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Dietary weight-loss and exercise effects on serum biomarkers of angiogenesis in overweight postmenopausal women: a randomized controlled trial

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Abstract

Obese and sedentary persons have an increased risk for cancer, but underlying mechanisms are poorly understood. Angiogenesis is common to adipose tissue formation and remodeling, and to tumor vascularization. 439 overweight/obese, healthy, postmenopausal women (body mass index (BMI)>25 kg/m^2) aged 50-75 years, recruited between 2005-2008 were randomized to a 4-arm 12-month randomized controlled trial, comparing a caloric restriction diet arm (goal: 10% weight-loss, N=118), aerobic exercise arm (225 min/week of moderate-to-vigorous activity, N=117), a combined diet+exercise arm (N=117), or control (N=87) on circulating levels of angiogenic biomarkers. Vascular endothelial growth factor (VEGF), plasminogen activator inhibitor-1 (PAI-1); and pigment epithelium-derived factor (PEDF) were measured by immunoassay at baseline and 12-months. Changes were compared using generalized estimating equations, adjusting for baseline BMI age, and race/ethnicity. Participants randomized to the diet+exercise arms had statistically significantly greater reductions in PAI-1 at 12-months compared to controls (-19.3% vs. +3.48% respectively, P<0.0001). Participants randomized to the diet and diet+exercise arms had statistically significantly greater reductions in PEDF (-9.20%, -9.90% respectively, both P<0.0001) and VEGF (-8.25%, P=0.0005; -9.98%, P<0.0001, respectively) compared to controls. There were no differences in any of the analytes in participants randomized to the exercise arm compared to controls. Increasing weight-loss was statistically significantly associated with linear trends of greater reductions in PAI-1, PEDF and VEGF. Weight-loss is significantly associated with reduced circulating VEGF, PEDF and PAI-1, and could provide incentive for reducing weight as a cancer prevention method in overweight and obese individuals.

Keywords

Angiogenesis; exercise; weight-loss VEGF; PAI-1; PEDF

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Introduction

Overweight and obesity is associated with increased risk of a variety of cancers,(1) and regular physical activity is associated with reduced cancer risk.(2) A largely unexplored mechanism that could link obesity with cancer risk is angiogenesis, a process where new blood vessels form from pre-existing vessels allowing tissues to expand, regulated by maintaining a balance between pro- and anti-angiogenic factors.(3) Angiogenesis plays an important role in obesity; adipose tissue is highly plastic, and requires vascularization in order to expand.(4). Here, we investigated the pro-angiogenic Vascular Endothelial Growth Factor (VEGF) and Plasminogen Activator Inhibitor type-1 (PAI-1), and the anti-angiogenic Pigment Epithelium-Derived Factor (PEDF), as they have been extensively studied in overweight/obesity, and have also been implicated in tumorigenesis in both animal models and in epidemiological studies, extending our previous investigation of physical activity on these biomarkers.(5)

Angiogenesis blockade is an active area of clinical and translational research.(6,7) Most of the developed drugs target VEGF and its related pathways, including bevacizumab, a monoclonal antibody that inhibits VEGF-A, which has been approved for use by the FDA for treatment of a number of cancers.(8) However, given the potential adverse effects of these compounds,(9) they have not been proposed for the cancer prevention setting. Low-risk and low-cost methods for reducing angiogenesis, therefore, could have important public health benefits.

VEGF is a key regulator of angiogenesis and vascular permeability.(10) VEGF is elevated in the obese state (11,12), although mice overexpressing VEGF were protected against diet-included obesity and insulin resistance.(13) VEGF can also promote the growth, survival, migration and invasion of cancer cells.(14) PAI-1 is a serine protease inhibitor (serpin) and is elevated in overweight and type-2 diabetic patients.(15,16) It promotes angiogenesis,(17,18) and is a prognostic marker for poor outcome in a number of cancers including breast.(19) PEDF, an adipokine and serpin, has anti-angiogenic properties and is active against a wide range of angiogenic stimuli, including VEGF.(20) It has broad anti-tumor activity, and reduced levels of PEDF are associated with a worse prognosis in a variety of different cancers.(21) However, it is also associated with the presence of the metabolic syndrome, and contributes to the development of insulin resistance in obesity.(22,23)

Excessive accumulation of adipose tissue creates a pro-tumorigenic environment, characterized by inflammation, macrophage invasion and increased angiogenesis.(24) In this environment, tumor cells can circumvent inhibitory signals and harness these dysregulated processes to proliferate, and form new blood vessels resulting in inappropriate growth and tissue invasion.(24)(25) Further expansion of established dormant avascular tumors requires initiation of angiogenesis, or the ‘angiogenic switch’, allowing the tumor to transition to exponential growth.(26)

Previously, we found that women who lost at least 1.85% of baseline fat-mass (corresponding to median levels) in a 12-month exercise intervention experienced significant reductions in some biomarkers of angiogenesis,(5) compared with sedentary controls. To

extend this research, we investigated the independent and combined effects of dietary weight-loss and exercise on circulating levels of VEGF, PAI-1 and PEDF, in the context of a completed 12-month randomized controlled trial, the Nutrition & Exercise for Women (NEW) trial. We randomly assigned 439 postmenopausal overweight/obese women to a reduced calorie dietary weight-loss program, an aerobic exercise program, a combined dietary weight-loss plus exercise program, or to a control arm. We hypothesized that women randomized to dietary weight-loss, with or without exercise, would have significant decreases in VEGF and PAI-1, and increases in PEDF, compared with controls.

Methods

This study is ancillary to the NEW (www.clinicaltrials.gov NCT00470119) study, a 12-month randomized controlled trial that test the effects of caloric restriction and/or exercise on circulating sex steroid hormones in healthy overweight postmenopausal women. The study was carried out in the FHCRC, Seattle, Washington, USA, and performed with the approval of the FHCRC Institutional Review Board, in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. Written informed consent was obtained from each participant.

Study Population

The trial is described in detail elsewhere.(27) Briefly, 439 postmenopausal, healthy overweight (BMI>25 kg/m^2), sedentary women, aged 50-75 years, not taking hormonal therapy, were recruited through media and mass mailings and were enrolled in the study between 2005-2008; 12-month follow-up for all participants was completed in 2009. Eligible participants were randomly assigned to a (i) reduced-calorie dietary modification intervention (N=118); (ii) moderate-to-vigorous intensity aerobic exercise intervention (N=117); (iii) combined diet and exercise intervention (N=117); or (iv) control (no intervention) (N=87). Exclusion criteria included: >100 min/week of moderate physical activity; diagnosed serious medical condition(s); postmenopausal hormone use; consumption of >2 alcoholic drinks/day; current smoking; participation in another structured weight-loss program; contraindication to participation (e.g. abnormal exercise tolerance test). Permuted block randomization was used to achieve a proportionally smaller control group, stratified according to BMI (≥/<30 kg/m^2) and race/ethnicity. The random assignment was generated by a computerized program, written by the study statistician, and run by the study manager to assign eligible participants to study arms. Investigators and laboratory staff were blinded to randomization arm.

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 dietician 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.

The exercise intervention goal was 45 minutes of moderate-to-vigorous (≥4 metabolic equivalents [METs]) intensity exercise at a target heart rate of 70-85% observed maximum, 5 days/week by week 7. Participants attended three facility-based supervised sessions/week and exercised 2 days/week at home. They recorded exercise mode, duration, peak heart-rate, and perceived exertion at each session. Activities of ≥4 METs(28) counted towards the prescribed target.

Controls were asked not to change their diet or exercise habits.

Blood Specimen Collection and Processing

Fasting (12 hours) venous blood samples (50 mL) were collected during clinic visits at baseline (pre-randomization) and 12-months, when the study was completed. 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 1 hour, and stored at -70°C.

Assays

VEGF, PEDF (serum) and PAI-1 (plasma) were assayed at the Clinical and Epidemiologic Research Laboratory, at the Department of Laboratory Medicine, Boston Children's Hospital, Boston, MA, using Enzyme Linked Immunosorbent Assays from R&D Systems (Minneapolis, MN). Duplicate pooled blood samples were included for quality assurance (QA) purposes and to assess inter and intra-assay coefficient of variation (CV). Baseline and 12-month samples from each individual were included in the same batch, and participants' samples were randomly placed across batches. Laboratory personnel were blinded with regard to subject and QA sample identity. Inter- and intra-assay CVs for each assay were: VEGF 7.3% and 5.9%; PAI-1 8.2% and 7.9%; and PEDF 9.3% and 6.0%.

Covariates

All study measures were obtained at baseline and 12 months by trained personnel blinded to participants' randomization status. Height and weight were measured and body mass index (BMI, kg/m^2) calculated. Body composition (fat mass and percent body fat) was measured by DXA (Dual-energy X-ray absorptiometry) whole-body scanner (GE Lunar, Madison, WI). Cardiorespiratory fitness (VO_2max) was assessed using a maximal graded treadmill test according to a modified branching protocol.(29)

Questionnaires collected information on demographics, medical history, dietary intake, supplement use and physical activity patterns. Other covariates including adipokines, sex steroid hormones, biomarkers of inflammation, lipids, blood counts, and telomere length were measured as described previously. (30-35)

Statistical Analyses

Partial Pearson correlation coefficients were calculated between baseline biomarker measures, with Bonferroni correction for multiple testing (0.05/99=significant at $P<0.0005$).

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. The intervention effects on these variables were examined based on the assigned treatment at randomization, regardless of adherence or study retention (i.e., intent-to-treat). Mean 12-month changes in the intervention arm 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 α=0.05/3=0.02 for 3 comparisons) for the primary analysis. We used the method of last observation carried forward (LOCF) to deal with missing data at 12-months.

Changes in body composition and VO_2max levels were calculated and used to stratify observed changes in analytes between arms at 12 months. Weight-loss was categorized as no change/gained any weight (referent); lost <5% of baseline weight; and lost >5% of baseline weight. Participants with missing 12-month data were categorized as no change/gained weight. Changes in VO_{2max} were calculated and categorized as tertiles (increased <3.5%; increased >3.5-14.3%; increased >14.3%); participants with missing VO_2max data at 12 months were classified as increased <3.5%. Percent fat loss was categorized as: no change/ gaining any fat; decreasing <2.6%; decreasing 2.6-6.4%; and decreasing >6.4%, corresponding to tertiles of change. Participants missing 12-month percent body-fat, were categorized as no change/gaining fat. Fat loss, weight change and VO_{2max} levels in the control group were added as separate categories.

In secondary analyses, we examined changes in analytes for each intervention arm in subgroups of participants compared to controls by the above categories of weight-loss, change in VO_2max, and change in % body fat.

All models were adjusted for age, baseline BMI (<30kg/m^2, >30kg/m^2) and race/ethnicity. All statistical tests were two sided. Statistical analyses were performed using SAS software (version 8.2, SAS Institute Inc., Cary, NC).

Results

Participants

At 12-months, 399 of 439 participants completed physical exams and provided a blood sample, 397 underwent a DXA scan, and 371 completed a VO_2max test; 39 did not complete the study (Fig. 1). One participant randomized to diet+exercise was excluded from analysis due to missing baseline blood measures. At baseline, participants were on average 57.9 years, with an average BMI of 30.9 kg/m^2, and were predominantly non-Hispanic Whites; (Table 1).

Intervention Fidelity

Data on intervention adherence, weight-loss, and body composition changes in this trial have been previously reported.(27) The mean weight change was -2.4% (p=0.03; exercise arm), -8.5% (p<0.001; diet), and -10.8% (p<0.001; diet+exercise) vs. -0.8% among controls.

Women in all intervention groups significantly reduced % body fat (all p<0.001) compared to controls.

Percent of daily calories from fat decreased in both the diet and diet+exercise arms (-6.7% and -8.0%, respectively). In both diet groups, women attended an average of 27 diet counseling sessions (86%). Women randomized to exercise participated in moderate-to-vigorous activity for a mean (SD) of 163.3 (70.6) minutes/week, while women randomized to diet+exercise participated for 171.5 (62.9) minutes/week. Both groups significantly increased average pedometer steps/day and VO_2max compared to baseline.

Baseline Correlations

After Bonferroni correction, both PAI-1 and PEDF correlated strongly and statistically significantly (hereon 'significantly') (all P<0.0005) with the majority of markers associated with overweight (anthropometrics), triglyceride levels and insulin dysregulation, and negatively with adiponectin, ghrelin and HDL cholesterol (Table 2). PEDF correlated positively and significantly with CRP and SAA. Both had significant and negative associations with SHBG; with red blood cell counts and hematocrit levels (PAI-1 only); and with white blood cell counts (PEDF only); all P<0.001. VEGF did not correlate significantly with any baseline variables, with the exception of blood platelets (P<0.0001).

Intervention effects

Participants randomized to the diet+exercise arms had statistically significantly greater reductions in PAI-1 at 12-months compared to controls (-19.3% vs. +3.48% respectively, P<0.0001; Table 3). Participants randomized to the diet (-9.20%) and diet+exercise (-9.90%) arms had significantly greater reductions in PEDF compared to those randomized to the control arm (+0.18%), all P<0.0001. Finally, participants randomized to the diet (-8.25%, P=0.0005) and diet+exercise (-9.98%, <0.0001) arms had significantly greater reductions in VEGF compared to those randomized to the control arm (-1.21%). There were no statistically significant differences in any of the analytes in participants randomized to the exercise arm, compared to controls.

There was a statistically significant linear trend of reductions in PAI-1, PEDF and VEGF with increasing weight-loss among participants randomized to both the diet and diet +exercise arm: PAI-1, P_{trend}=0.0003; P_{trend}<0.0001 respectively; PEDF both P_{trend}<0.0001; and VEGF both P_{trend}<0.0001 (Table 4). In addition, participants who were randomized to the exercise arm had a statistically significant linear trend of reductions in PEDF with increasing levels of weight-loss (P_{trend}=0.002), but not in PAI-1 or VEGF.

Participants randomized to the diet+exercise arm and who increased their VO_2max levels by any level (Table 5) had significantly greater decreases in PAI-1 compared to controls (P_{trend}=0.0006). Similar trends were seen for PEDF and VEGF (both P_{trend}<0.0001). There were no statistically significant changes in any analyte in participants who were randomized to the exercise arm only and who increased their VO_2max compared to controls.

Finally, similar to weight-loss, increasing quantities of percent body-fat loss were associated with a statistically significant linear trend in reductions in PAI-1 in the diet and diet+exercise

arms (both $P_{trend}<0.0001$); in PEDF ($P_{trend}<0.0001$ (diet); $P_{trend}=0.009$ (exercise); $P_{trend}<0.0001$ (diet+exercise)); and in VEGF in the diet and diet+exercise arms (both $P_{trend}<0.0001$; Supplementary Table 1).

Discussion

This study compared the effects of dietary weight-loss, exercise, or their combination on circulating levels of regulators of angiogenesis, in a large sample of healthy, overweight/obese postmenopausal women. While correlation does not imply causality, the strong and statistically significant associations between PAI-1, PEDF and the majority of the anthropometric markers and circulating adipokines, suggest that they are interlinked with other factors associated with overweight and obesity. Of interest, despite PEDF's categorization as an anti-angiogenic factor, all of the correlations were in the same direction as for PAI-1, confirming similar results from a study in 125 men.(36) In contrast, VEGF levels did not correlate significantly with any variable, with the exception of platelet levels and white blood cell counts. Platelets are themselves regulators of angiogenesis; have a proliferative effect on cancer cells both *in vitro* and *in vivo*, (37) and can guide formation of early metastatic niches.(38)

Women randomized to the diet and diet+exercise arms had statistically significant reductions in PEDF (-9.20% and -9.90% respectively) and VEGF (-8.35% and -9.98% respectively); and statistically significant reductions in PAI-1 in women randomized to the diet+exercise arm only (-19.3%). Weight-loss appeared to account for the majority of these changes, with increasing quantities of weight-loss and reductions in percent body-fat both associated with statistically significant linear decreases in all analytes. This effect was seen only in women randomized to the diet and diet+exercise arms for PAI-1 and VEGF, but in all 3 intervention arms for PEDF. However, changes in VO_2max in the exercise arm alone were not associated with statistically significant reductions in any analyte. We confirmed that despite being an anti-angiogenic factor, weight-loss is significantly associated with reductions in PEDF.

While some studies have also reported effects of exercise and weight-loss on these angiogenic factors,(39,40) the studies have been small,(41,42) cross-sectional,(43) or limited to men.(44) In one study, 79 participants were randomized to a 12-week trial comparing an exercise intervention, a hypocaloric diet, and a combined arm, found that VEGF-A was non-significantly reduced by 10-22% in the weight-loss groups. However, the study lacked a control arm.(39) In our previous exercise trial in overweight/obese postmenopausal women, a 12 month exercise intervention produced a significantly greater reduction in PEDF levels (-3.7%), compared to control condition (+3.0%; P=0.009), and that above-median loss of body-fat was associated with greater reduction in PEDF.(5) The reasons for this difference are unclear – baseline levels of analytes in the present study were similar to those in our earlier investigation (444.9 pg/mL, VEGF; 11.8 μg/mL, PEDF; and 6.1 ng/mL, PAI-1).(5) Dietary weight-loss was not tested in that trial. A small study of 33 obese men and women also reported that weight-loss was statistically significantly associated with reductions in circulating PEDF levels.(36) A variety of studies have characterized the anti-tumorigenic effects of PEDF, (21) so the reductions in PEDF in response to weight-loss appear to be counterintuitive. However, its actions appear to be tissue-specific, having for example, anti-

inflammatory effects in the retina, and pro-inflammatory effects in adipose tissue and macrophages.(21)

In our previous studies investigating the role of weight-loss and exercise on circulating biomarkers, the majority of the biomarkers which were reduced (or in some cases elevated) in response to weight-loss were also reduced or elevated – albeit to a lesser degree- by exercise alone.(30-32,34,35) Our findings here indicate that exercise alone – either by intervention arm, or when stratified by changes in VO2max – had no effect on PEDF, PAI-1 or VEGF. With the exception of PEDF, weight-loss in the exercise arm was not associated with alterations in levels of these analytes. Exercise increases both skeletal muscle mass and circulation; both processes require upregulation of angiogenesis. It is unclear why the exercise intervention or increases in VO_{2max} in the exercise arm alone, did not affect circulating levels of VEGF, PAI-1 or PEDF. However while there are few data on this topic in the literature, it appears that expression of angiogenic factors may be localized to muscle tissue, and may contribute little to circulating levels. A small RCT of 79 obese men and women randomized to a diet, exercise or control arm found no statistically significant differences in circulating VEGF levels in the exercise arm at 6-months.(39) Finally the degree of weight-loss experienced by participants in our study in the exercise arm (-2.4%) compared to -8.5% in the diet arm, and -10.8% in the diet + exercise arm,(27) may have been insufficient to influence circulating levels of these analytes. While it is impossible to ascertain whether reductions in circulating levels of VEGF and other angiogenic factors could impact tumor-level angiogenesis, systemic reductions in these markers might conceivably be associated with a less favorable milieu for tumor growth and proliferation. It has been hypothesized that, as a consequence of metabolic syndrome, up-regulation of PAI-1 expression predisposes breast cancer to more aggressive stages, partially by affecting angiogenesis.(45,46) Pro-angiogenic factors such as VEGF secreted by adipose stem cells have been implicated in tumor growth by promoting vascularization.(47,48) Indeed a review suggested that a chemopreventive approach targeting both angiogenesis and inflammation in healthy individuals (termed angioprevention) may prevent tumor cell growth and progression by blocking vascularization of indolent tumors.(6) Suggested interventions include metabolic regulators such as metformin, anti-inflammatory agents, and a variety of phytochemicals and their derivatives.(6) However many anti-angiogenic drugs –including metformin (49)- have significant side effects. Weight-loss may represent a safe and effective method of improving the angiogenic profile in both cancer patients and in healthy individuals.

Study strengths include the randomized trial design, the inclusion of dietary weight-loss, exercise, and combined weight-loss and exercise interventions, the excellent adherence to interventions, and high quality and valid biomarker assays. Limitations include the relatively homogenous population which may limit generalizability, assays of only a select number of angiogenesis markers, and measurement of angiogenesis only in blood rather than in target tissue. Linear analyses may potentially incorporate observational study weaknesses, such as confounding. However we used the GEE modification of linear regression analyses, which has been demonstrated to overcome potential observational study weaknesses such as confounding, effect modification, and correlation within individuals over time.

In conclusion, we report that weight-loss reduced circulating VEGF, PEDF, and PAI-1, and suggest that weight-loss in overweight or obese postmenopausal women may reduce risk for cancer in part through altering angiogenesis. Further investigations into the unexpected reductions of PEDF levels with weight-loss are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

CD and AMcT had full access to all data and take responsibility for its integrity and accuracy of the data analysis

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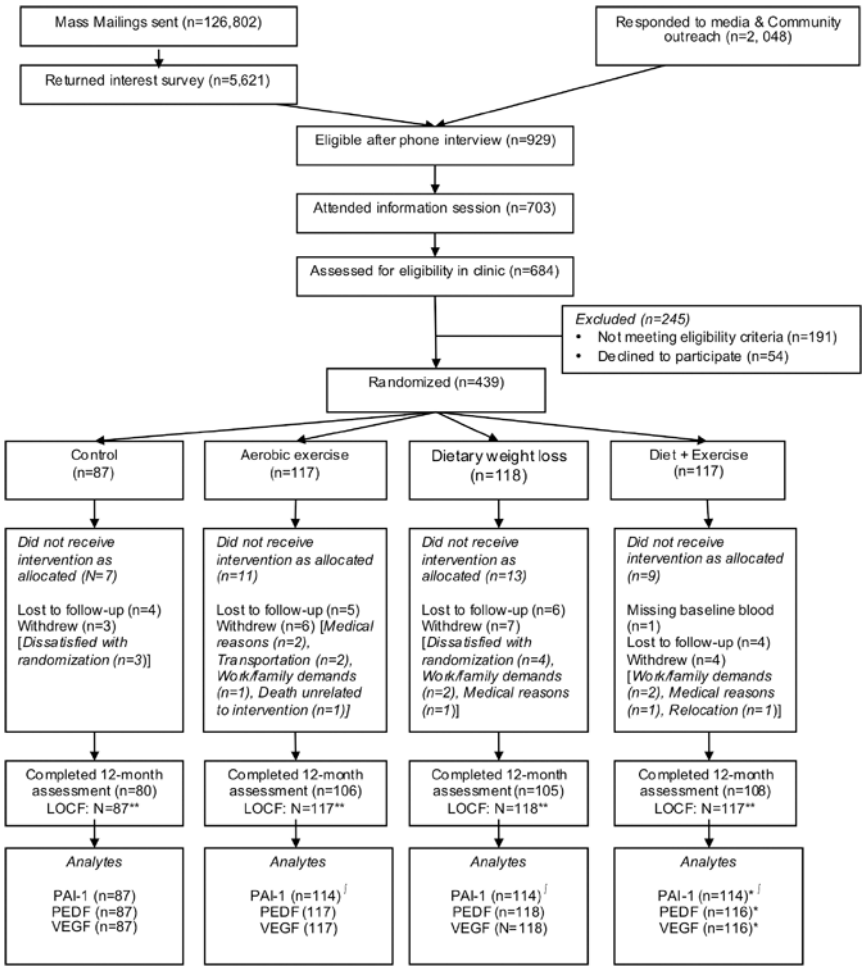

Figure 1. CONSORT diagram of the Nutrition and Exercise for Women (NEW) trial

*Missing baseline blood sample (N=1): omitted from analysis

∫Missing 12-month plasma sample (N=10 in total)

**LOCF: Missing data at 12 months were imputed using the method last observation carried forward for VO_2max, percent body fat, and weight

Table 1

Baseline characteristics of NEW study participants

	Control (N=87)	Diet (N=118)	Exercise (N=117)	Diet+Exercise (N=117)	All Participants (N=438)
	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_2max (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 (%)				
Race/Ethnicity					
Non-Hispanic White	74 (85.1)	101 (85.6)	98 (83.8)	100 (85.4)	372 (84.9)
African American	6 (6.9)	9 (7.6)	15 (12.8)	5 (4.3)	35 (8.0)
Hispanic/Latino	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					
College Graduate and Above	59 (67.8)	76 (64.4)	70 (59.8)	81 (69.8)	286 (65.3)
Smoker (ever)	32 (36.8)	55 (46.6)	47 (40.2)	47 (40.5)	181 (41.3)
	Mean (SD)				
PAI-1 (ng/mL)	8.9 (4.6)	8.1 (5.0)	7.6 (4.3)	7.9 (4.9)	8.1 (4.7)
PEDF (μg/mL)	10.9 (1.9)	10.9 (2.2)	10.6 (1.8)	10.7 (2.3)	10.8 (2.1)
VEGF (pg/mL)	391.4 (248.2)	369.7 (255.6)	377.3 (229.9)	393.9 (266.0)	382.5 (249.7)

Table 2

Baseline correlations between angiogenesis biomarkers and other study covariates

	PAI-1*		PEDF**		VEGF**	
	Rho	**P-value**	**Rho**	**P-value**	**Rho**	**P-value**
Anthropometrics						
Percent body-fat	0.13	0.007	0.20	<0.0001	0.06	0.22
BMI	0.29	<0.0001	0.36	<0.0001	0.07	0.19
Weight	0.24	<0.0001	0.33	<0.0001	0.09	0.09
Adipokines/Insulin etc.						
Homeostatic Model Assessment	0.40	<0.0001	0.36	<0.0001	-0.05	0.33
C-Peptide	0.48	<0.0001	0.45	<0.0001	-0.02	0.65
Adiponectin	-0.23	<0.0001	-0.16	0.0009	-0.04	0.44
Ghrelin	-0.27	<0.0001	-0.20	<0.0001	-0.13	0.009
Leptin	0.25	<0.0001	0.38	<0.0001	0.11	0.03
Insulin-like grown factor-1	-0.04	0.38	-0.06	0.22	-0.07	0.17
IGF- binding protein-3	0.20	<0.0001	0.13	0.007	0.02	0.72
Inflammation associated biomarkers						
C-reactive protein (CRP)	0.14	0.004	0.26	<0.0001	0.08	0.12
Serum amyloid protein A (SAA)	0.07	0.15	0.17	0.0004	0.02	0.66
TNF-alpha	0.05	0.34	0.07 N=437	0.12	0.02 N=437	0.73
IL-10	-0.07	0.19	-0.07 N=435	0.17	-0.02 N=435	0.73
IL-6	0.04	0.38	0.15	0.002	0.06	0.19
Lipoproteins and triglycerides						
Triglycerides	0.32	<0.0001	0.26 N=434	<0.0001	0.02 N=434	0.69
Low density lipoprotein	0.03	0.66	-0.04 N=434	0.47	-0.07 N=434	0.12
High density lipoprotein (HDL)	-0.21	<0.0001	-0.20 N=434	<0.0001	-0.02 N=434	0.69
Oxidized LDL	0.09	0.07	-0.04 N=434	0.46	-0.05 N=434	0.33
Sex steroid hormones						
Androstenedione	0.08	0.12	0.02	0.63	0.06	0.19

	PAI-1*		PEDF**		VEGF**	
	Rho	**P-value**	**Rho**	**P-value**	**Rho**	**P-value**
Estrone	0.13	0.004	0.19	0.0001	0.09	0.05
Estradiol	-0.01	0.77	0.07	0.15	0.06	0.19
Testosterone	-0.07	0.15	-0.02	0.61	0.01	0.80
Sex hormone binding globulin	-0.28	<0.0001	-0.24	<0.0001	-0.11	0.02
Blood cell counts and indices						
Hematocrit	0.23 N=427	<0.0001	0.11 N=436	0.02	-0.01 N=436	0.92
Mean corpuscular volume	-0.10	0.03	-0.18	0.0002	0.01	0.88
Red blood cell count	0.27 N=428	<0.0001	0.22 N=437	<0.0001	-0.01 N=437	0.97
White blood cell count	0.12	0.02	0.17	0.0004	0.16	0.0009
Lymphocytes	0.04 N=428	0.39	-0.03 N=437	0.48	-0.02 N=437	0.61
Mean corpuscular hemoglobin	-0.12 N=428	0.02	-0.14	0.004	-0.04	0.36
Platelets	0.11 N=428	0.03	0.02 N=437	0.78	0.23 N=437	<0.0001
Mean corpuscular hemoglobin concentration	-0.07	0.16	0.04	0.36	-0.14	0.003
Telomere length						
Telomere length	0.04N=427	0.48	0.03N=436	0.56	0.01 N=436	0.78

Correlations between continuous variables were estimated using the Pearson correlation. Significance was set at P<0.0005 after Bonferroni correction (P=0.05/99, for 33 × 3 comparisons).

*N=429, except where indicated by superscript

**All N=438 except where indicated by superscript

Table 3

Effects of dietary weight-loss, exercise, and combined dietary weight-loss and exercise on PAI-1, PEDF and VEGF in overweight and obese postmenopausal women

Biomarker	Study Arm	Time-point				Change		P-value[a]	
		Baseline		12 Month				Unadjusted	Adjusted[b]
		N	Mean (95% CI)	N	Mean (95% CI)	Absolute change (%)	Relative change 12MΔ (I-C)[c]	P	P
PAI-1 (ng/mL)	Control	87	7.88 (7.09-8.76)	87	8.15 (7.30-9.11)	0.27 (3.48)			
	Diet	114	6.97 (6.28-7.73)	114	6.32 (5.71-7.01)	-0.65 (-9.31)	-0.92	0.04	0.04
	Exercise	114	6.49 (5.82-7.24)	114	7.03 (6.371-7.75)	0.53 (8.23)	0.26	0.45	0.59
	Diet+Ex	114	6.74 (6.08-7.47)	114	5.45 (4.87-6.07)	-1.30 (-19.3)	-1.58	<0.0001	<0.0001
PEDF (μg/mL)	Control	87	10.75 (10.35-11.17)	87	10.77 (10.37-11.20)	19.63 (0.18)			
	Diet	118	10.68 (10.31-11.07)	118	9.70 (9.33-10.08)	-0.98 (-9.20)	-1.00	<0.0001	<0.0001
	Exercise	117	10.46 (10.16-10.79)	117	10.20 (9.89-10.52)	-0.27 (-2.59)	-0.29	0.12	0.07
	Diet+Ex	116	10.44 (10.02-10.88)	116	9.41 (9.04-9.79)	-1.03 (-9.90)	-1.05	<0.0001	<0.0001
VEGF (pg/mL)	Control	87	325.0 (284.6-371.1)	87	321.0 (279.3-369.0)	-3.92 (-1.21)			
	Diet	118	294.4 (259.7-333.7)	118	270.1 (238.6-305.8)	-24.3 (-8.25)	-20.4	0.0004	0.0005
	Exercise	117	307.5 (272.1-347.5)	117	297.9 (263.7-336.5	-9.65 (-3.14)	-5.73	0.33	0.24
	Diet+Ex	116	310.9 (272.2-355.1)	116	279.9 (244.7-320.2)	-31.0 (-9.98)	-27.1	<0.0001	<0.0001

[a] P: p-values for comparing the 12-month changes in intervention groups vs. Control group.

[b] GEE models adjusted for age, baseline BMI (<30kg/m^2, >30kg/m^2) and race/ethnicity

[c] Relative difference of absolute change at 12 months from baseline between intervention and control arms. P-values<0.008 are considered significant

Table 4

Change in PAI-1, VEGF, and PEDF by percent weight-loss in intervention groups compared with controls

Analyte and Weight change categories[b]	Diet					Exercise					Diet+Exercise				
	N	Baseline GM[a] (95% CI)	12 Month GM[a] (95% CI)	Abs. Change (%)	P[c]	N	Baseline GM[a] (95% CI)	12 Month GM[a] (95% CI)	Abs. Change (%)	P[c]	N	Baseline GM[a] (95% CI)	12 Month GM[a] (95% CI)	Abs. Change (%)	P[c]
PAI-1															
Control	87	7.88 (7.09-8.76)	8.15 (7.29---9.11)	0.27 (3.5)		87	7.88 (7.09-8.76)	8.15 (7.29-9.11)	0.27 (3.5)		87	7.88 (7.09-8.76)	8.15 (7.29-9.11)	0.27 (3.5)	
No Change/Gained Weight	22	7.05 (5.61---8.87)	8.29 (6.75---10.18)	1.24 (17.6)	0.15	41	5.87 (4.88-7.05)	6.67 (5.69-7.82)	0.81 (13.7)	0.20	12	6.39 (5.22-7.84)	6.87 (5.43-8.69)	0.48 (7.4)	0.68
Lost ≥5%	18	7.72 (5.58---10.69)	8.56 (6.78---10.80)	0.84 (10.8)	0.56	44	6.66 (5.65-7.85)	7.46 (6.38-8.72)	0.80 (12.0)	0.38	14	8.12 (6.20-10.63)	7.55 (5.72-9.96)	-0.57 (-7.0)	0.28
Lost >5%	74	6.78 (6.00---7.66)	5.42 (4.80---6.12)	-1.36 (-20.0)	0.0002	29	7.22 (5.73-9.09)	6.91 (5.61-8.53)	-0.30 (-4.2)	0.32	88	6.59 (5.83-7.45)	5.00 4.40-5.68	-1.59 (-24.2)	<0.0001
$^{d}P_{trend}$					0.0003					0.79					<0.0001
PEDF															
Control	87	10.75 (10.35---11.17)	10.77 (10.37---11.20)	19.63 (0.2)		87	10.75 (10.35-11.17)	10.77 (10.37-11.20)	0.02 (0.2)		87	10.75 (10.35-11.17)	10.77 (10.37-11.20)	0.02 (0.2)	
No Change/Gained Weight	23	11.84 (11.12---12.60)	12.21 (11.47---13.00)	373.6 (3.2)	0.33	41	10.46 (9.97-10.97)	10.58 (10.09-11.05)	0.09 (0.9)	0.88	12	9.36 (7.88-11.11)	9.48 (8.06-11.16	0.13 (1.3)	0.87
Lost <5%	19	11.22 (10.25---12.29)	10.72 (9.83---11.70	-0.50 (-4.5)	0.16	46	10.44 (9.95-10.96)	10.28 (9.82-10.76)	-0.164 (-1.6)	0.27	14	11.08 (10.03-12.23)	10.61 (9.84-11.45	-0.47 (-4.2)	0.18
Lost ≥5%	76	10.23 (9.79---10.68)	8.82 (8.49, 9.16)	-1.40 (-13.7)	<0.0001	30	10.51 (9.84-11.23)	9.60 (8.92-10.33)	-0.91 (-8.7)	0.001	90	10.50 (10.03-10.99)	9.23 (8.82-9.64)	-1.27 (-12.1)	<0.0001
$^{d}P_{trend}$					<0.0001					0.002					<0.0001
VEGF															
Control	87	325.0 (284.6---371.1)	321.0 (279.3---369.0)	-3.92 (-1.2)		87	325.0 (284.6-371.1)	321.0 (279.3-369.0)	-3.92 (-1.2)		87	325.0 (284.6-371.1)	321.0 (279.3-369.0)	-3.92 (-1.2)	
No Change/Gained Weight	23	288.9 (220.7---378.0)	292.8 (222.9---384.6)	3.97 (1.4)	0.13	41	265.9 (212.9-332.2	262.2 (211.0-325.9)	-3.69 (-1.4)	0.89	12	381.8 (282.6-515.8)	377.6 (269.0-530.0)	-4.21 (-1.1)	0.86
Lost <5%	19	296.4 (227.4---386.3)	284.2 (219.3---368.3)	-12.2 (-4.1)	0.36	46	302.6 (253.4-361.4	292.0 (243.8-349.6)	-10.7 (-3.5)	0.39	14	369.4 (257.6-529.7)	337.4 (229.0 497.0)	-32.0 (-8.7)	0.07
Lost ≥5%	76	295.6 (250.5,348.7)	260.2 (221.1---306.2)	-35.3 (-12.0)	<0.0001	30	384.3 (304.2-485.6	365.5 (287.7-464.4)	-18.8 (-4.9)	0.25	90	294.5 (251.9-344.2)	261.2 (223.8 (304.9)	-33.3 (-11.3)	<0.0001
$^{d}P_{trend}$					<0.0001					0.20					<0.0001

[a] GM: geometric mean

[b] Analyses stratified by weight-loss percentage and using all available data. All models adjusted for age, baseline BMI (<30kg/m^2, >30kg/m^2), race/ethnicity

P[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.

$^{d}P_{trend}$: 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 levels of percent weight-loss.

Table 5

Change in PAI-1, VEGF, and PEDF by tertiles of percent change in VO_2max levels (mL/kg/min)

Analyte and Change in VO_{2max}, stratified by tertiles[b]	Exercise						Diet+Exercise					
	Baseline		12-month		Abs. Change (%)[e]	P**	Baseline		12-month		Abs. Change (%)[e]	P [c]
	N	GM[a] (95% CI)	N	GM[a] (95% CI)			N	GM[a] (95% CI)	N	GM[a] (95% CI)		
PAI-1												
Control	87	7.88 (7.09-8.76)	87	8.15 (7.29-9.11)	0.27 (3.5)		87	7.88 (7.09-8.76)	87	8.15 (7.29-9.11	0.27 (3.5)	
Increased <3.5%	47	6.20 (5.28-7.27)	47	6.59 (5.62-7.74)	0.40 (6.4)	0.71	52	6.86 (5.76-8.14)	52	5.77 (4.93-6.76	-1.08 (-15.7)	0.005
Increased ≥3.5-14.3%	35	7.45 (6.09-9.10)	35	7.49 (6.24-8.98)	0.04 (0.6)	0.75	31	6.68 (5.58-7.99)	31	4.78 (3.70-6.18	-1.90 (-28.4)	0.0001
Increased≥14.3%	32	5.99 (4.83-7.43)	32	7.20 (6.08-8.52)	1.21 (20.3)	0.10	31	6.63 (5.62-7.84)	31	5.60 (4.74-6.61)	-1.04 (-15.6)	0.02
P_{trend}[d]	0.24						0.0006					
PEDF												
Control	87	10.75 (10.35-11.17)	87	10.77 (10.37-11.20)	1.96 (0.2)		87	10.75 (10.35-11.17)	87	10.77 (10.37-11.20)	19.63 (0.2)	
Increased <3.5%	47	10.78 (10.32-11.25)	47	10.52 (10.04-11.02)	-0.25 (-2.4)	0.13	53	10.45 (9.87-11.07)	53	9.52 (9.00-10.07)	-0.93 (-8.9)	<0.0001
Increased ≥3.5-14.3%	37	10.49 (9.92-11.09)	37	10.17 (9.63-10.73)	-0.32 (-3.1)	0.19	32	10.55 (9.80-11.34)	32	9.41 (8.73-10.14)	-1.14 (-10.8)	0.0002
Increased≥14.3%	33	10.02 (9.43-10.64)	33	9.78 (9.20,10.41)	-0.24 (-2.3)	0.31	31	10.32 (9.37-11.36)	31	9.22 (8.45-10.05)	-1.11 (-10.7)	<0.0001
P_{trend}[d]	0.18						<0.0001					
VEGF												
Control	87	325.0 (284.6-371.1)	87	321.0 (279.3,369.0)	-3.92 (-1.2)		87	325.0 (284.6-371.1)	87	321.0 (279.3-369.0)	-3.92 (-1.2)	
Increased <3.5%	47	347.2 (285.0-423.1)	47	340.6 (281.5-412.0)	-6.66 (-1.9)	0.66	53	323.5 (258.4-404.9)	53	301.8 (242.5-375.7)	-21.6 (-6.7)	0.07
Increased ≥3.5-14.3%	37	333.0 (273.9-404.9)	37	324.6 (263.2-400.2)	-8.43 (-2.5)	0.81	32	272.4 (218.2-340.0)	32	237.5 (188.6-299.1)	-34.9 (-12.8)	<0.0001
Increased≥14.3%	33	236.5 (188.1-297.5)	33	223.5 (179.7-278.0)	-13.0 (-5.5)	0.07	31	333.0 (267.7-414.4)	31	291.5 (230.2-369.0	-41.6 (-12.5)	0.0009
P_{trend}[d]	0.15						<0.0001					

[a] GM: geometric mean.

[b] Analyses stratified by change in VO_2max by tertiles, using all available data, adjusted for age, baseline BMI (<30kg/m^2, >30kg/m^2), race/ethnicity

P[c]: p-value obtained from GEE model comparing difference in change of the biomarkers from baseline to 12-month in intervention group vs control within tertiles of % change in VO_2max.

[d] 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 %change in VO_2max.