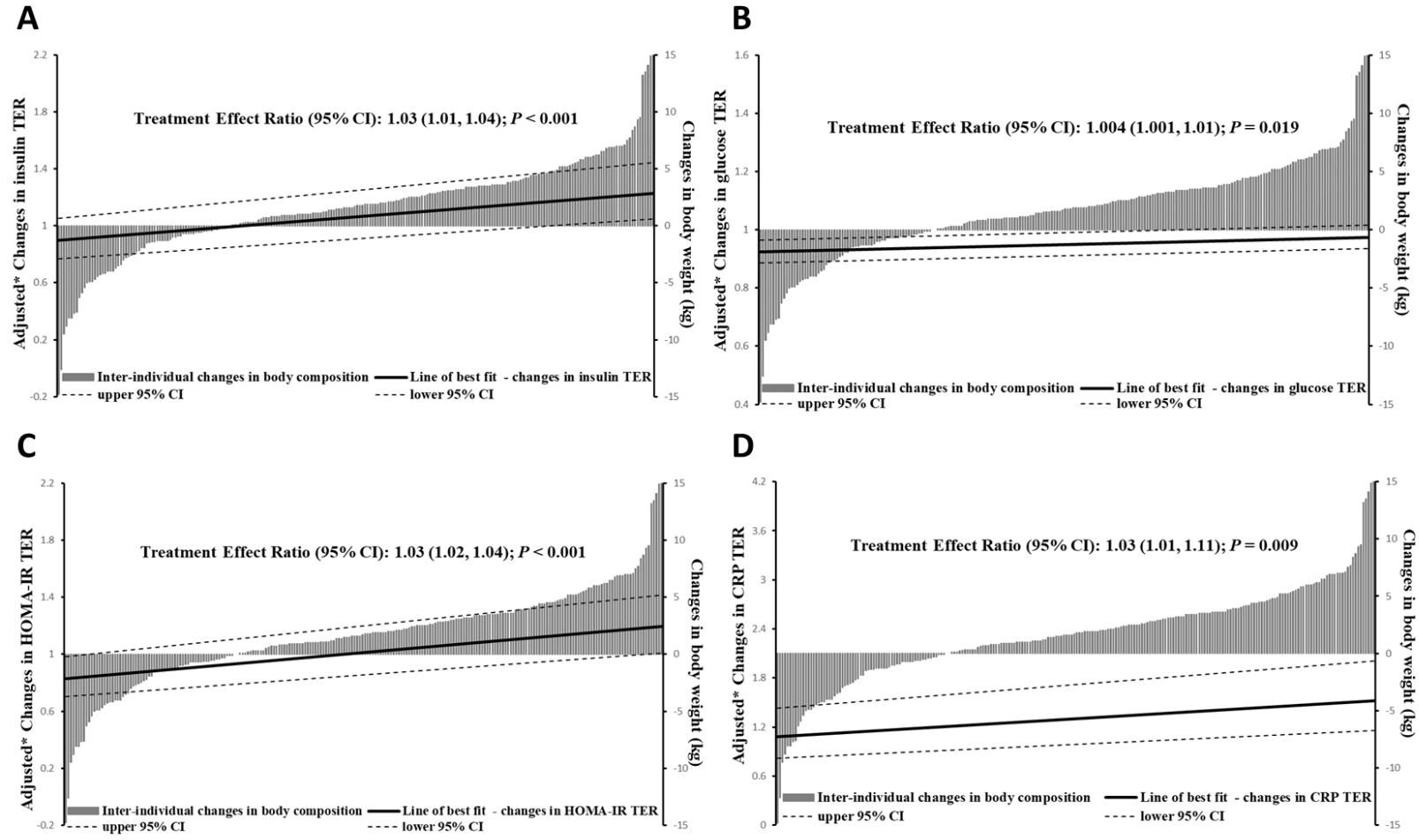

A
Treatment Effect Ratio (95% CI): 1.03 (1.01, 1.04); P < 0.001
Adjusted* Changes in estradiol TER
Changes in body weight (kg)
Inter-individual changes in body composition
Line of best fit - changes in estradiol TER
upper 95% CI
lower 95 % CI
B
Treatment Effect Ratio (95% CI): 1.02 (1.01, 1.03); P < 0.001
Adjusted* Changes in estrone TER
Changes in body weight (kg)
Inter-individual changes in body composition
Line of best fit - changes in estrone TER
upper 95% CI
lower 95% CI
C
Treatment Effect Ratio (95% CI): 1.04 (1.02, 1.05); P < 0.001
Adjusted* Changes in free estradiol TER
Changes in body weight (kg)
Inter-individual changes in body composition
Line of best fit - changes in free estradiol TER
upper 95% CI
lower 95% CI
D
Treatment Effect Ratio (95% CI): 0.98 (0.97, 0.99); P < 0.001
Adjusted* Changes in SHBG TER
Changes in body weight (kg)
Inter-individual changes in body composition
Line of best fit - changes in SHBG TER
upper 95% CI
lower 95% CI

Figure 3


A
Treatment Effect Ratio (95% CI): 1.03 (1.01, 1.04); P < 0.001
Adjusted* Changes in insulin TER
Changes in body weight (kg)
Inter-individual changes in body composition
Line of best fit - changes in insulin TER
upper 95% CI
lower 95% CI
B
Treatment Effect Ratio (95% CI): 1.004 (1.001, 1.01); P = 0.019
Adjusted* Changes in glucose TER
Changes in body weight (kg)
Inter-individual changes in body composition
Line of best fit - changes in glucose TER
upper 95% CI
lower 95% CI
C
Treatment Effect Ratio (95% CI): 1.03 (1.02, 1.04); P < 0.001
Adjusted* Changes in HOMA-IR TER
Changes in body weight (kg)
Inter-individual changes in body composition
Line of best fit - changes in HOMA-IR TER
upper 95% CI
lower 95% CI
D
Treatment Effect Ratio (95% CI): 1.03 (1.01, 1.11); P = 0.009
Adjusted* Changes in CRP TER
Changes in body weight (kg)
Inter-individual changes in body composition
Line of best fit - changes in CRP TER
upper 95% CI
lower 95% CI