Published in final edited form as:

Cancer Prev Res (Phila). 2016 November ; 9(11): 835–843. doi:10.1158/1940-6207.CAPR-16-0163.

Dietary Weight Loss, Exercise and Oxidative Stress in Postmenopausal Women: a Randomized Controlled Trial

Catherine Duggan¹, Jean de Dieu Tapsoba¹, Ching-Yun Wang^{1,2}, Kristin L. Campbell³, Karen Foster-Schubert⁴, Myron Gross⁵, and Anne McTiernan^{1,2}

¹Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

²School of Public Health, University of Washington, Seattle, WA

³Faculty of Medicine, University of British Columbia, Vancouver, BC

⁴School of Medicine, University of Washington, Seattle, W

⁵University of Minnesota, Minneapolis, Minnesota, USA

Abstract

Oxidative stress, a potential mechanism linking obesity and cancer, results from an imbalance between activation/inactivation of reactive oxygen species, byproducts of cellular metabolism. In a randomized controlled trial we investigated effects of diet and/or exercise on biomarkers of oxidative stress. 439 overweight/obese (body mass index (BMI) >25 kg/m²) postmenopausal women, 50–75 years, were randomized to 12-months of (1) reduced-calorie weight loss diet ('diet'; n=118), (2) moderate- to-vigorous intensity aerobic exercise ('exercise'; n=117), (3) combined diet and exercise intervention ('diet+exercise'; n=117), or (4) control (n=87). Outcomes were circulating markers of oxidative stress including Fluorescent Oxidation Products (FOP), F₂-isoprostanes, and oxidized-LDL. On average participants were 57.9 years, with a BMI of 30.9 kg/m². F₂-Isoprostanes were significantly reduced in the diet (–22.7%, P=0.0002), and diet +exercise (–23.5%, P<0.0001) arms vs. controls (–2.99%); and non-significantly reduced in the exercise arm (–14.5%, P=0.01). Participants randomized to the diet and diet+exercise arms had significant increases in levels of FOP (control –5.81%; diet +14.77% (P=0.0001); diet+exercise +17.45%, (P=0.0001)). In secondary analyses, increasing weight loss was statistically significantly associated with linear trends of greater reductions in oxidized-LDL and in F₂-isoprostanes, and increases in FOP. Compared to controls, exercise participants whose maximal oxygen consumption increased had significant decreases in levels of F₂-isoprostanes and in oxidized-LDL, and increases in FOP. Dietary weight loss, with or without exercise, significantly reduced some markers of oxidative stress in postmenopausal women.

Keywords

Oxidative stress; weight loss; exercise; randomized control trial

Corresponding author Catherine Duggan, PhD, Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA, cduggan@fredhutch.org, Tel. 206 667 2323.

Disclosures: The authors declare that they have no conflicts of interest to report.

Trial registration: Clinicaltrials.gov identifier NCT00470119

INTRODUCTION

An estimated 25% of cancers are due to obesity and sedentary lifestyles.(1) Low-grade chronic inflammation is a hallmark of obesity, and has been implicated in the etiology of cancer via a number of processes, possibly mediated by alterations in levels of adipokines, cytokines, and release of reactive oxygen species (ROS) from a number of sources, including infiltrating macrophages.(2) ROS, byproducts of cellular metabolism, can act as signaling molecules,(3) which at low-to-moderate concentrations are essential in a number of physiological processes.(4,5) At higher levels, imbalances between ROS production and inactivation lead to oxidative stress. Evidence from animal models and cell lines support the role of oxidative stress and ROS in cancer initiation and promotion,(6,7) via inactivation of tumor suppressors,(8) increasing expression of pro-inflammatory cytokines,(9) and inducing signaling pathways.(10) Oxidative stress also causes peroxidation of lipids and proteins, and is a source of DNA damage.(11)

Oxidative stress can be assessed via circulating levels of its byproducts. Levels of lipid peroxidation have been widely used as an indicator of ROS-mediated damage to cell membranes, and oxidized low-density lipoprotein (ox-LDL) is a circulating global marker of oxidative stress. F₂-isoprostanes are a complex family of isomeric F₂-prostaglandin-like compounds derived from free radical-catalyzed non-enzymatic peroxidation of arachidonic acid.(12) Finally, fluorescent oxidation products (FOP) result from oxidation of a variety of sources including DNA proteins and lipids.(13,14)

Studies have reported conflicting associations between oxidative stress biomarkers and risk of breast and other cancers in women. In the Long Island Breast Cancer Project case-control study, there was a statistically significant trend in breast cancer risk with increasing quartiles of urinary 15-F(2t)-isoprostanes.(15) A nested case-control study from the Shanghai Women's Health Study, reported that levels of F₂-isoprostanes were significantly associated with increased risk of breast cancer, but only among women with a BMI ≥ 29 kg/m². (16)

For other cancers, a case-control study reported a 2-fold increase in risk of lung cancer for men in the second and third tertiles of urinary 15-F(2t)-isoprostane, but not in women.(17) Positive associations were also reported between increasing urinary levels of F₂-isoprostanes and gastric cancer,(18) and incident colorectal adenomas in one study,(19) but not in another. (20) There are limited data on associations between ox-LDL or FOP and cancer risk. Increased levels of ox-LDL are associated with risk of colorectal cancer,(21) and FOPs with increased risk for incident colorectal adenomas.(19)

Mixed associations with premenopausal breast cancer risk were seen in a nested case-control analysis of the Nurses' Health Study. Three fluorescent oxidation products were measured in a subset of participants from a case control study of premenopausal breast cancer patients nested in the Nurses' Health study: there was no association with one FOP, and a trend towards an inverse association with breast cancer risk was observed for the other two.(22)

Markers of oxidative stress are higher in obese individuals, and are associated with presence of the metabolic syndrome, and diabetes (23–25) both associated with cancer risk.(26,27)

Two cross-sectional studies reported correlations between BMI, waist circumference and increased serum F₂-isoprostane levels.(3,28,29) There are few data on the role of weight loss or physical activity on markers of oxidative stress, and studies tend to be small and of short duration.(30–33) While we and others have found that exercise can lower markers of oxidative stress,(34–36) to our knowledge, no previous studies have assessed the effect of amount of weight loss on oxidative stress measures.

The purpose of this study was to examine, in a randomized controlled clinical trial (RCT), effects of 12-months dietary weight loss and moderate-to-vigorous exercise programs, alone and in combination, on circulating markers of oxidative stress (ox-LDL, plasma F₂-isoprostane, and FOP) in 439 overweight/obese postmenopausal women. Secondary aims were to assess dose-response effects of weight loss and exercise, the latter measured by change in cardiorespiratory fitness (VO₂max).

MATERIALS AND METHODS

This study is ancillary to the NEW study, a 12-month RCT testing the effects of caloric restriction and/or exercise vs. control on circulating sex-steroid hormones and other biomarkers in healthy overweight/obese postmenopausal women. The study was performed with the approval of the FHCRC Institutional Review Board according to an assurance filed with and approved by the U.S. Department of Health and Human Services. Written informed consent was obtained from each participant.

Study Population

The study is described in detail elsewhere.(37) Briefly, 439 postmenopausal, overweight/obese (BMI ≥ 25 kg/m²), sedentary women, 50–75 years, not taking hormonal therapy, were enrolled between 2005–2008, and randomly assigned to a reduced-calorie dietary modification intervention (N=118); moderate-to-vigorous intensity aerobic exercise intervention (N=117); combined diet and exercise intervention (N=117); or control (N=87). Exclusion criteria were: 100 min/week of moderate physical activity; diabetes/other serious medical condition(s); >2 alcoholic drinks/day; current smoker; participation in another structured weight loss program; contraindications e.g. abnormal exercise tolerance test, or inability to attend sessions. Permuted block randomization was used to achieve a proportionally smaller control group, stratified according to BMI (<30 kg/m²) and race/ethnicity.

Interventions

The dietary intervention was a modification of the Diabetes Prevention Program and LookAHEAD lifestyle behavior-change programs with goals of 1200–2000 kcal/day, <30% daily calories from fat, 10% weight loss by 6-months, and weight maintenance thereafter. Participants had at least two individual meetings with a dietitian followed by weekly group meetings for 6-months; thereafter they attended monthly, with biweekly phone/email contact. Intervention adherence was defined by percent of in-person nutrition session attendance, by tertiles.

Exercise intervention goals were 45 minutes of moderate-to-vigorous (4 metabolic equivalents [METs]) intensity exercise at a target heart rate of 70–85% observed maximum, five days/week by week 7. Participants attended three facility-based supervised sessions/week, and exercised two days/week at home. They recorded exercise mode, duration, peak heart-rate, and perceived exertion at each session. Activities of 4 METs (38) counted towards the prescribed target.

Controls were asked not to change their diet or exercise habits, and were offered four weight loss classes and eight weeks of exercise training at study completion.

Covariates

All study measures were obtained and analyzed by trained personnel blinded to participants' randomization status at baseline and 12-months, at study completion. Height, weight, and BMI were measured as previously described.(37) Body composition was measured by DXA (dual-energy X-ray absorptiometry) whole-body scanner (GE Lunar, Madison, WI). Cardiorespiratory fitness (VO₂max; mL/kg/min) was assessed using a maximal graded treadmill test according to a modified branching protocol. Questionnaire data included demographics, medical history, dietary intake, supplement use and physical activity patterns. Medication use known affect inflammatory markers, including systemic corticosteroids, ACE (angiotensin-converting enzyme) inhibitors, statins, or non-steroidal anti-inflammatories,(39) were coded as use/non-use at baseline and 12-months.

Blood Specimen Collection and Processing

Participants refrained from alcohol (48 hours), vigorous exercise or NSAID use (24 hours) prior to fasting venous blood collection (50mL) at baseline and 12-months. Blood was processed within one hour, and stored at -70°C.

Assays

Oxidative stress assays were performed at the Advanced Research and Diagnostic laboratory, University of Minnesota. FOPs were measured using a spectrofluorometer at an excitation/emission wavelength of 360/380 nm from plasma extracted by ethanol-ether, and fluorescence measured as relative fluorescent intensity units/milliliter (FI/mL) of plasma. (40) Ox-LDL was measured from serum samples by ELISA (Mercodia, Uppsala, Sweden). Plasma F₂-isoprostanes were pre-purified using thin-layer chromatography. Samples were excluded if the purification step failed, assessed using QC standards; in total 33 samples were excluded (Fig. 1). F₂-isoprostane levels were assayed by GC/MS using an Agilent 6890 Series GC and an Agilent 5973N Mass Selective Detector as previously described, with modifications.(41,42)

QA samples were included in each assay to assess coefficients of variation (CV). Participant's baseline and 12-month samples were included in the same batch; paired samples were randomly placed across batches. Inter- and intra-assay CVs for the analytes were 6.62% and 4.42% (FOP); 27.3% and 22.76% (ox-LDL); and 31.1% and 29.4% (F₂-isoprostane).

Statistical Analyses

Logarithmic transformations were applied to outcome variables to improve normality of the distribution. Descriptive data are presented as geometric means (95% confidence intervals (CI)). Mean changes in analytes from baseline to 12-months, stratified by arm, were computed. Intervention effects on variables were examined based on assigned treatment at randomization, regardless of adherence or study retention (i.e. intent-to-treat). Mean 12-month changes in intervention arms were compared to controls using the generalized estimating equations modification of linear regression to account for intra-individual correlation over time. Intervention effects are presented as both absolute and relative change. Bonferroni correction adjusted for multiple comparisons (2-sided $\alpha=0.05/6=0.008$ for 6 comparisons) for the primary analysis. Analyses were adjusted for baseline age, race/ethnicity, BMI and NSAIDs/statin use. We used the method of last observation carried forward (LOCF) to deal with missing data at 12-months. In preplanned analyses, we examined changes in analytes in each intervention arm in subgroups of participants by 1) weight loss (no change/gained weight; lost >0 – $<5\%$ of baseline weight; lost $>5\%$ of baseline weight); 2) change in $VO_2\text{max}$ stratified by tertiles. Analyses stratified by changes in body-fat stratified by tertiles, and dietary adherence assessed by percentage of support groups attended (tertiles) were also performed (Supplementary tables 1 and 2). Participants with missing 12-month weight, $VO_2\text{max}$ or body-fat data were classified as ‘no change’ (LOCF). For these subgroup analyses, a p-value of <0.05 was considered significant.

Results

Baseline characteristics of participants are presented in Table 1. On average, participants were 57.9 years, with a BMI of 30.9 kg/m^2 , and were predominantly non-Hispanic White (84.9%). At 12-months, 399 women (91%) returned for clinical measures and blood draws (Figure 1). One participant randomized to the diet+exercise arm was missing a baseline blood sample (Fig. 1); and three 12-month samples were missing from the exercise ($n=2$) and diet arms ($n=1$). As described above a total of 33 samples were excluded from the F_2 -isoprostane assay for failing QC inclusion criteria.

Weight, body composition, and other inflammation-related biomarker changes were previously reported. Participants in the diet arm lost a mean of 8.5% of their baseline weight, ($P<0.0001$); those in the exercise arm, -2.4% ($P=0.03$); and those in the diet+exercise arm, 10.8% ($P<0.0001$); all compared to -0.8% decrease among controls.(37) Adherence to the diet and aerobic exercise interventions was excellent. Estimated relative fat intake (% total kcal/day) decreased by 18% and 20% in the diet and diet+exercise arm respectively; women in both arms attended an average of 86% of behavior change sessions. By 12-months, women randomized to exercise and diet+exercise arms achieved an average of 80% and 85% respectively of the target 225 min/week aerobic exercise, and significantly increased average pedometer steps/day (exercise, 42% increase; diet+exercise, 58% increase) and $VO_2\text{max}$ (exercise 9% increase; diet+exercise, 7% increase), compared to baseline.

In fully adjusted models, participants randomized to the diet+exercise intervention non-significantly reduced levels of ox-LDL (-7.49% ; $P=0.03$) compared to controls ($+2.25\%$) at 12-months (Table 2). Participants randomized to the diet and the diet+exercise arms had

significant increases in levels of FOP (control -5.81%; diet +14.77% ($P=0.0001$); diet +exercise +17.45%, ($P=0.0001$)). F₂-Isprostanes were significantly reduced in the diet (-22.7%, $P=0.0002$), and diet+exercise (-23.5%, $P<0.0001$) arms compared to controls (-2.99%). Exercise reduced levels of F₂-Isprostanes but this was not significant after Bonferroni correction (-14.5%, $P=0.01$).

Participants randomized to the diet+exercise arm who lost 5% of their baseline body weight had significantly greater reductions in levels of ox-LDL (-8.7% $P=0.02$) compared to controls (+2%); there was a statistically significant overall trend of decreasing levels of ox-LDL with increasing weight loss ($P_{\text{trend}}=0.02$; Table 3). In all three intervention arms there was a statistically significant linear association between increasing levels of weight loss and reductions in F₂-isoprostanes ($P_{\text{trend}}<0.0001$ (diet); $P_{\text{trend}}=0.0002$ (exercise); $P_{\text{trend}}<0.0001$ (diet+exercise)). In contrast, there was a significant trend between increasing levels of weight loss and increases in FOP compared to controls in the diet+exercise arm ($P_{\text{trend}}<0.0001$) and in the diet arm ($P_{\text{trend}}<0.0001$), but not in the exercise arm ($P_{\text{trend}}=0.93$). Among participants who were randomized to exercise intervention arms (exercise and diet +exercise), those who increased their VO₂max had significant decreases in levels of F₂-isoprostanes compared to controls (Table 4) in both exercise ($P_{\text{trend}}=0.003$) and diet +exercise ($P_{\text{trend}}=0.0006$) arms. Participants randomized to the diet+exercise arm had statistically significant increases in FOP levels with increasing VO₂max ($P_{\text{trend}}=0.0002$), but not for those participants randomized to the exercise arm. Participants who increased their VO₂max by 3.5–14.3% above baseline in the diet+exercise arm had a marginally significant decrease in ox-LDL compared to controls (-11.0% vs. +2.0% respectively, $P=0.05$). We compared changes in tertiles of percent body fat change vs. controls across the three intervention arms (Supplementary Table 1). Increasing levels of fat-loss were statistically significantly associated with decreasing levels of ox-LDL in the diet+exercise arms only ($P_{\text{trend}}=0.006$). There were no consistent associations for changes in ox-LDL by fat loss in the diet or exercise arms. FOP were significantly increased in the diet and diet+exercise arms with increasing levels of fat loss ($P_{\text{trend}}<0.0001$ (diet) and $P_{\text{trend}}=0.0001$ (diet +exercise)) compared to controls, but not in the exercise arm. F₂-isoprostanes levels significantly decreased with increasing fat loss in each of the three arms compared to controls: ($P_{\text{trend}}<0.0001$ (diet); $P_{\text{trend}}=0.002$ (exercise); $P_{\text{trend}}<0.0001$ (diet+exercise)). Finally, in participants receiving the dietary intervention, increased adherence as measured by attendance at the support groups, stratified by tertiles, was associated with significant reductions in ox-LDL (diet+exercise $P_{\text{trend}}=0.03$), increases in FOP in both diet and diet +exercise arms $P_{\text{trend}}=0.0001$ and $P_{\text{trend}}=0.0007$ respectively); and reductions in F₂-isoprostanes ($P_{\text{trend}}=0.0003$, $P_{\text{trend}}<0.0001$, respectively; supplementary Table 2). Repeating the analyses using only available data (i.e. missing data were not imputed) did not meaningfully affect any of the findings. Exclusion of outliers (values >99th percentile) had no effect on results for any analyte (data not shown).

Discussion

Women randomized to a 12-month dietary weight loss program, with or without exercise, significantly reduced levels of F₂-isoprostanes and ox-LDL compared to controls. In contrast, FOPs increased in women randomized to either diet or diet+exercise arms. Further

analyses showed that higher levels of weight loss were associated with greater reductions in F₂-isoprostanes and ox-LDL, and to greater increases in FOP. Levels of F₂-isoprostanes were significantly reduced in participants who increased their VO₂max in the exercise and diet+exercise arms, suggesting that exercise may have effects on F₂-isoprostanes independent of adiposity changes.

A variety of uncontrolled small studies reported significantly reduced levels of oxidative stress after bariatric surgery-induced weight loss,(30,43) or moderate-intensity exercise interventions in women.(31,32,44) A 12-week dietary intervention among 20 women found that F₂-isoprostane decreased by 32% among women who lost 5kg of body-weight.(45) However, another 12-week weight loss intervention produced no change in F₂-isoprostanes vs. controls in 42 individuals with metabolic syndrome .(46) Fifty-six overweight healthy individuals randomized to a dietary weight loss intervention with an average 10.4% weight loss in the intervention arm, significantly reduced ox-LDL vs. controls.(47) Significant reductions in ox-LDL were observed in overweight women with abnormal metabolic profiles who underwent a 12-week dietary intervention; there were no reductions among women who were overweight but metabolically healthy.(48)

In 318 sedentary premenopausal women randomized to 4-months of 150 min/week of aerobic exercise vs. control, F₂-isoprostanes were significantly reduced only in women who were in the highest quartile of plasma F₂-isoprostanes at baseline.(36) We previously reported significant reductions in F₂-isoprostane levels which decreased linearly with gain in VO₂max relative to controls among 173 overweight postmenopausal women in a year-long exercise RCT.(34) However, exercise had no effect on levels in 55 individuals with diabetes, randomized to a low-fat diet, exercise, (30 minutes/day, 50–65% VO₂max, three days/week), a combination, or to a control arm for eight weeks.(49)

F₂-isoprostanes are formed by free-radical induced peroxidation of arachidonic acid in both skeletal muscle and plasma. These data support reductions of levels of this marker of oxidative stress via weight loss. In addition, of the analytes measured, only F₂-isoprostane levels were significantly altered in participants randomized to the exercise intervention, or in participants who increased their VO₂max. Exercise is known to promote ROS production in contracting muscle, and there has been concern about the effects of this oxidative stress both at a cellular and tissue level. (50) However, our data demonstrates that moderate-to-vigorous physical activity, associated with increases in VO₂max, significantly decreased circulating levels of markers of oxidative stress, compared to controls.

The increase in levels of FOP in the diet and diet+exercise arm is surprising. In a case-control study of individuals with colon rectal adenomatous polyps, CRP and F₂-isoprostanes were inversely associated with an oxidative balance score calculated by combining pro- and anti-oxidant factors, but there was an unexpected positive association with FOP.(19)

Strengths of our study include: 1) the RCT design allowing us to test the independent and combined effects of dietary weight loss and exercise on markers of oxidative stress; 2) use of a number of measures associated with oxidative stress, which measure lipid peroxidation (ox-LDL), F₂-isoprostanes, and oxidation from several sources (FOP); 3) the long-term

intervention with excellent study retention and intervention adherence; 4) a large sample size which provided excellent power and the ability to examine dose-response of change in outcomes with change in weight and VO₂max.

Limitations include: 1) the relatively homogenous study population which may limit generalizability; and 2) a selection of global markers of oxidative stress which limits our ability to determine the exact components of the oxidative stress ‘profile’ affected by the intervention.. In conclusion, a dietary weight loss intervention significantly reduced F₂-isoprostanes and ox-LDL, and increased FOP, while an exercise intervention significantly reduced F₂-isoprostanes. These results suggest further mechanisms through which obesity and sedentary lifestyles could increase risk of several cancers, and support testing weight loss and exercise interventions as cancer prevention methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support:

This work was supported by grants from the National Cancer Institute at the National Institutes of Health (R01 CA105204-01A1 and U54-CA116847 AMcT, CD, CYW, KC, KFS; R01 CA161131-01A1, AMcT, CD, CYW, MG); and grants from the Breast Cancer Research Foundation (AMcT, CD, CYW, JDT).

References

1. McTiernan A. Mechanisms linking physical activity with cancer. *Nat Rev Cancer*. 2008; 8(3):205–11. [PubMed: 18235448]
2. Park J, Morley TS, Kim M, Clegg DJ, Scherer PE. Obesity and cancer--mechanisms underlying tumour progression and recurrence. *Nature reviews Endocrinology*. 2014; 10(8):455–65.
3. Njajou OT, Kanaya AM, Holvoet P, Connelly S, Strotmeyer ES, Harris TB, et al. Association between oxidized LDL, obesity and type 2 diabetes in a population-based cohort, the Health, Aging and Body Composition Study. *Diabetes Metab Res Rev*. 2009; 25(8):733–9. [PubMed: 19780064]
4. Holmstrom KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nat Rev Mol Cell Biol*. 2014; 15(6):411–21. [PubMed: 24854789]
5. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007; 39(1):44–84. [PubMed: 16978905]
6. Castellani P, Balza E, Rubartelli A. Inflammation, DAMPs, tumor development, and progression: a vicious circle orchestrated by redox signaling. *Antioxidants & redox signaling*. 2014; 20(7):1086–97. [PubMed: 23373831]
7. Gupta RK, Patel AK, Shah N, Chaudhary AK, Jha UK, Yadav UC, et al. Oxidative stress and antioxidants in disease and cancer: a review. *Asian Pac J Cancer Prev*. 2014; 15(11):4405–9. [PubMed: 24969860]
8. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology*. 2007; 132(6):2169–80. [PubMed: 17498510]
9. Nguyen XM, Lane J, Smith BR, Nguyen NT. Changes in inflammatory biomarkers across weight classes in a representative US population: a link between obesity and inflammation. *J Gastrointest Surg*. 2009; 13(7):1205–12. [PubMed: 19415399]

10. Leslie NR, Bennett D, Lindsay YE, Stewart H, Gray A, Downes CP. Redox regulation of PI 3-kinase signalling via inactivation of PTEN. *The EMBO journal*. 2003; 22(20):5501–10. [PubMed: 14532122]
11. Imlay JA, Linn S. DNA damage and oxygen radical toxicity. *Science*. 1988; 240(4857):1302–09. [PubMed: 3287616]
12. Milne GL, Dai Q, Roberts LJ 2nd. The isoprostanes-25 years later. *Biochimica et biophysica acta*. 2015; 1851(4):433–45. [PubMed: 25449649]
13. Wu T, Willett WC, Rifai N, Rimm EB. Plasma fluorescent oxidation products as potential markers of oxidative stress for epidemiologic studies. *Am J Epidemiol*. 2007; 166(5):552–60. [PubMed: 17615091]
14. Wu T, Rifai N, Willett WC, Rimm EB. Plasma fluorescent oxidation products: independent predictors of coronary heart disease in men. *Am J Epidemiol*. 2007; 166(5):544–51. [PubMed: 17615090]
15. Rossner P Jr, Gammon MD, Terry MB, Agrawal M, Zhang FF, Teitelbaum SL, et al. Relationship between urinary 15-F2t-isoprostane and 8-oxodeoxyguanosine levels and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2006; 15(4):639–44. [PubMed: 16614103]
16. Dai Q, Gao YT, Shu XO, Yang G, Milne G, Cai Q, et al. Oxidative stress, obesity, and breast cancer risk: results from the Shanghai Women's Health Study. *J Clin Oncol*. 2009; 27(15):2482–8. [PubMed: 19380446]
17. Epplein M, Franke AA, Cooney RV, Morris JS, Wilkens LR, Goodman MT, et al. Association of plasma micronutrient levels and urinary isoprostane with risk of lung cancer: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev*. 2009; 18(7):1962–70. [PubMed: 19531680]
18. Asombang AW, Kayamba V, Mwanza-Lisulo M, Colditz G, Mudenda V, Yarasheski K, et al. Gastric cancer in Zambian adults: a prospective case-control study that assessed dietary intake and antioxidant status by using urinary isoprostane excretion. *Am J Clin Nutr*. 2013; 97(5):1029–35. [PubMed: 23535107]
19. Kong SY, Bostick RM, Flanders WD, McClellan WM, Thyagarajan B, Gross MD, et al. Oxidative balance score, colorectal adenoma, and markers of oxidative stress and inflammation. *Cancer Epidemiol Biomarkers Prev*. 2014; 23(3):545–54. [PubMed: 24443405]
20. Siamakpour-Reihani S, Scarbrough PM, Wang F, Spasojevic I, Base K, Sedjo R, et al. Systemic markers of oxidative status and colorectal adenomatous polyps. *Ann Epidemiol*. 2012; 22(8):587–91. [PubMed: 22695388]
21. Suzuki K, Ito Y, Wakai K, Kawado M, Hashimoto S, Toyoshima H, et al. Serum oxidized low-density lipoprotein levels and risk of colorectal cancer: a case-control study nested in the Japan Collaborative Cohort Study. *Cancer Epidemiol Biomarkers Prev*. 2004; 13(11 Pt 1):1781–7. [PubMed: 15533907]
22. Sisti JS, Lindstrom S, Kraft P, Tamimi RM, Rosner BA, Wu T, et al. Premenopausal plasma carotenoids, fluorescent oxidation products, and subsequent breast cancer risk in the nurses' health studies. *Breast Cancer Res Treat*. 2015
23. Holvoet P, Lee DH, Steffes M, Gross M, Jacobs DR Jr. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. *JAMA*. 2008; 299(19):2287–93. [PubMed: 18492970]
24. Kim M, Paik JK, Kang R, Kim SY, Lee SH, Lee JH. Increased oxidative stress in normal-weight postmenopausal women with metabolic syndrome compared with metabolically healthy overweight/obese individuals. *Metabolism*. 2013; 62(4):554–60. [PubMed: 23142163]
25. Van Guilder GP, Hoetzer GL, Greiner JJ, Stauffer BL, Desouza CA. Influence of metabolic syndrome on biomarkers of oxidative stress and inflammation in obese adults. *Obesity (Silver Spring)*. 2006; 14(12):2127–31. [PubMed: 17189537]
26. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet*. 2008; 371(9612):569–78. [PubMed: 18280327]
27. Keum N, Greenwood DC, Lee DH, Kim R, Aune D, Ju W, et al. Adult weight gain and adiposity-related cancers: a dose-response meta-analysis of prospective observational studies. *J Natl Cancer Inst*. 2015; 107(2)

28. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest*. 2004; 114(12):1752–61. [PubMed: 15599400]
29. Keaney JF Jr, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol*. 2003; 23(3):434–9. [PubMed: 12615693]
30. Marfella R, Barbieri M, Ruggiero R, Rizzo MR, Grella R, Mozzillo AL, et al. Bariatric surgery reduces oxidative stress by blunting 24-hours acute glucose fluctuations in type 2 diabetic obese patients. *Diabetes Care*. 2009
31. Schmitz KH, Warren M, Rundle AG, Williams NI, Gross MD, Kurzer MS. Exercise effect on oxidative stress is independent of change in estrogen metabolism. *Cancer Epidemiol Biomarkers Prev*. 2008; 17(1):220–3. [PubMed: 18199727]
32. Attipoe S, Park JY, Fenty N, Phares D, Brown M. Oxidative stress levels are reduced in postmenopausal women with exercise training regardless of hormone replacement therapy status. *J Women Aging*. 2008; 20(1–2):31–45. [PubMed: 18581699]
33. Elosua R, Molina L, Fito M, Arquer A, Sanchez-Quesada JL, Covas MI, et al. Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. *Atherosclerosis*. 2003; 167(2):327–34. [PubMed: 12818416]
34. Campbell PT, Gross MD, Potter JD, Schmitz KH, Duggan C, McTiernan A, et al. Effect of exercise on oxidative stress: a 12-month randomized, controlled trial. *Med Sci Sports Exerc*. 2010; 42(8):1448–53. [PubMed: 20139793]
35. Nikolaidis MG, Kyparos A, Vrabas IS. F(2)-isoprostane formation, measurement and interpretation: the role of exercise. *Prog Lipid Res*. 2011; 50(1):89–103. [PubMed: 20951733]
36. Arikawa AY, Thomas W, Gross M, Smith A, Phipps WR, Kurzer MS, et al. Aerobic training reduces systemic oxidative stress in young women with elevated levels of F2-isoprostanes. *Contemp Clin Trials*. 2013; 34(2):212–7. [PubMed: 23178737]
37. Foster-Schubert KE, Alfano CM, Duggan CR, Xiao L, Campbell KL, Kong A, et al. Effect of diet and exercise, alone or combined, on weight and body composition in overweight-to-obese postmenopausal women. *Obesity (Silver Spring)*. 2012; 20(8):1628–38. [PubMed: 21494229]
38. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc*. 2000; 32(9 Suppl):S498–504. [PubMed: 10993420]
39. Prasad K. C-reactive protein (CRP)-lowering agents. *Cardiovascular drug reviews*. 2006; 24(1):33–50. [PubMed: 16939632]
40. Wu T, Rifai N, Roberts LJ 2nd, Willett WC, Rimm EB. Stability of measurements of biomarkers of oxidative stress in blood over 36 hours. *Cancer Epidemiol Biomarkers Prev*. 2004; 13(8):1399–402. [PubMed: 15298964]
41. Morrow JD, Roberts LJ 2nd. Mass spectrometry of prostanoids: F2-isoprostanes produced by non-cyclooxygenase free radical-catalyzed mechanism. *Methods in enzymology*. 1994; 233:163–74. [PubMed: 8015454]
42. Antoni MH, Lutgendorf SK, Cole SW, Dhabhar FS, Sephton SE, McDonald PG, et al. The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. *Nat Rev Cancer*. 2006; 6(3):240–8. [PubMed: 16498446]
43. Cabrera JE, Valezi AC, Delfino VD, Lavado EL, Barbosa DS. Reduction in Plasma Levels of Inflammatory and Oxidative Stress Indicators After Roux-En-Y Gastric Bypass. *Obes Surg*. 2009
44. Devries MC, Hamadeh MJ, Glover AW, Raha S, Samjoo IA, Tarnopolsky MA. Endurance training without weight loss lowers systemic, but not muscle, oxidative stress with no effect on inflammation in lean and obese women. *Free Radic Biol Med*. 2008; 45(4):503–11. [PubMed: 18502211]
45. Davi G, Guagnano MT, Ciabattini G, Basili S, Falco A, Marinopiccoli M, et al. Platelet activation in obese women: role of inflammation and oxidant stress. *Jama*. 2002; 288(16):2008–14. [PubMed: 12387653]

46. Tsai IJ, Croft KD, Mori TA, Falck JR, Beilin LJ, Puddey IB, et al. 20-HETE and F2-isoprostanes in the metabolic syndrome: the effect of weight reduction. *Free Radic Biol Med*. 2009; 46(2):263–70. [PubMed: 19013235]
47. Pierce GL, Beske SD, Lawson BR, Southall KL, Benay FJ, Donato AJ, et al. Weight loss alone improves conduit and resistance artery endothelial function in young and older overweight/obese adults. *Hypertension*. 2008; 52(1):72–9. [PubMed: 18504322]
48. Shin MJ, Hyun YJ, Kim OY, Kim JY, Jang Y, Lee JH. Weight loss effect on inflammation and LDL oxidation in metabolically healthy but obese (MHO) individuals: low inflammation and LDL oxidation in MHO women. *Int J Obes (Lond)*. 2006; 30(10):1529–34. [PubMed: 16552406]
49. Mori TA, Dunstan DW, Burke V, Croft KD, Rivera JH, Beilin LJ, et al. Effect of dietary fish and exercise training on urinary F2-isoprostane excretion in non-insulin-dependent diabetic patients. *Metabolism*. 1999; 48(11):1402–8. [PubMed: 10582548]
50. Slattery K, Bentley D, Coutts AJ. The role of oxidative, inflammatory and neuroendocrinological systems during exercise stress in athletes: implications of antioxidant supplementation on physiological adaptation during intensified physical training. *Sports Med*. 2015; 45(4):453–71. [PubMed: 25398224]

Figure 1.

CONSORT diagram of the Nutrition and Exercise for Women (NEW) trial

‡ Omitted 33 analytes from the F₂-isoprostanes assay, as they failed assay quality control (n=4, control arm; n=9 exercise arm; n=12 diet arm; n=8 diet+exercise arm)

*Missing baseline blood sample (n=1)

‡ Missing 12-month blood sample

** LOCF: Missing data at 12 months were imputed using the method last observation carried forward.

Table 1

Baseline characteristics of randomized participants

	Control (N=87)		Diet (N=118)		Exercise (N=117)		Diet+Exercise (N=117)		All Participants (N=438) ^a	
	Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)	
Age (years)	57.4 (4.4)		58.1 (6.0)		58.1 (5.0)		58.0 (4.5)		57.9 (5.0)	
BMI (kg/m ²)	30.7 (3.9)		31.1 (3.9)		30.7 (3.7)		31.0 (4.3)		30.9 (4.0)	
Waist circumference (cm)	94.83 (10.2)		94.61 (10.2)		95.05 (10.1)		93.71 (9.9)		94.5 (10.1)	
VO ₂ max (kg/mL/min))	23.1 (4.1)		22.7 (3.8)		22.5 (4.1)		23.6 (4.1)		22.9 (4.03)	
Usual physical activity (min/wk)	23.8 (41.2)		33.6 (45.5)		37.7 (43.7)		32.4 (42.9)		32.4 (43.6)	
Total calories (kcal/d)	1988 (669)		1884 (661)		1986 (589)		1894 (638)		1935 (637.9)	
	N (%)		N (%)		N (%)		N (%)		N (%)	
Race/Ethnicity										
	74 (85.1)		101 (85.6)		98 (83.8)		100 (85.4)		372 (84.9)	
	6 (6.9)		9 (7.6)		15 (12.8)		5 (4.3)		35 (8.0)	
	3 (3.4)		2 (1.7)		2 (1.7)		5 (4.3)		12 (2.7)	
Other	4 (4.6)		6 (5.1)		2 (1.7)		7 (6.0)		19 (4.3)	
Education										
	59 (67.8)		76 (64.4)		70 (59.8)		81 (69.8)		286 (65.3)	
Medication Use										
	19 (21.8)		10 (8.5)		19 (16.2)		25 (21.6)		73 (16.7)	
	26 (29.9)		51 (43.2)		36 (30.8)		44 (37.9)		157 (35.8)	
Smoker (ever)	32 (36.8)		55 (46.6)		47 (40.2)		47 (40.5)		181 (41.3)	
Analytes										
	Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)	
Ox-LDL (U/L)	104.9 (40.45) ^{N=87}		112.4 (36.40) ^{N=118}		111.3 (46.20) ^{N=116}		105.9 (32.89) ^{N=116}		108.9 (39.20) ^{N=437}	

	Control (N=87)	Diet (N=118)	Exercise (N=117)	Diet+Exercise (N=117)	All Participants (N=438) ^a
FOP (fI milliunits/mL)	64.03 (175.9) ^{N=87}	45.71 (99.28) ^{N=118}	81.35 (217.6) ^{N=116}	47.41 (73.53) ^{N=116}	59.27 (151.3) ^{N=437}
F ₂ -isprostane (pg/mL)	62.93 (31.09) ^{N=83}	64.91 (32.77) ^{N=106}	67.79 (32.32) ^{N=108}	61.47 (28.75) ^{N=108}	64.36 (31.26) ^{N=405}

^aDifferences in the final number of analytes are due to the following:

- Omitted 33 analytes from the F2-isoprostanes assay, as they failed assay quality control (n=4, control arm; n=9 Exercise arm; n=12 Diet arm; n=8 Diet+Exercise arm);
- One participant randomized to the Diet+Exercise arm was missing a baseline blood sample (n=1)
- Two participants randomized to the diet arm and to the Exercise arm were missing a 12-month serum sample (n=2). Finally, one participant randomized to the Exercise arm was missing a 12 month plasma sample (n=1)

Table 2

Change in levels of circulating levels of oxidative stress biomarkers, by intervention arm, using last observation carried forward.

Biomarker	Study Arm	Time-points				Change		P-value ^d	
		Baseline		12 Month		Absolute change ^c (%)	Relative change 12M (1-C)/ ^d	Un-adjusted	Adjusted ^b
		N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)				
Oxidized LDL (U/L)	Control	87	97.22 (89.33, 105.8)	87	99.41 (91.97, 107.4)	2.19 (2.25)	.	.	.
	Diet	118	106.5 (100.3, 113.1)	118	100.2 (94.11, 106.7)	-6.32 (-5.93)	-8.51	0.08	0.09
	Exercise	116	102.6 (95.32, 110.5)	116	101.3 (94.63, 108.4)	-1.33 (-1.29)	-3.52	0.47	0.60
	Diet+Ex	116	100.9 (95.22, 106.8)	116	93.31 (87.88, 99.08)	-7.56 (-7.49)	-9.75	0.03	0.03
FOP ^e (fI milliunits/mL)	Control	87	39.7 (35.1, 44.9)	87	37.4 (35.0, 39.9)	-2.31 (-5.81)	.	.	.
	Diet	118	36.2 (33.6, 39.1)	118	41.6 (38.6, 44.9)	5.35 (14.77)	0.01	0.0002	0.0001
	Exercise	116	44.3 (38.8, 50.6)	116	42.2 (37.1, 48.0)	-2.1 (-4.70)	0.00	0.86	0.85
	Diet+Ex	116	39.3 (36.3, 42.6)	116	46.2 (42.0, 50.8)	6.9 (17.45)	0.01	0.0001	0.0001
F2-Isoprostane (pg/mL)	Control	83	56.23 (50.71, 62.36)	83	54.55 (49.15, 60.54)	-1.68 (-2.99)	.	.	.
	Diet	106	57.41 (52.06, 63.31)	106	44.37 (39.26, 50.14)	-13.0 (-22.7)	-11.4	0.0004	0.0002
	Exercise	108	60.49 (55.07, 66.45)	108	51.71 (46.56, 57.4)	-8.78 (-14.5)	-7.10	0.03	0.01
	Diet+Ex	108	54.10 (48.67, 60.13)	108	41.38 (37.31, 45.89)	-12.7 (-23.5)	-11.0	<0.0000	<0.0000

^a P-values for comparing the 12-month changes vs. Control group. P-values <0.008 are considered significant due to multiple comparisons (Bonferroni correction)

^b GEE model adjusted for age, baseline BMI (<30kg/m², 30kg/m²) and race/ethnicity and NSAID and statin use

^c Change at 12-month from baseline

^d Relative difference of absolute change at 12 month from baseline between intervention and control arms.

^e Absolute and relative changes appear as zero due to rounding

Table 3

Change in levels of analytes stratified by percent weight loss compared to controls, by randomization arm, where missing data on weight was imputed using LOCF

Analyte and Weight change categories ^b	Diet				Exercise				Diet+Exercise			
	BASELINE		12 MONTH		Abs. Change (%) ^e	P ^c	BASELINE		12 MONTH		Abs. Change (%) ^e	P ^c
	N	GM ^a (95% CI)	N	GM ^a (95% CI)			N	GM ^a (95% CI)				
Oxidized LDL (U/L)												
Control	87	97.22 (89.33, 105.8)	87	99.41 (91.97, 107.4)	2.19 (2.3)	.	87	97.22 (89.33, 105.8)	87	99.41 (91.97, 107.4)	2.19 (2.3)	.
No Change/ Gained Weight	23	112.0 (98.38, 127.6)	23	107.6 (94.24, 122.9)	-4.41 (-3.9)	0.26	41	106.4 (96.28, 117.6)	41	109.1 (98.19, 121.2)	2.67 (2.5)	0.93
Lost <5%	19	103.3 (88.90, 120.0)	19	97.35 (80.90, 117.1)	-5.93 (-5.7)	0.28	46	104.7 (91.52, 119.7)	46	99.22 (88.98, 110.6)	-5.43 (-5.2)	0.43
Lost 5%	76	105.7 (97.93, 114.1)	76	98.77 (91.52, 106.6)	-6.95 (-6.6)	0.08	29	94.51 (81.43, 109.7)	29	94.26 (81.38, 109.2)	-25 (-0.3)	0.92
P _{trend} ^d	0.06				0.62				0.02			
FOP (fI milliunits/mL)												
Control	87	39.7 (35.1, 44.9)	87	37.4 (35.0, 39.9)	-2.3 (-5.8)	.	87	39.7 (35.1, 44.9)	87	37.4 (35.1, 39.9)	-2.3 (-5.8)	.
No Change/ Gained Weight	23	41.5 (30.2, 57.1)	23	42.7 (31.3, 58.4)	1.2 (2.9)	0.15	41	46.3 (35.1, 61.1)	41	43.9 (33.1, 58.4)	-2.3 (-5.1)	0.90
Lost <5%	19	35.3 (31.5, 39.5)	19	36.8 (32.8, 41.2)	1.5 (4.2)	0.05	46	43.2 (35.6, 52.3)	46	40.8 (33.7, 49.5)	-2.3 (-5.4)	0.98
Lost 5%	76	35.0 (32.8, 37.4)	76	42.6 (39.9, 45.4)	7.5 (21.5)	<0.0001	29	43.4 (35.5, 53.0)	29	42.0 (37.0, 47.7)	-1.3 (-3.1)	0.88
P _{trend} ^d	<0.0001				0.93				<0.0001			
F2-Isoprostanes (pg/mL)												
Control	83	56.23 (50.71, 62.36)	83	54.55 (49.15, 60.54)	-1.68 (-3.0)	.	83	56.23 (50.71, 62.36)	83	54.55 (49.15, 60.54)	-1.68 (-3.0)	.
No Change/ Gained Weight	19	66.94 (55.02, 81.45)	19	57.39 (36.49, 90.26)	-9.55 (-14.3)	0.54	39	62.35 (54.88, 70.85)	39	59.28 (52.16, 67.36)	-3.08 (-4.9)	0.60
Lost <5%	16	56.17 (43.95, 71.80)	16	47.43 (38.00, 59.19)	-8.75 (-15.6)	0.15	42	60.80 (51.51, 71.77)	42	50.80 (42.06, 61.35)	-10.0 (-16.5)	0.05
Lost 5%	71	55.36 (48.89, 62.69)	71	40.80 (36.00, 46.23)	-14.6 (-26.3)	<0.0001	27	57.44 (46.73, 70.61)	27	43.64 (34.80, 54.72)	-13.8 (-24.0)	0.0002
P _{trend} ^d	<0.0001				0.0002				<0.0001			

^a GM: geometric mean

^b Analyses stratified by weight loss percentage and using all available data (participants randomized to intervention arms with missing 12-month weight were classified as having no change/gained)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

^c p-value obtained from GEE model comparing the difference in change of the biomarkers from baseline to 12- month in intervention group vs control within strata of percent weight loss, adjusted for age, baseline BMI (<30kg/m², 30kg/m²), race/ethnicity (White, Black, and others), NSAID use and statins use

^d ^Ptrend*: p-value obtained from GEE model testing the linear trend in the change of the biomarkers from baseline to 12-month from Control through all levels of percent weight loss, adjusted for age, baseline BMI (<30kg/m², 30kg/m²), race/ethnicity (White, Black, and others), NSAID use and statins use

^e Change at 12-month from baseline

Table 4

Change in levels of analytes by tertiles of percent change in VO₂max levels (mL/kg/min), compared to controls

Analyte and Change in VO _{2max} stratified by tertiles	Exercise only				Diet +Exercise							
	BASELINE		12 MONTH		BASELINE		12 MONTH					
	N	GM (95% CI)	N	GM (95% CI)	Abs. Change (%)*	Pa	N	GM (95% CI)				
									Abs. Change (%)*	Pa		
Oxidized LDL (U/L)												
Control	87	97.22 (89.33, 105.8)	87	99.41 (91.97, 107.4)	2.19 (2.3)	.	87	97.22 (89.33, 105.8)	87	99.41 (91.97, 107.4)	2.19 (2.3)	.
Increased <3.5%	47	107.1 (95.28, 120.4)	47	110.2 (99.89, 121.6)	3.10 (2.9)	0.49	53	98.91 (91.64, 106.8)	53	93.92 (86.31, 102.2)	-5.00 (-5.1)	0.12
Increased 3.5–14.3%	36	99.33 (87.75, 112.4)	36	92.14 (80.41, 105.6)	-7.19 (-7.2)	0.19	32	111.0 (100.0, 123.1)	32	98.77 (88.97, 109.7)	-12.2 (-11.0)	0.05
Increased 14.3%	33	100.0 (86.28, 116.0)	33	99.59 (88.45, 112.1)	-43 (-0.4)	0.84	31	94.52 (82.98, 107.7)	31	87.03 (76.16, 99.44)	-7.50 (-7.9)	0.11
P _{trend} ^b	0.49				0.04							
FOP (fI milliunits/mL)												
Control	87	39.7 (35.1, 44.9)	87	37.4 (35.0, 39.9)	-2.3 (-5.8)	.	87	37.7 (35.10, 44.9)	87	37.4 (35.0, 39.9)	-2.3 (-5.8)	
Increased <3.5%	47	40.2 (33.8.0, 47.8)	47	36.0 (32.6, 39.8)	-4.2 (-10.5)	0.57	53	40.8 (35.2, 47.3)	53	48.3 (40.1, 58.1)	7.4 (18.2)	0.002
Increased 3.5–14.3%	36	51.1 (37.2, 70.4)	36	54.9 (37.8, 79.7)	3.7 (7.3)	0.22	32	40.9 (36.5, 45.8)	32	48.2 (42.1, 55.2)	7.3 (17.9)	0.0004
Increased 14.3%	33	43.5 (35.9, 52.6)	33	39.8 (35.9, 44.0)	-3.7 (-8.5)	0.69	31	35.4 (31.6, 39.7)	31	41.0 (38.0, 44.2)	5.6 (15.7)	0.001
P _{trend} ^b	0.84				0.0002							
F2-Isoprostanes (pg/mL)												
Control	83	56.23 (50.71, 62.36)	83	54.55 (49.15, 60.54)	-1.68 (-3.0)	.	83	56.23 (50.71, 62.36)	83	54.55 (49.15, 60.54)	-1.68 (-3.0)	.
Increased <3.5%	45	67.56 (59.35, 76.90)	45	62.27 (55.17, 70.27)	-5.29 (-7.8)	0.13	49	53.12 (44.22, 63.81)	49	40.22 (34.14, 47.38)	-12.9 (-24.3)	0.002
Increased 3.5–14.3%	34	57.09 (48.18, 67.64)	34	51.29 (43.81, 60.05)	-5.80 (-10.2)	0.30	28	55.27 (46.38, 65.87)	28	44.76 (36.01, 55.64)	-10.5 (-19.0)	0.03
Increased 14.3%	29	54.54 (44.64, 66.65)	29	39.13 (30.10, 50.85)	-15.4 (-28.3)	0.001	31	54.62 (46.10, 64.72)	31	40.32 (34.36, 47.31)	-14.3 (-26.2)	0.002
P _{trend} ^b	0.003				0.0006							

^a p-value obtained from GEE model comparing the difference in change of the biomarkers from baseline to 12-month in intervention group vs control within tertiles of percent change in VO₂max.

^b P_{trend}*: p-value obtained from GEE model testing the linear trend in the change of the biomarkers from baseline to 12-month from Control through all tertiles of percent change in VO₂max. All models adjusted for age, baseline BMI (<30kg/m², 30kg/m²), race/ethnicity (White, Black, and others), NSAID use and statins use.

^c Change at 12-month from baseline