Exercise Induced Dose Response Alterations in Adiponectin and Leptin Levels are Dependent on Body Fat Changes in Women at Risk for Breast Cancer

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Abstract

**Background:** Dysregulation of adipokines, such as adiponectin and leptin, is associated with a variety of chronic diseases, including cancer. Physical activity protects against breast cancer and one of the mechanisms which may underlie this association is exercise induced changes in adipokine levels. The WISER Sister Trial was a three-armed randomized controlled trial in pre-menopausal women (n=137) with an elevated risk for breast cancer. **Methods:** A 5-menstrual cycle long dosed aerobic exercise intervention compared low dose exercise (150 min/week) (n=44), or high dose exercise (300 min/week) (n=48), to a control group asked to maintain usual activity levels (n=45). Exercise intensity progressed to and was maintained at 70-80% of age predicted heart rate max. Body composition and adipokine levels were measured at baseline and follow up. **Results:** We observed significant linear trends for increased fitness capacity (Δ%: -2.0% control, 10.1% low dose, 13.1% high dose), decreased fat tissue-to-total tissue mass (Δ%: 0.7% control, -2.9% low dose, -3.7% high dose), increased body fat adjusted adiponectin (Δ%: -0.6% control, 0.6% low dose, 0.9% high dose), and decreased body fat adjusted leptin (Δ%: 0.7% control, -8.2% low dose, -10.2% high dose). **Conclusions:** In this randomized clinical trial of pre-menopausal women at risk for breast cancer, we demonstrate a dose response effect of exercise on adiponectin and leptin, and that dose response is dependent on changes in body fat. **Impact:** Improved adipokine levels, achieved by aerobic exercise training induced decreases in body fat, may decrease breast cancer risk for high risk pre-menopausal women.
Introduction

Physical activity protects against both pre and post-menopausal breast cancer. In a meta-analysis which quantified data from 31 studies, breast cancer risk was found to decrease by 2% for every 25 MET hr/week increment in non-occupational activity, 3% for every 10 MET hr/week increment in recreational activity, and 5% for every 2 hr/week increment in moderate plus vigorous recreational activity (1). Specific to pre-menopausal physical activity, exercise (39+ MET hr/week on average) before menopause is associated with a 23% lower risk of pre-menopausal breast cancer (2). Mechanisms proposed to explain the ability of higher levels of physical activity to prevent breast cancer include the beneficial effects of activity on hormonal exposures, metabolic hormones, immune function, inflammation, and adipokines (3). The adipose tissue microenvironment is an important element in breast cancer development; therefore we investigated the effect of physical activity on circulating adipokine levels (adiponectin and leptin).

Both leptin and adiponectin are adipose derived proteins which have pathological signaling cascades in relationship to breast cancer (4). Leptin stimulates proliferation, migration, and invasion of MDA-MB-231 and MCF-7 breast cancer cell lines (5, 6). Further, leptin and leptin receptors are overexpressed in 70-80% of breast cancer tumors (7). While elevated leptin appears to be detrimental, blunted serum adiponectin levels are associated with aggressive tumor phenotypes (8). Mice with reduced adiponectin expression show earlier tumor onset (9). Indeed, adiponectin has been shown to block proliferation of several breast cancer cells lines (10).

Exercise is a systemic therapy that can modulate biomarkers potentially involved in breast cancer pathways. Pre-clinical models demonstrate improved leptin sensitivity and enhanced adiponectin expression (11, 12). Clinical trials utilizing exercise interventions report decreased leptin levels, but mixed results with regard to increasing adiponectin levels (13-17). Additionally, it is difficult to
determine the independent effects of exercise on adipokine levels as alterations in body composition are often not controlled. Further, there have been no dose-response randomized clinical trials to date. Changes in adiponectin and leptin levels may be dependent on the population being investigated, the dose of exercise, and possible concomitant changes in body composition. Therefore, due to the link between adipokines and breast cancer, we investigated the effect of exercise on adiponectin and leptin levels and their relationship with changes in body composition in pre-menopausal women at elevated risk for breast cancer. We conducted a randomized controlled trial in a high risk population utilizing a dosed exercise intervention (150 minutes/week and 300 minutes/week).

Materials and Methods

Complete study design and methods are available and have been previously published (18). In the Women In Steady Exercise Research (WISER) Sister study, conducted at the University of Pennsylvania, pre-menopausal women with an elevated risk for breast cancer were recruited nationally for a 5-menstrual cycle long exercise intervention. Recruitment for the study was conducted from December 2008 to March 2012, and occurred through the Facing Our Risk of Cancer Empowered (FORCE) organization, the Cancer Genetics Network, genetic counselors, dissemination of brochures through survivorship conferences, and maintaining a web presence. Through these efforts 1025 women contacted the WISER Sister study team with interest in participating and were eligible based on initial screening. Following full screening for eligibility, 217 women were eligible for the WISER Sister study, 162 women consented, 139 women were randomized, and 137 had available blood samples for adipokine analysis (18). Of the 137 women, 23% were local participants (residing within 75 miles of the University of Pennsylvania) and 77% were long distance participants. Eligibility criteria consisted of: age ≥ 18 years, eumenorrheic, non-smokers, BMI between 18 and 50 kg/m², no history of fibroids, endometriosis, or polycystic ovary syndrome, no recent use of hormonal contraception, no contraindications for exercise training, controlled hypertension, weight stable, and sedentary (less than 75 min...
of aerobic exercise per week). Additionally, elevated risk for breast cancer was defined as > 18% lifetime risk of breast cancer according to Gail or Claus predication models, or documented deleterious mutation of BRCA1 or BRCA2, or documentation of a family member with a known deleterious mutation which would confer a 25% or greater probability of a deleterious mutation in the participant. This study was approved by the University of Pennsylvania Human Subjects Review Committee, and written informed consent was obtained from all subjects prior to beginning study activities.

Women were randomized with equal chance into one of three parallel-arms. Randomization was done within the strata formed by BMI (baseline BMI was divided by above versus below 30 kg/m²) and menstrual age (years since starting menstruation was dichotomized to above and below 10 years). Treatment groups consisted of a control group and 2 intervention groups. The control group was asked to maintain their usual level of physical activity and to not engage in any new exercise program during study participation. The low dose exercise group completed 150 minutes per week and the high dose group completed 300 minutes per week. Following orientation on proper use of the treadmill, heart rate monitors, and instruction on warm-up, cool-down, stretches, and exercise log completion, a treadmill was shipped to the participant’s home. Participants engaged in the 5-menstrual cycle long intervention at home and were monitored for adherence through several methods. Exercise logs (date, time, location of workout, and type of equipment used, average heart rate from their heart rate monitor, duration of workout and stretching) were over read by the study staff. Objective heart rate monitors were worn during exercise. Data was downloaded to the University of Pennsylvania. Study staff reviewed exercise logs and heart rate data weekly and would contact women after missed sessions to encourage adherence. Exercise intensity was set at 65-70% of age predicted maximum heart rate (220-age) for the first four weeks and 70-80% for the remainder of the study. The high dose exercise group started at 150 minutes per week and increased 20-25 minutes every two weeks until reaching 300 minutes. Further
detailed descriptions of the intervention and study parameters have been presented by Schmitz et al (18).

Physical activity was assessed by the Modifiable Activity Questionnaire and fitness level was determined using the Bruce protocol (19-21). Body composition was evaluated by dual-energy x-ray absorptiometry (DXA) (Hologic, Bedford MA) and all scans were analyzed by APEX 3.3. Dietary intake was measured through 3-day dietary records and participants were asked to maintain their normal caloric intake throughout the study. These metrics were completed at baseline and follow up as previously described (18).

All assessments were completed at the University of Pennsylvania on the same day. Baseline and follow up visits were scheduled between days 6 and 10 of the menstrual cycle. Plasma from blood draws was stored at -80°C until analysis of adipokines by the University of Pennsylvania Diabetes Core. Adiponectin and leptin were measured by ELISA (R&D Systems and Diagnostic Systems labs, respectively). Adiponectin intra and inter-assay coefficients of variation were 16.9 and 15.4, respectively. Leptin intra and inter-assay coefficients of variation were 7.5 and 8.4, respectively.

Paired t-tests were used to assess change within group. A one-way ANOVA was used to test for differences in percent change of variables between intervention groups. Linear regression using percent change variables was used to evaluate intervention effects on adipokines following adjustment for percent change in body fat. An extension of the Wilcoxon rank-sum test was used to test for linear trends across groups. Statistical analyses were conducted using STATA version 12 (Stata Corp., College Station, TX) and statistical significance was set at an alpha level of p < 0.05.

Results

Demographic characteristics of the study participants are depicted in Table 1. The age of the 137 participants ranged from 18 to 49 years of age. The majority of women were overweight, non-Hispanic
white, married, and college graduates. Women randomized to the low dose group were more likely to be married and have children, but this difference between groups did not affect other outcomes.

Sixteen women did not complete the study. We observed the following attrition: 1 woman from the control group, 6 women from the low dose group, and 9 women from the high dose group. In total, the intervention groups completed over 80% of the prescribed minutes (low dose, 85%; high dose, 81%). 76% of participants in the low dose group had greater than 80% adherence and 75% of participants in the high dose group had greater than 80% adherence.

Following the 5-menstrual cycle long intervention, we observed a significant dose response effect on fitness capacity (p < 0.001) as both low dose and high dose participants significantly increased their fitness capacity compared to baseline levels and compared to the control group (Table 2). There were no differences in caloric intake. Energy expenditure decreased in the control group compared to baseline levels and there was a significant between group difference in the percent change in physical activity which was significant for a dose response effect (p < 0.001). The control group increased body weight (mass) compared to baseline levels and there was a significant difference between groups for mass as both low and high dose exercise groups lost 0.6% of their body weight (p = 0.03 linear trend). The decrease in body weight for the low and high dose groups was not due to loss of lean tissue as we observe increased lean tissue mass compared to baseline for low and high dose groups. The low dose and high dose participants did lose fat tissue (kg) and decreased % body fat (fat tissue-to-total tissue mass) compared to baseline levels and compared to the control group. Both of these variables also demonstrated strong linear trends (p < 0.001) for a dose response effect.

Among the 121 women that completed the study, adiponectin levels decreased in the low dose exercise group compared to their baseline levels and compared to the control group (Table 2 and Figure 1A – white bars). Leptin levels decreased in the high dose group compared to their baseline levels. The percent change in leptin levels was not significantly different between any two groups (Table 2 and
Given the mechanistic relationship between adipocytes and adipokine levels, we sought to explore the effect of exercise on this relationship. Therefore, we adjusted the observed percent change in adipokines by the percent change in fat tissue-to-total tissue mass for each group. We observed that after controlling for the percent change in body fat, there was a significant increase in adiponectin levels for both exercise groups compared to the control group which decreased adiponectin levels \((p < 0.001)\). Additionally, after controlling for the percent change in fat tissue-to-total tissue mass, there was a significant decrease in leptin levels for both exercise groups compared to the control group which increased leptin levels \((p < 0.001)\). Furthermore, there was an exercise induced dose response effect as there was a significant linear trend across all groups for both adipokines \((p = 0.001\) for both adiponectin and leptin).

On additional analysis of body composition effects on the adipokines, we observed that a decrease in fat tissue \((\beta = -0.8)\), as well as fat tissue-to-total tissue mass \((\beta = -1.5)\), was associated with a significant increase in adiponectin levels only in the control group \((\text{fat tissue } p = 0.02; \text{ fat tissue-to-total tissue mass } p = 0.02)\). Decreased mass and fat tissue were significantly associated with decreased leptin levels in all groups, but decreased fat tissue-to-total tissue mass \((\text{control } \beta = 1.7; \text{ low dose } \beta = 3.0; \text{ high dose } \beta = 3.2)\) was significantly associated with decreased leptin levels only in the exercise intervention groups \((\text{control } p = 0.09; \text{ low dose } p = 0.01; \text{ high dose } p = 0.001)\).

**Discussion**

This study investigated the effect of a dosed exercise training intervention (150 min/week or 300 min/week) on body composition and adipokine levels in pre-menopausal women at high risk for breast cancer. We observed a significant dose response for enhanced fitness capacity, decreased body fat, and beneficial changes in body fat-adjusted-adipokine levels. This study is the first dose response randomized clinical trial to date which assesses the effect of volume of exercise on adipokine levels and
controls for changes in body composition. Additionally, we investigated this relationship in a population in which augmentation of adiponectin, and attenuation of leptin, may be critically important for long term health outcomes, particularly breast cancer.

Adiponectin levels have an inverse relationship with adiposity levels. Growing evidence suggests that adiponectin inhibits the growth of cancer cells and reduces cancer risk (22). We observed a significant difference between control and low dose exercise groups, with control participants on average increasing adiponectin levels by 7.3%, while participants in the low dose exercise group decreased adiponectin levels by 6.1%. These results are counterintuitive as lower levels of adiponectin are associated with increased risk for chronic diseases and an decrease was seen in the low dose exercise group.

Previous reports indicate there is variation with regards to the response of adiponectin levels to exercise, and a review by Simpson reports only 38% of RCTs demonstrate an exercise induced increase in adiponectin levels (13-15, 23-25). Specific to this investigation, Friedenreich et al reported that a year-long aerobic-exercise intervention of 225 minutes/week (70-85% of observed maximal heart rate) did not lead to any differences in adiponectin levels for 320 previously sedentary postmenopausal women (26). Further, Abbenhardt et al utilized the same exercise intervention in a 4 armed RCT that included dietary restriction, exercise, dietary restriction + exercise, and control. Overall they observed no significant effect of exercise on adiponectin levels yet the exercise group appears to have decreased adiponectin levels by 7.2% (15). Several other studies which have observed increases in adiponectin levels have observed these changes with concomitant reductions in weight through a weight loss intervention arm and not through exercise (13, 15, 23). In our study, we did not observe between group differences for decreased mass. However, we did observe an exercise induced dose response for decreased body fat (fat tissue-to-total tissue mass). Given the relationship between adiponectin and adiposity, we adjusted for changes in body fat. Exercise induced reduction in body fat led to increased
adjusted adiponectin levels in a dose dependent manner. However, a predicted increase of less than 1% in adiponectin levels may have limited clinical utility. Plasma adiponectin levels in normal subjects has been reported at 5-20 µg/ml (27), and adiponectin concentrations of 5-25 µg/ml show inhibitory effects on TNF-α and adhesion molecule expression (28). Obese subjects have plasma adiponectin levels < 6 µg/ml (29). Thus, exercise induced changes in body fat which impart a < 1% increase in plasma adiponectin levels for healthy, pre-menopausal women have an unknown effect for long term protection against breast cancer in this at risk cohort.

Another adipokine that is linked epidemiologically with breast cancer risk (30), and also mechanistically to carcinogenesis (31), is leptin. This protein is produced by adipocytes, fibroblasts, and also breast cancer cells once malignancy is present. Therefore, leptin can act in an endocrine, paracrine, as well as autocrine manner. There are complex biological networks involved with leptin signaling in the breast. Thus, therapeutic interventions to lower leptin levels, and keep it low, are necessary for women at risk for breast cancer.

We observed very similar patterns of decreased leptin levels for unadjusted and adjusted percent change in leptin. The dose response to exercise was apparent with body fat adjustment, but we also observed a significant difference between unadjusted baseline and final levels in leptin for the high dose exercise group. Our findings are in line with others that have investigated the effect of exercise and changes in body composition on leptin levels (32). Following 12 month aerobic exercise interventions Abbenhardt et al observed a 13% reduction in leptin levels with exercise, and Frank et al a 7% reduction (15, 33). It appears that leptin demonstrates a much more reliable association with body fatness and this association may be due to the high degree of association seen between exercise induced changes in leptin expression levels and changes in adipocyte size (11).

Our study investigated the adipokines, adiponectin and leptin, as they are recognized for their influence on breast cancer risk and tumor biology. Adipocytes primarily secrete these proteins, but
different fat depots play contrasting physiological roles. Adiponectin and leptin are predominantly secreted by subcutaneous adipose tissue (34, 35). Yet, aerobic exercise training reduces visceral adipose tissue to a greater extent than subcutaneous adipose tissue (36, 37). We saw a significant dose dependent decrease in body fat as measured by DXA. However, we did not investigate differences between subcutaneous and visceral fat. Given our unexpected unadjusted adiponectin results, the small body fat-adjusted dose response, and the variability seen in other studies in response to exercise, there are likely other systemic and metabolic factors influencing adiponectin expression and plasma concentrations (38). For instance, Fatouros et al demonstrated a large effect size for increased adiponectin levels with high intensity resistance training which was not seen with low or moderate intensities (39). This study highlights how different intensities of exercise and different modalities of exercise (resistance vs. aerobic) may affect adipocyte dysfunction given the unique cross talk between subcutaneous adipose tissue and skeletal muscle.

Our study is the first randomized controlled trial to examine the effect of a dosed 5-menstrual cycle long exercise intervention on circulating adiponectin and leptin in a national cohort of women at risk for breast cancer. A key strength of our study is our high adherence level, particularly in the high dose exercise group. Additionally, this dose response study investigates volume of exercise through increased duration of exercise. We did not alter exercise intensity between groups, thus allowing for easy adoption for public health recommendations. We also recognize that there are limitations to the current study. While altering volume of exercise through duration is advantageous for implementation, it also leaves the question of intensity unanswered. Further, we measured plasma adiponectin and leptin levels. The relative importance of adipokines as endocrine, paracrine, or autocrine factors is unknown. Thus, the extent to which circulating adipokine levels reflect the potential interaction with the pre-neoplastic epithelium is also unknown (40).
Overall, pre-clinical and clinical research points towards a role for adiponectin and leptin in carcinogenesis. Adipose tissue dysfunction affects the production of these adipokines. Thus, keeping body fat at healthy levels is of vital importance for pre-menopausal women at risk for an obesity linked disease such as breast cancer. In this study we demonstrate a dose response effect of exercise on these adipokines, and that dose response is dependent on changes in body fat.

Acknowledgments

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References


Table 1. Demographic characteristics of randomized women.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Sample (n=137)</th>
<th>Control (n=45)</th>
<th>Low Dose (n=44)</th>
<th>High Dose (n=48)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>34.3 ± 6.92</td>
<td>34.5 ± 7.54</td>
<td>35.1 ± 6.45</td>
<td>33.5 ± 6.75</td>
<td>0.46</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>26.8 ± 6.21</td>
<td>27.0 ± 6.13</td>
<td>26.7 ± 6.06</td>
<td>26.6 ± 6.53</td>
<td>0.96</td>
</tr>
<tr>
<td>Caloric intake (kcal/d)</td>
<td>1732.8 ± 737.35</td>
<td>1629.2 ± 526.53</td>
<td>1883.7 ± 775.67</td>
<td>1691.6 ± 854.34</td>
<td>0.24</td>
</tr>
<tr>
<td>Children, no. (%)</td>
<td>82 (59.9%)</td>
<td>23 (51.1%)</td>
<td>34 (77.3%)</td>
<td>25 (52.1%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Marital status, no. (%)</td>
<td>83 (60.6%)</td>
<td>22 (48.9%)</td>
<td>34 (77.3%)</td>
<td>27 (56.2%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Race, no. (%)</td>
<td>116 (84.7%)</td>
<td>38 (84.4%)</td>
<td>39 (88.6%)</td>
<td>39 (81.2%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Hispanic</td>
<td>10 (7.3%)</td>
<td>3 (6.7%)</td>
<td>3 (6.8%)</td>
<td>4 (8.3%)</td>
<td>0.94</td>
</tr>
<tr>
<td>Education, no. (%)</td>
<td>4 (2.9%)</td>
<td>3 (6.6%)</td>
<td>1 (2.3%)</td>
<td>0 (0%)</td>
<td>0.43</td>
</tr>
<tr>
<td>High school or less</td>
<td>40 (29.2%)</td>
<td>12 (26.7%)</td>
<td>13 (29.5%)</td>
<td>15 (31.2%)</td>
<td></td>
</tr>
<tr>
<td>Some college</td>
<td>93 (67.9%)</td>
<td>30 (66.7%)</td>
<td>30 (68.2%)</td>
<td>33 (68.8%)</td>
<td></td>
</tr>
</tbody>
</table>

137 women with elevated risk for breast cancer (defined as > 18% lifetime risk of breast cancer)

according to Gail or Claus predication models, documented deleterious mutation of BRCA1 or BRCA2, or
documentation of a family member with a known deleterious mutation which would confer a 25% or
greater probability of a deleterious mutation in the participant) were randomized to control, low dose
exercise (150 min/week), or high dose exercise (300 min/week) groups. Means ± standard deviation.
Table 2. Energetics, body composition, and adipokines before and following the exercise training intervention.

<table>
<thead>
<tr>
<th></th>
<th>Control (N=44)</th>
<th>Low Dose(N=38)</th>
<th>High Dose (N=39)</th>
<th>P-value</th>
<th>P-value linear trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Final</td>
<td>% Δ</td>
<td>Baseline</td>
<td>Final</td>
</tr>
<tr>
<td>Exercise test (min)</td>
<td>8.0 ± 1.6</td>
<td>7.8 ± 1.8</td>
<td>-2.0% ± 9.2</td>
<td>8.0 ± 1.6</td>
<td>9.0 ± 1.6a</td>
</tr>
<tr>
<td>Caloric intake (kcal/d)</td>
<td>1845.6 ± 559.3</td>
<td>1821.4 ± 450.3</td>
<td>4.1 ± 30.8</td>
<td>1822.3 ± 559.3</td>
<td>1800.0 ± 542.4</td>
</tr>
<tr>
<td>Energy expenditure (MET-hr/week)</td>
<td>8.4 ± 6.6</td>
<td>2.2 ± 2.3a</td>
<td>-63.2 ± 39.4</td>
<td>9.0 ± 9.3</td>
<td>9.0 ± 5.3</td>
</tr>
<tr>
<td>Whole body total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>74.6 ± 16.2</td>
<td>75.7 ± 17.4a</td>
<td>1.2 ± 4.5</td>
<td>72.7 ± 17.7</td>
<td>72.2 ± 17.7</td>
</tr>
<tr>
<td>Lean tissue (kg)</td>
<td>45.4 ± 5.8</td>
<td>45.8 ± 6.3</td>
<td>0.8 ± 3.3</td>
<td>44.1 ± 7.0</td>
<td>44.5 ± 6.7a</td>
</tr>
<tr>
<td>Fat tissue (kg)</td>
<td>29.2 ± 11.2</td>
<td>29.8 ± 11.9</td>
<td>2.0 ± 8.4</td>
<td>28.5 ± 11.4</td>
<td>27.7 ± 11.7a</td>
</tr>
<tr>
<td>Fat tissue/total tissue mass (%)</td>
<td>39.1 ± 6.8</td>
<td>39.3 ± 6.9</td>
<td>0.7 ± 4.5</td>
<td>39.2 ± 6.2</td>
<td>38.1 ± 6.7a</td>
</tr>
<tr>
<td>Adipokines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>11.9 ± 6.6</td>
<td>12.3 ± 6.0</td>
<td>7.3 ± 19.6</td>
<td>12.3 ± 4.7</td>
<td>11.7 ± 5.1a</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>21.9 ± 13.1</td>
<td>20.5 ± 12.5</td>
<td>-2.5 ± 30.9</td>
<td>18.4 ± 11.2</td>
<td>16.9 ± 11.0</td>
</tr>
</tbody>
</table>

Data presented as means ± standard deviation, a P < 0.05 within group. b P < 0.05 between group on post-hoc Bonferroni testing.
Figure Legend

Figure 1. Percent change values for adiponectin (A) and leptin (B) are presented as mean ± SD. White bars represent the unadjusted percent change calculation and black bars represent predicted percent change when (†) adjusted for percent change in fat tissue-to-total tissue mass. The mediation of adipokine levels by body composition demonstrates a dose-response effect across intervention groups as we observed significant linear trends. *† P < 0.05.
A

Adiponectin (Δ%)

Control  Low Dose  High Dose

Unadjusted

Adjusted

Linear trend

P = 0.001,
adjusted model

B

Leptin (Δ%)

Control  Low Dose  High Dose

Unadjusted

Adjusted

Linear trend

P = 0.001,
adjusted model