Research Article

Cancer Epidemiology, Biomarkers & Prevention

Long-Term Effects of Weight Loss and Exercise on Biomarkers Associated with Angiogenesis

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Abstract

Background: We tested the effect of weight loss on circulating levels of the angiogenic factors VEGF and pigment epithelium-derived factor (PEDF) in postmenopausal overweight/obese women, 18 months after completing a year-long 4-arm randomized controlled trial of behavioral weight loss and/or exercise versus control (i.e., 30 months postrandomization).

Methods: The 439 overweight/obese, postmenopausal women, ages 50 to 75 years, were randomized to: diet (goal: 10% weight loss, $N = 118$), exercise (225 min/wk moderate-to-vigorous activity, $N = 117$), diet + exercise ($N = 117$), or control ($N = 87$). At 12 months, 399 women gave a blood sample; 156 returned at 30 months. Biomarkers were measured by immunoassay. Changes were compared using generalized estimating equations, adjusting for baseline BMI, age, and race/ethnicity.

Results: Participants randomized to diet, exercise, and diet + exercise arms had greater reductions in VEGF at 30 months (−14.1% $P = 0.02$; −19.7% $P = 0.003$; −14.5% $P = 0.002$, respectively) versus controls (−4.5%). There were no statistically significant changes in PEDF in any intervention arm. Participants maintaining ≥10% of baseline weight loss at 30 months had greater reductions in VEGF versus those who gained weight/had no weight change (−22.3% vs. −10.2% respectively, $P = 0.002$). Participants maintaining any weight loss had significantly lower levels of PEDF at 30 months versus those who gained weight/no weight change.

Conclusions: Sustained weight loss via diet and/or exercise results in reductions in angiogenic factors, and can be maintained up to 30-month follow-up. Limitations include relatively small numbers, and possible bias toward more successful weight loss among women who returned at 30 months.

Impact: Maintaining weight loss can achieve long-term reductions in biomarkers of angiogenesis that can persist up to 18 months after completion of a weight loss intervention. *Cancer Epidemiol Biomarkers Prev; 26(12); 1788–94. ©2017 AACR.*

Introduction

Excess body fat is associated with increased risk of a variety of cancers (1–3). The exact mechanisms linking adiposity and excess cancer risk are unknown but may include altered sex hormone metabolism, increased insulin levels, and adipokine pathophysiology combined with systemic elevated inflammation (4, 5). These candidate mechanisms have a variety of effects on downstream processes, including on angiogenesis, a process where new blood vessels are formed. Angiogenesis is tightly regulated by a number of pro- and antiangiogenic factors in healthy tissue, but during tumorigenesis these pathways are dysregulated, and new blood vessels form, allowing dormant avascular tumors to form new blood vessels and to proliferate (6). Adipose tissue expansion also requires angiogenesis, and many of the angiogenic factors upregulated during adipogenesis are involved in the "angiogenic switch" (7–9).

We have previously reported that women who lost at least 1.85% of baseline fat mass in a 12-month exercise intervention experienced significant reductions in some biomarkers of angiogenesis (10) compared with sedentary controls. Subsequently, in a completed randomized control trial—the Nutrition and Exercise in Women (NEW) study—of 439 overweight/obese, postmenopausal women, we investigated the independent and combined effects of a behavioral dietary weight loss intervention and an exercise intervention on circulating levels of pro- and antiangiogenic factors, compared with controls. VEGF and plasminogen activator inhibitor type-1 (PAI-1), proangiogenic factors, and pigment epithelium-derived factor (PEDF), a potent inhibitor of angiogenesis (11), were measured in participants at baseline and 12 months post-randomization. Participants randomized to the diet + exercise arms had statistically significantly greater reductions in PAI-1, VEGF, and PEDF at 12 months compared with controls (all $P < 0.0001$). Increasing weight loss was statistically significantly associated with linear trends of greater reductions in PAI-1, PEDF, and VEGF (12). However, it is currently unknown whether beneficial changes in these and other cancer-related biomarkers persist long-term. Some studies on diabetes prevention indicate that, despite weight regain, risk of developing diabetes is reduced on average for several years (13). If biomarker changes associated with decreased cancer risk can be maintained, with or without sustained weight loss, it would support weight loss interventions for cancer prevention in overweight or obese individuals.

In this study, we investigate the effects of weight loss on long-term circulating levels of VEGF and PEDF in 156 women who were

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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enrolled in the NEW study, and who returned at 30 months post-randomization; that is, 18 months after discontinuing the year-long intervention, to give a blood sample. To our knowledge, this study will be the first to investigate the long-term effects of dietary weight loss on circulating levels of these angiogenic factors.

Materials and Methods

This study is ancillary to the NEW (www.clinicaltrials.gov NCT00470119) study, a 12-month randomized controlled trial that tested the effects of caloric restriction and/or exercise on circulating sex steroid hormones in healthy overweight, postmenopausal women. The parent and ancillary studies were carried out in the Fred Hutchinson Cancer Research Center (FHCRC; Seattle, Washington), and performed with the approval of the FHCRC Institutional Review Board, in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. Written informed consent was obtained from each participant. The parent trial is described in detail elsewhere (14). Briefly, 439 postmenopausal, healthy, overweight (BMI $\geq$ 25 kg/m^2), sedentary women, ages 50 to 75 years, not taking hormonal therapy, were recruited through media and mass mailings and were enrolled in the study between 2005 and 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/wk of moderate physical activity; diagnosed diabetes or other serious medical condition(s); postmenopausal hormone use; consumption of >2 alcoholic drinks/d; current smoking; participation in another structured weight loss program; contraindication to participation (e.g., abnormal exercise tolerance test, inability to attend sessions). Permuted block randomization was used to achieve a proportionally smaller control arm, stratified according to BMI ($\geq$/<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.

At 12 months, 399 of 439 participants from the NEW study completed physical exams and provided a blood sample (Fig. 1). For the ancillary study, women were identified from study records as they approached their 30-month anniversary of randomization. Seventy-five women had already passed the 30-month anniversary at the time recruitment began, and were thus ineligible. To assess interest, women were contacted by study staff, and invited to be part of the follow-up study. Of the 324 eligible (i.e., those who were still within the 30-month time frame) women contacted from the parent trial, 221 women agreed to provide questionnaire-based follow-up data and, of these, 156 provided fasting blood samples.

Blood specimen collection and processing

Fasting (12 hours) venous blood samples (50 mL) were collected during clinic visits at baseline (prerandomization), 12 months, when the study was completed and at 30 months post-randomization. Participants refrained from alcohol (48 hours), vigorous exercise or NSAID use (24 hours) before fasting venous blood collection (50 mL) at baseline and 12 months. Blood was processed within one hour and stored at −70°C.

Assays

VEGF and PEDF were assayed from 30-month blood samples 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). Laboratory personnel were blinded with regard to subject and QA sample identity. Inter- and intra-assay CVs for each assay were VEGF 3.5% and 3.5%; PEDF 7.5% and 5.8%, respectively. To correct for assay drift over time and to incorporate these data into the baseline and 12-month data, we included a series of baseline and 12-month samples that matched samples that had been previously assayed in each batch of 30-month samples (12). We compared the results from these samples with previously measured results, and used mean of the difference to normalize the newly assayed 30-month data.

Covariates

All study measures were obtained at baseline, 12-month, and 30-month time points by trained personnel blinded to participants' randomization status. Height and weight were measured and body mass index (BMI, kg/m^2) calculated. 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 (15). Questionnaires collected information on demographics, medical history, dietary intake, supplement use, and physical activity patterns.

Statistical analyses

The Kolmogorov-Smirnov test was used for the baseline comparisons between participants versus nonparticipants because the assumption of homogeneous variances between the two samples may not hold for all variables. Descriptive data are presented as geometric means [95% confidence intervals (CI)].

We compared average changes in outcomes from baseline to 30 months by randomization arm, using the generalized estimating equations (GEE) modification of linear regression to account for the correlation within individuals over time. The main analyses were performed according to the intention-to-treat principle, where outcome for patients are analyzed by treatment assignment, regardless of adherence.

For secondary, preplanned analyses, we combined data from participants who had been randomized to the control and exercise arms to the "No Diet" group ($N = 56$), and data from participants randomized to the diet and diet + exercise arms to the "Diet" group ($N = 87$). Participants were divided into four categories based on change from baseline to 30 months: (i) gained weight or remained weight stable; (ii) lost < 5% of baseline weight; (iii) lost 5–< 10% of baseline weight; and (iv) lost $\geq$ 10% of baseline weight. These four weight-loss categories were used to stratify observed changes in analytes between the Diet and No Diet groups at 30 months. We then compared 30-month analyte changes by these 30-month weight loss groups. We next combined data for all women regardless of randomization group, and compared analytes changes from baseline to 30 months stratified by weight-loss categories.

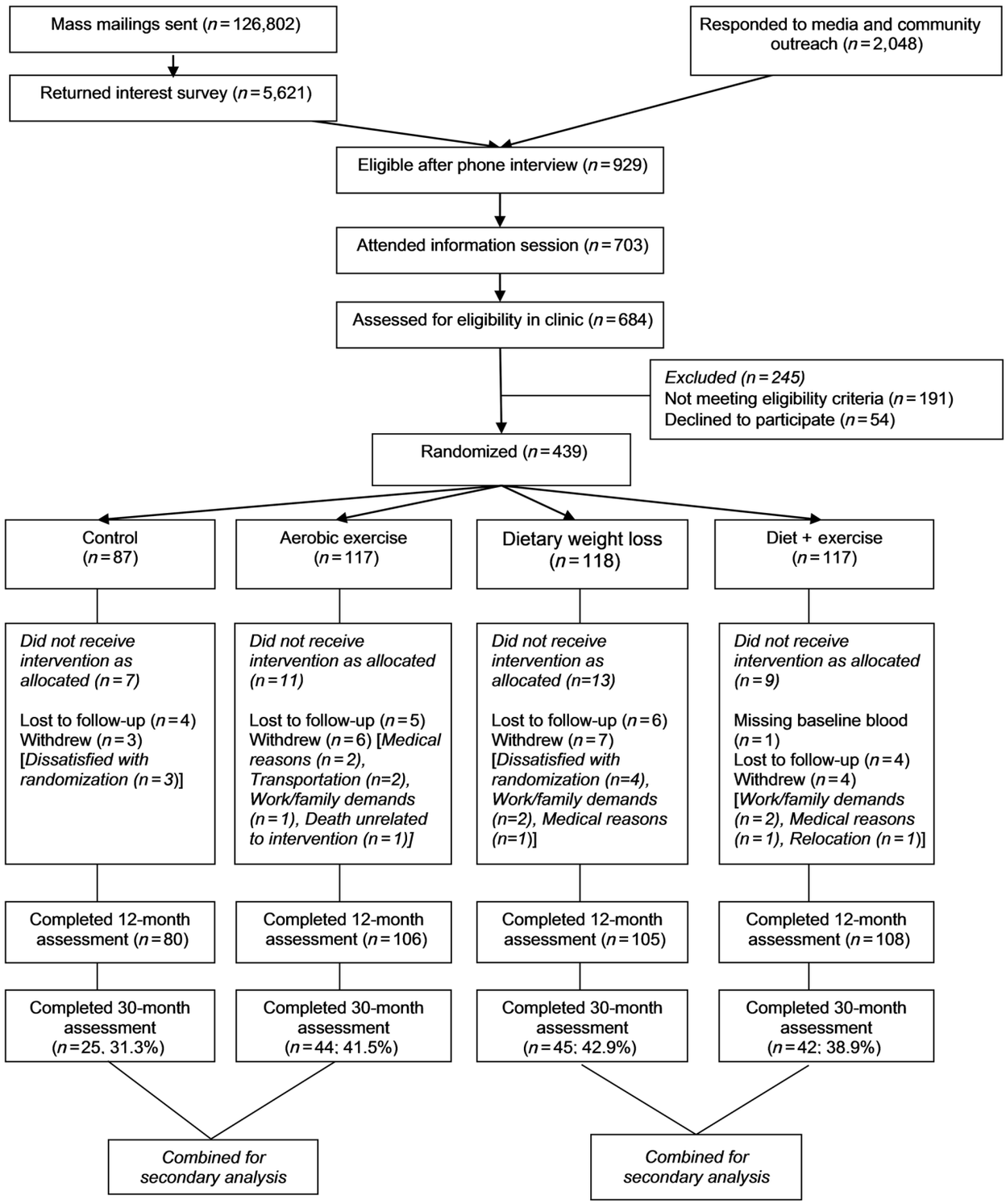

Figure 1.
CONSORT diagram. It illustrates the participant progress through the phases of the NEW study, a four-arm randomized control trial, including enrollment, intervention allocation and follow-up at 12 and 30 months postrandomization.

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

Results

Participants

At 30 months, 156 (39.1%) of 399 women who had completed the 12-month assessment provided a blood sample and completed questionnaires. At baseline, participants were on average 58.0 years, with an average BMI of 30.1 kg/m^2, and were predominantly non-Hispanic whites. Compared with nonparticipants, women who agreed to take part in the ancillary study had lower baseline BMIs (30.1 vs. 31.3 kg/m^2, $P = 0.007$), and a smaller waist circumference ($P < 0.001$), but did not differ by baseline VEGF or PEDF levels, or percent weight lost during the 12-month intervention period (Table 1).

Although a pattern of weight regain is seen, at 30 months the mean reduction in BMI in the diet + exercise arm was −7.92% at 30 months versus −13.7% loss at 12 months. Weight loss at 30 months and 12 months in the control, diet, and exercise arms, were −2.88% and +0.62% (control); −6.64% and −12.0% (diet) and −2.11% and −1.93% (exercise), respectively.

Intervention and weight-loss effects on angiogenic factors in the parent study (NEW study)

Briefly, in the parent trial (the NEW study), participants randomized to the diet and diet + exercise arms only had statistically significantly greater reductions in both VEGF and PEDF at 12 months compared with those randomized to the control arm (all $P \leq 0.0005$). There was a statistically significant linear trend of reductions in PEDF and VEGF with increasing weight loss among participants randomized to both the diet and diet + exercise arm (all $P_{trend}<0.0001$). Participants randomized to the exercise arm also had statistically significant linear trend of reductions in PEDF with increasing levels of weight loss (12)

Intervention effects on angiogenic factors at 30-month follow-up (NOW study)

At the 30-month follow-up, participants randomized to any of the three intervention arms had statistically significant reductions in levels of VEGF between baseline and 30 months compared to controls (controls −4.54%; diet −14.1%, $P = 0.02$; exercise −19.7%, $P = 0.003$; diet + exercise −14.5%, $P = 0.002$; Table 2). In contrast, there were no statistically significant changes in levels of PEDF between baseline and 30 months in any randomization arm, compared with controls.

Weight loss effects on angiogenic factors at 30-month follow-up

In the parent study, weight loss appeared to account for the majority of the reductions in both PEDF and VEGF, with increasing weight loss associated with statistically significant linear decreases in all analytes. Because of the relatively small numbers, we were unable to stratify randomization arm by weight-loss categories. However, when we combined the diet and diet + exercise arms into a "Diet" group, and control and exercise arms into a "No Diet" group, and stratified by weight loss, there were no associations between changes in analytes from baseline to 30-months by intervention grouping. This lack of association may be due to the greater increase in percent weight loss in the control and exercise arms at 30 months (−2.88% and −2.11% respectively), compared with 12 months (0.62% and −1.93%, respectively; Supplementary Table S1).

When we combined all participants' data regardless of intervention arm, and stratified by four categories of percentage of weight loss, only women who maintained ≥10% of baseline weight at 30 months had significantly greater reductions in VEGF levels compared to those who lost no weight or gained weight (−22.3% vs. −10.2% respectively, $P = 0.002$; Table 3).

Table 1. Baseline covariates by the study group

	No diet (Control and Exercise), $N = 69$ Mean (SD)	Diet (Diet, and Diet + Exercise), $N = 87$ Mean (SD)	Study participants, $N = 156$ Mean (SD)	Study nonparticipants, $N = 282$ Mean (SD)	P[a]
Age	58.54 (5.22)	58.10 (5.75)	58.3 (5.5)	57.74 (4.74)	0.48
BMI kg/m^2	29.63 (3.64)	30.39 (3.85)	30.06 (3.77)	31.31 (4.0)	0.007
Waist circumference (cm)	91.47 (8.51)	92.29 (8.82)	91.93 (8.66)	95.97 (10.53)	<0.001
Physical activity	38.32 (46.90)	35.99 (48.46)	37.02 (47.64)	29.90 (41.12)	0.16
% calories intake from fat	33.45 (6.66)	33.97 (6.22) $^{N=85}$	33.74 (6.41) $^{N=154}$	34.63 (7.19)$^{N=272}$	0.57
VO_2max	22.78 (4.11)	22.70 (4.38)	22.74 (4.25)	23.05 (3.90)	0.92
Total calories intake	2,108 (668.6)	1,886 (563.2)$^{N=85}$	1,985 (620.5)$^{N=154}$	1,907 (647)$^{N=272}$	0.32
Weight loss at 12 months (%)	−0.92 (4.21)	−12.60 (6.33)	−7.43 (7.99)	−6.02 (7.34)	0.19
VEGF	347.6 (220.6)	383.2 (228.9)	367.4 (225.2)	390.8 (262.3)	0.74
PEDF	10.62 (2.03)	10.81 (2.16)	10.73 (2.10)	10.80 (2.07)	0.73
	N(%)	*N*(%)	*N*(%)	*N*(%)	P[b]
Race/ethnicity					
Non-Hispanic white	61 (88.4)	76 (87.4)	137 (87.8)	235 (83.3)	0.09
African American	4 (5.8)	2 (2.3)	6 (3.8)	29 (10.3)	
Hispanic/Latino	2 (2.9)	4 (4.6)	6 (3.8)	6 (2.1)	
Other	2 (2.9)	5 (5.7)	7 (4.6)	12 (4.3)	
Education	46 (66.67)	60 (68.97)	106 (67.95)	180 (63.83)	0.39

[a]A P value from a nonparametric Kolmogorov-Smirnov two-sample test for difference between study participants versus nonparticipants.
[b]A P value from a χ^2 test for a difference in proportions between study participants versus nonparticipants.

Table 2. Twelve- and 30-month changes in BMI and in angiogenesis biomarkers by randomization arm

	Time point							P			
	Baseline		12 Months		30 Months						
Randomization arm	*N*	GM (95% CI)	*N*	GM (95% CI)	*N*	GM (95% CI)	0–12 Months change (%)	0–30 Months change (%)	P_{12}^{unadj}	P_{12}^{adj}	P_{30}^{unadj}	P_{30}^{adj}
BMI												
Control	25	30.42 (28.99–31.93)	25	30.61 (29.03–32.28)	24	29.55 (27.77–31.43)	0.19 (0.62)	−0.88 (−2.88)	.	.	.	.
Diet	45	29.88 (28.85–30.94)	45	26.30 (25.14–27.51)	45	27.89 (26.69–29.15)	−3.58 (−12.0)	−1.98 (−6.64)	<0.001	<0.001	0.05	0.05
Exercise	44	28.87 (27.95–29.83)	44	28.32 (27.35–29.32)	44	28.27 (27.24–29.33)	−0.56 (−1.93)	−0.61 (−2.11)	0.02	0.02	0.47	0.45
Diet+Ex	42	30.46 (29.28–31.69)	42	26.28 (25.13–27.47)	42	28.05 (26.86–29.29)	−4.19 (−13.7)	−2.41 (−7.92)	<0.001	<0.001	0.005	0.005
VEGF												
Control	25	304.4 (236.7–391.4)	25	318.0 (243.2–415.9)	23	290.6 (221.1–381.8)	13.63 (4.48)	−13.8 (−4.54)	.	.	.	.
Diet	45	321.3 (268.6–384.3)	45	289.1 (241.4–346.3)	43	276.1 (224.0–340.2)	−32.1 (−10.0)	−45.2 (−14.1)	<0.001	<0.001	0.02	0.02
Exercise	44	269.4 (218.5–332.2)	44	267.1 (215.3–331.4)	39	216.5 (171.3–273.5)	−2.29 (−0.85)	−52.9 (−19.7)	0.13	0.126	0.002	0.003
Diet+Ex	42	312.2 (252.1–386.6)	42	284.2 (230.2–350.8)	39	267.0 (210.5–338.7)	−28.0 (−8.97)	−45.2 (−14.5)	<0.001	<0.001	0.002	0.002
PEDF												
Control	25	11.37 (10.60–12.20	25	11.24 (10.50–12.04	23	11.79 (10.75–12.93	−0.13 (-1.16)	0.42 (3.68)	.	.	.	.
Diet	45	10.68 (10.06–11.34	45	9.19 (8.66–9.77	43	10.59 (9.92–11.32	−1.49 (−13.9)	−0.09 (−0.84)	<0.001	<0.001	0.41	0.42
Exercise	44	9.95 (9.46–10.46	44	9.94 (9.46–10.45)	39	10.98 (10.32–11.68)	−0.01 (−0.08)	1.03 (10.35)	0.72	0.72	0.21	0.19
Diet+Ex	42	10.50 (9.88–11.16)	42	9.25 (8.77–9.75)	39	10.53 (10.02–11.07)	−1.25 (−11.9)	0.03 (0.29)	<0.001	<0.001	0.46	0.48

NOTE: P_{12}^{unadj} was obtained from a GEE model comparing changes in the biomarkers from baseline with 12 months between intervention versus control arm, unadjusted. P_{12}^{adj} was obtained from a GEE model comparing changes in the biomarkers from baseline with 12 months between intervention versus control arm, adjusted for baseline age, baseline BMI (<30 kg/m^2, ≥30 kg/m^2), and race/ethnicity. P_{30}^{unadj} was obtained from a GEE model comparing changes in the biomarkers from baseline with 30 months between intervention versus control arm, unadjusted. P_{30}^{adj} was obtained from a GEE model comparing changes in the biomarkers from baseline with 30 months between intervention vs control arm, adjusted for baseline age, baseline BMI (<30 kg/m^2, ≥30 kg/m^2), and race/ethnicity.

Participants who maintained any level of weight loss at 30 months had significantly lower levels of PEDF compared with those who had either gained weight or had no weight change. However, for participants who lost 0% to 5% and 5% to 10% of weight, these differences were represented by comparatively smaller increases in PEDF levels (+3.67%, 3.26%, respectively) compared with no weight loss/weight gain (+15.76%). Participants who lost ≥10% of baseline weight reduced PEDF levels by −7.73% ($P < 0.001$).

Discussion

This is the first study to examine the associations between maintaining long-term weight loss in participants 18 months after completing a structured randomized controlled dietary weight loss and/or exercise intervention, on circulating levels of biomarkers of angiogenesis—VEGF and PEDF. At 30 months, participants randomized to any of the three intervention arms had statistically significant reductions in levels of VEGF between baseline and 30 months compared with controls. In contrast, there were no statistically significant changes in levels of PEDF between baseline-30 months in any randomization arm, compared with controls.

Like the parent study, weight loss appeared to account for the majority of the reductions in both PEDF and VEGF: only women who maintained ≥10% of baseline weight at 30 months had significantly greater reductions in VEGF levels compared with those who lost no weight or gained weight. Participants who maintained any level of weight loss at 30 months had significantly lower levels of PEDF compared with those who had either gained weight or had no weight change.

Studies such as the Diabetes Prevention Program, which examined long-term weight maintenance, have primarily focused on diabetes prevention and have reported that participants who undertook lifestyle changes to reduce caloric intake and increase physical activity maintained some weight loss for a number of years after participants completed the intervention. These long-term benefits also included a reduction in the cumulative incidence of diabetes, and lower fasting glucose levels, among others (13, 16–18).

Randomized controlled studies have demonstrated that weight loss is associated with beneficial changes in cancer-associated biomarkers including adipokines (19), sex-steroid hormones (20), and markers of inflammation (21), among others. However, to our knowledge, few studies have examined long-term weight loss maintenance and its association with changes in cancer-related biomarkers. In the present study, the long-term reduction in levels of VEGF and PEDF appears to be related to the extent to which weight loss persisted, with the greatest reductions seen in participants who maintained a ≥10% reduction of baseline weight.

VEGF and its receptors have been identified as one of the main molecular drivers of tumor angiogenesis (22), and the VEGF pathway is a primary target for antiangiogenic drugs due to its "ubiquitous and increased expression in most human cancers" (23, 24). Adipogenesis is dependent upon angiogenesis (25–27), and VEGF is elevated in the obese state (28, 29). Expansion of fat mass induces hypoxia, a driver of VEGF expression, via HIF-1 stabilization (30).

Weight loss in our studies is consistently associated with reductions in the anti-angiogenic PEDF. In mouse models, PEDF

Table 3. Change from baseline to 30 months in the log-transformed (normalized) biomarkers by category of weight loss (all participants combined)

	Baseline		30 Months			
Weight change Baseline to 30 months	***N***	**GM (95% CI)9**	***N***	**GM (95% CI)**	**Absolute change (%)**	***P*[a]**
VEGF						
Lost ≤0%	33	286.7 (233.1–352.7)	30	257.4 (202.2–327.7)	−29.3 (−10.2)	.
Lost 0–5%	53	312.0 (266.6–365.1)	51	279.1 (233.0–334.3)	−32.9 (−10.5)	0.84
Lost 5–10%	37	302.1 (239.4–381.2)	31	266.3 (201.5–351.9)	−35.8 (−11.9)	0.13
Lost ≥10%	32	291.1 (219.6–385.9)	32	226.1 (169.5–301.6)	−65.0 (−22.3)	0.002
PEDF						
Lost ≤0%	33	10.32 (9.59–11.11)	30	11.95 (11.04–12.94)	1.63 (15.76)	.
Lost 0–5%	53	10.93 (10.37–11.51)	51	11.33 (10.79–11.89)	0.40 (3.67)	0.02
Lost 5–10%	37	10.30 (9.73–10.91)	31	10.64 (10.02–11.29)	0.34 (3.26)	0.01
Lost ≥10%	32	10.34 (9.68–11.05)	32	9.54 (8.94–10.19)	−0.80 (−7.73)	<0.001

[a]A *P* value obtained from a GEE model comparing the change from baseline to 30 months in the (log-transformed) biomarkers between higher levels of weight loss percentage to lowest level (Loss ≤ 0%).

is a key inhibitor of stromal vasculature and epithelial tissue growth in mouse prostate and pancreas, and exogenous PEDF induced tumor epithelial apoptosis *in vitro* and limited *in vivo* tumor xenograft growth, triggering endothelial apoptosis (31). However, it also has a significant role as an adipokine that induces insulin resistance and inflammatory signaling in muscle and fat cells (32), is associated with obesity (33) and with increased risk of developing the metabolic syndrome (34).

In our prior studies of the impact of weight loss on reducing levels of circulating biomarkers of angiogenesis (10, 12), we discussed the potential utility of weight loss as a chemopreventive approach targeting both angiogenesis and inflammation in healthy individuals (35). Again, although it is currently impossible to ascertain whether reductions in circulating levels of angiogenic factors might inhibit tumor cell growth and progression by blocking the vascularization of indolent tumors, the long-term maintenance of reduced levels of VEGF and PEDF can only reflect a more beneficial angiogenic profile. A structured weight loss program may thus represent a safe and effective method of improving the long-term angiogenic profile in overweight individuals.

Limitations of our study include the relatively small numbers of women that returned for the 30-month follow-up visit, which limit the power to investigate effects in subgroups of data. However, these numbers are broadly similar to other studies where participants returned for follow-up approximately 2-years post enrollment, after the intervention was completed (17). In addition, there may have been bias in women who returned—women who were more successful at maintaining their weight might have been more likely to return to the clinic. We also investigated only two angiogenic biomarkers.

Strengths of our study include the well-characterized study population, a focus on postmenopausal women, a group at high risk for obesity-related breast cancer, and excellent participant adherence to interventions. In addition, our focus on long-term effects of weight loss and exercise biomarkers of angiogenesis, builds on our prior work in the parent trial.

In conclusion, our findings support that post-intervention, maintaining weight loss can achieve statistically significant reductions in biomarkers of angiogenesis, that can be maintained up to 18 months after a diet and/or exercise intervention was completed. These findings should be investigated further in other RCTs of weight loss with extended follow-up.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C. Duggan, C.-Y. Wang, K.E.F. Schubert, A. McTiernan
Development of methodology: C. Duggan, K.E.F. Schubert
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.E.F. Schubert, A. McTiernan
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Duggan, J.de D. Tapsoba, C.-Y. Wang
Writing, review, and/or revision of the manuscript: C. Duggan, J.de D. Tapsoba, C.-Y. Wang, A. McTiernan
Study supervision: K.E.F. Schubert, A. McTiernan

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References

1. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K. Body fatness and cancer — viewpoint of the IARC working group. N Eng J Med 2016;375:794–8.
2. 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;371:569–78.
3. Kyrgiou M, Kalliala I, Markozannes G, Gunter MJ, Paraskevaidis E, Gabra H, et al. Adiposity and cancer at major anatomical sites: umbrella review of the literature. BMJ 2017;356.
4. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. J Clin Invest 2017;127:1–4.
5. Lohmann AE, Goodwin PJ, Chlebowski RT, Pan K, Stambolic V, Dowling RJ. Association of obesity-related metabolic disruptions with cancer risk and outcome. J Clin Oncol 2016;34:4249–55.
6. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003;3:401–10.
7. Lemoine AY, Ledoux S, Larger E. Adipose tissue angiogenesis in obesity. Thromb Haemost 2013;110:661–8.

8. Cao Y. Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. Cell Metab 2013;18:478–89.
9. Rupnick MA, Panigrahy D, Zhang CY, Dallabrida SM, Lowell BB, Langer R, et al. Adipose tissue mass can be regulated through the vasculature. Proc Natl Acad Sci USA 2002;99:10730–5.
10. Duggan C, Xiao L, Wang CY, McTiernan A. Effect of a 12-month exercise intervention on serum biomarkers of angiogenesis in postmenopausal women: a randomized controlled trial. CEBP 2014;23:648–57.
11. Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu HJ, Benedict W, et al. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. Science 1999;285:245–8.
12. Duggan C, Tapsoba Jde D, Wang CY, McTiernan A. Dietary weight loss and exercise effects on serum biomarkers of angiogenesis in overweight postmenopausal women: a randomized controlled trial. Cancer Res 2016;76:4226–35.
13. Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ, Brenneman AT, et al. 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. Lancet 2009;374:1677–86.
14. 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;20:1628–38.
15. Pate RR, Blair SN, Durstine JL, Eddy DL, Hanson P, Painter P, et al. Guidelines for exercise testing and prescription. Philadelphia, PA: Lea & Febiger; 1991. p. 70–2.
16. Lindstrom J, Ilanne-Parikka P, Peltonen M, Aunola S, Eriksson JG, Hemio K, et al. Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the finnish diabetes prevention study. Lancet 2006;368:1673–9.
17. Gilis-Januszewska A, Lindstrom J, Tuomilehto J, Piwonska-Solska B, Topor-Madry R, Szybinski Z, et al. Sustained diabetes risk reduction after real life and primary health care setting implementation of the diabetes in Europe prevention using lifestyle, physical activity and nutritional intervention (DE-PLAN) project. BMC Public Health 2017;17:198.
18. Wing RR. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. Arch Intern Med 2010;170:1566–75.
19. Abbenhardt C, McTiernan A, Alfano CM, Wener MH, Campbell KL, Duggan C, et al. Effects on adiponectin and leptin after individual and combined diet and exercise interventions in postmenopausal women. J Intern Med 2013;274:163–75.
20. Campbell KL, Foster-Schubert KE, Alfano CM, Wang CC, Wang CY, Duggan CR, et al. Reduced-calorie dietary weight loss, exercise, and sex hormones in postmenopausal women: randomized controlled trial. J Clin Oncol 2012;30:2314–26.
21. Imayama I, Ulrich CM, Alfano CM, Wang C, Xiao L, Wener MH, et al. Effects of a caloric restriction weight loss diet and exercise on inflammatory biomarkers in overweight/obese postmenopausal women: a randomized controlled trial. Cancer Research 2012;72:2314–26.
22. Kerbel RS. Tumor angiogenesis. N Eng J Med 2008;358:2039–49.
23. Jayson GC, Kerbel R, Ellis LM, Harris AL. Antiangiogenic therapy in oncology: current status and future directions. Lancet 2016;388:518–29.
24. Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. Nature 2005;438:967–74.
25. Christiaens V, Lijnen HR. Angiogenesis and development of adipose tissue. Mol Cell Endocrinol 2010;318:2–9.
26. Voros G, Maquoi E, Demeulemeester D, Clerx N, Collen D, Lijnen HR. Modulation of angiogenesis during adipose tissue development in murine models of obesity. Endocrinology 2005;146:4545–54.
27. Corvera S, Gealekman O. Adipose tissue angiogenesis: impact on obesity and type-2 diabetes. Biochim Biophys Acta 2014;1842:463–72.
28. Doupis J, Rahangdale S, Gnardellis C, Pena SE, Malhotra A, Veves A. Effects of diabetes and obesity on vascular reactivity, inflammatory cytokines, and growth factors. Obesity 2011;19:729–35.
29. Silha JV, Krsek M, Sucharda P, Murphy LJ. Angiogenic factors are elevated in overweight and obese individuals. Int J Obes 2005;29:1308–14.
30. Early Breast Cancer Trialists' Collaborative Group Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 1998;351:1451–67.
31. Doll JA, Stellmach VM, Bouck NP, Bergh AR, Lee C, Abramson LP, et al. Pigment epithelium-derived factor regulates the vasculature and mass of the prostate and pancreas. Nat Med 2003;9:774–80.
32. Famulla S, Lamers D, Hartwig S, Passlack W, Horrighs A, Cramer A, et al. Pigment epithelium-derived factor (PEDF) is one of the most abundant proteins secreted by human adipocytes and induces insulin resistance and inflammatory signaling in muscle and fat cells. Int J Obes 2011;35:762–72.
33. Wang P, Smit E, Brouwers MC, Goossens GH, van der Kallen CJ, van Greevenbroek MM, et al. Plasma pigment epithelium-derived factor is positively associated with obesity in Caucasian subjects, in particular with the visceral fat depot. Eur J Endocrinol 2008;159:713–71.
34. Chen C, Tso AW, Law LS, Cheung BM, Ong KL, Wat NM, et al. Plasma level of pigment epithelium-derived factor is independently associated with the development of the metabolic syndrome in Chinese men: a 10-year prospective study. J Clin Endocrinol Metab 2010;95:5074–81.
35. Albini A, Tosetti F, Li VW, Noonan DM, Li WW. Cancer prevention by targeting angiogenesis. Nat Rev Clin Oncol 2012;9:498–509.