Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2014 April ; 23(4): 648–657. doi:10.1158/1055-9965.EPI-13-1155.

Effect of a 12-month Exercise Intervention on Serum Biomarkers of Angiogenesis in Postmenopausal Women: a randomized controlled trial

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Abstract

Background—Increased physical activity is associated with decreased risk of several types of cancer, but underlying mechanisms are poorly understood. Angiogenesis, where new blood vessels are formed, is common to adipose tissue formation/remodelling and tumor vascularization.

Methods—We examined effects of a 12-month 45 minutes/day, 5 days/week moderate-intensity aerobic exercise intervention, on four serum markers of angiogenesis in 173 sedentary, overweight, postmenopausal women, 50–75 years, randomized to intervention vs. stretching control. Circulating levels of positive regulators of angiogenesis (vascular endothelial growth factor (VEGF), osteopontin (OPN), plasminogen activator inhibitor-1 (PAI-1)); and the negative regulator pigment epithelium-derived factor (PEDF), were measured by immunoassay at baseline and 12-months. Changes were compared using generalized estimating equations, adjusting for baseline levels of analytes and BMI.

Results—VEGF, OPN or PAI-1 levels did not differ by intervention arm. Participants randomized to exercise significantly reduced PEDF (−3.7%) vs. controls (+3.0%; P=0.009). Reductions in fat-mass were significantly associated with reductions in PAI-1 (P_{trend}=0.03; P_{trend}=0.02) and PEDF (P_{trend}=0.002; P_{trend}=0.01) compared to controls, or to those who gained any fat-mass respectively. There was a significant association between decreases in VO_{2max}, and increased reductions in PEDF (P_{trend}=0.03), compared to participants who increased their level of fitness.

Conclusions—Fat-loss reduces circulating PAI-1 and PEDF. Changes in VO_{2max} are associated with alterations in PEDF, but these associations are complex.

Impact—Unexpected reductions in PEDF with decreasing fat-mass, and with decreasing VO_{2max}, warrants further study, including examining effects of different types and intensities of exercise; and role of dietary weight-loss with and without exercise.

Keywords

Angiogenesis; exercise; VEGF; PAI-1; PEDF; osteopontin

Introduction

A strong and consistent body of epidemiologic evidence supports an association between increased levels of physical activity and reduced risk for several cancers including breast,

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

colon, endometrium, lung, and others.(1, 2) The available epidemiologic data suggest that individuals engaging in aerobic physical activity for approximately 3–4 hours/week at moderate or greater levels of intensity, have on average a 30% reduction in colon cancer risk, a 20%–40% lower risk of breast cancer, and approximate reductions in risk of lung, endometrial, and ovarian cancers of 20%, 30%, and 20%, respectively, compared with those who are sedentary.(3) However, mechanisms linking risk reductions in cancer to physical activity have not been fully elucidated.

Studies suggest that exercise can exert its cancer-preventive effects at many stages during the process of carcinogenesis, by modifying carcinogen activation; increasing a variety of anti-oxidant enzymes; enhancing DNA repair systems; altering cell proliferation, apoptosis and differentiation; and decreasing inflammation.(4) Alterations in angiogenic pathways are another way whereby exercise can modulate its anti-tumorigenic effects; however few studies have examined the effect of exercise on expression of these factors.

Angiogenesis and revascularization are common to both tumor growth and to tissue remodeling during adipose tissue expansion in order to support increased adipocyte numbers.(5, 6) However, there is still relatively little known about how altered levels of these proteins from the stromal and adipose microenvironment in the obese state contribute to the early events in the progression to malignancy. Barriers to understanding the effect of obesity and physical activity on human cancer development include lack of appropriate model systems to assess complex stromal and tissue remodeling events during the premalignant stages of cancer formation in humans. Elucidation of changes in the expression of certain biomarkers, such as angiogenic factors in response to exercise, in healthy overweight, sedentary individuals may indicate a profile of these factors associated with a pro-tumorigenic environment.

In tumors, an avascular phase corresponds to a small occult lesion of 1–2 mm in diameter, growth limited by a lack of oxygen and nutrients,(7, 8) which remains dormant by reaching a steady state between proliferation and apoptosis. The ‘angiogenic switch’ is a critical process whereby a tumor is transformed from the dormant state to large, vascular tumor with metastatic potential, and is triggered by angiogenic factors. Inhibiting angiogenesis may, therefore, be of potential value in preventing progression from a dormant small, avascular tumor, to invasive cancer. Adult adipose tissue is one of the largest, most plastic and highly vascularized tissues in the body.(9, 10) Adipogenesis requires tissue expansion, remodeling and increased vascularization, and angiogenic factors are upregulated in the obese state to support these processes. Adipogenesis and vascularization are spatially and temporally linked:(9) inhibition of angiogenesis can regulate fat mass,(11) and can inhibit diet-induced obesity in mice.(12) Adipose tissues are highly vascularized and expansion or shrinkage of adipose tissue mass requires up- or down-regulation of adipogenesis in response to changes in energy input and expenditure.(13)

A variety of angiogenic factors are common to tumorigenesis and adipogenesis, including Vascular Endothelial Growth Factor (VEGF), a key mediator of angiogenesis;(14) osteopontin (OPN), an adipokine whose plasma levels are increased in obesity(15, 16) and in patients with type 2 diabetes;(17) and Plasminogen Activator Inhibitor type-1 (PAI-1), a serine protease inhibitor (serpin)(18) which can act as a positive switch for angiogenesis by promoting endothelial cell migration toward fibronectin-rich tumor tissue, and whose inhibitors prevent angiogenesis.(19, 20) Finally, Pigment Epithelium-Derived Factor (PEDF), an adipokine and serpin, is a multifunctional secreted glycoprotein that displays broad anti-tumor activity; is essential for maintaining avascularity(21, 22) and preventing aberrant neovascularization;(23) is a potent negative regulator of angiogenesis;(24) and is

active against wide range of angiogenic stimuli, including VEGF, basic fibroblast growth factor, platelet-derived growth factor-BB, and IL-8.(21)

Some studies have examined the effects of exercise on these angiogenic factors, but the studies have been small, (25, 26) cross-sectional,(27) or limited to men.(28) Here we investigate the effects of a 1-year randomized controlled trial (RCT) of a moderate-to-vigorous physical activity intervention vs. control, on serum levels of VEGF, PAI-1 OPN and PEDF in 173 postmenopausal, overweight or obese, previously sedentary women.

Methods

This study is ancillary to the Physical Activity for Total Health study (Clinicaltrials.gov NCT00668174), an RCT comparing the effect of a 12-month moderate-intensity aerobic exercise intervention vs. stretching control program on circulating levels of estrone measured at baseline (pre-randomization) and 12 months. Secondary endpoints included comparing intervention effects on other sex hormones and other cancer biomarkers(29–31). The study was performed with the approval of the Fred Hutchinson Cancer Research Center 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 subject.

Study Population

The study has been described in detail elsewhere.(29, 32) Briefly, 173 postmenopausal, healthy overweight (BMI>25 kg/m^2), defined as sedentary women (<60 minutes/week of moderate- or vigorous-intensity recreational activity and a maximal oxygen consumption (VO_{2max}) <25.0 ml/kg/min), aged 50–75, not taking hormonal therapy, were enrolled between 1998–2000, and randomly assigned to exercise (n=87) intervention or a stretching control group (n=86).(29). Randomization was stratified by BMI (<27.5 kg/m^2 vs. >27.5 kg/m^2).

Covariates

Demographics, lifestyle behaviors, and anthropometrics were measured at baseline and 12 months, and BMI was calculated as kg/m^2. Body fat was measured by a DXA whole-body scanner (GE Lunar, Madison, WI). Aerobic fitness was assessed using a modified branching treadmill protocol.(33, 34) Heart rate and oxygen consumption were monitored by a MedGraphics automated cart during the test (MedGraphics, St.Paul, MN).

Exercise Intervention

The intervention consisted of at least 45 minutes of moderate-intensity exercise, 5 days/week for 12 months. The training program gradually increased to 60–75% of maximal heart rate for 45 minutes per session by week 8, where it was maintained for the duration of the study. We used two measures of exercise adherence. We assessed baseline and 12-month $VO_{2\ max}$ in all participants, who kept daily activity logs. Briefly,at the end of the study, intervention participants completed a mean of 176 (s.d. 91) minutes/week of aerobic exercise (78% of the 225 min/week goal); lost an average of 1.3 kg vs. 0.1-kg weight-gain in controls (P=0.01), and lost 8.5 g/cm^2 of intra-abdominal body fat vs. a gain (0.1 g/cm2) among controls (P=0.045).(32) On average, $VO_{2\ max}$ increased from baseline to 12-months by 12.7% in exercisers, and by 0.8% in controls ($P < 0.0001$).

Blood Specimen Collection and Processing

At baseline and 12-months, participants provided a 12-hour fasting 50 ml sample of blood, which was processed within 1 hour of collection and stored at −80°C. Subjects were instructed to refrain from alcohol (48 hours) and vigorous exercise (24 hours) prior to clinic appointments. Of 173 participants randomized, baseline and 12-month serum was available for 169 participants (84 control; 85 intervention); and plasma for 164 (80 controls; 84 exercise). Samples were stored on average for 10 years before analysis for angiogenic factors. Samples had not been thawed prior to analysis.

Assays

VEGF, PEDF (serum) and PAI-1 and osteopontin (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 (ELISAs) from R&D Systems (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. The inter- and intra-assay CVs for each assay were as follows: VEGF 7.5% and 6.6%; OPN 9.8% and 6.8%; PAI-1 6.5% and 4.6%; and PEDF 10.4% and 4.4%. Other circulating biomarkers were measured as previously described, including sex steroid hormones (estradiol, testosterone, sex hormone binding globulin (SHBG)), insulin, ghrelin and IGF-1. (35–37)

Statistical Analyses

Partial Pearson correlation coefficients were calculated between baseline biomarker measures, corrected for multiple testing (Bonferroni correction: 0.05/20= significant at $P<0.0003$). A logarithmic transformation was applied to the outcome variables to improve the normality of the distribution. Generalized linear models were used to test for differences in baseline values across study arms. Descriptive data are presented as geometric means (95% confidence intervals (CI)). Mean changes in analytes from baseline to 12-months, stratified by group, were computed; 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 (GEE) modification of linear regression to account for intra-individual correlation over time. The analyses were adjusted by BMI and baseline levels of the outcome variables.

In preplanned analyses, changes in body composition, and VO_{2max} between baseline and 12-months were calculated, and used to predict observed change in analytes at 12-months by linear regression. Fat loss was categorized as gaining any fat; losing less than the median of percent change in total body fat; or more than the median of change in percentage total body fat (kg) (corresponding to </>1.85%). VO_{2max} was categorized as decreasing, or increasing less than or more than the median of percent change in VO_{2max} (</> 13.5%). Fat-loss and VO_{2max} levels in the control group were added as a separate category. All statistical tests were two sided. Statistical analyses were performed using SAS software (version 8.2, SAS Institute Inc., Cary, NC).

Results

At baseline, intervention and control groups were similar with regard to demographic characteristics, body composition, mean daily caloric intake, fitness levels, and hormone

concentrations (Table 1). Participants, on average, were 61 years old, obese, highly educated, and with a low level of fitness.

After correction for multiple testing, there were no significant associations observed between VEGF and any of the other covariates examined. OPN correlated significantly only with SHBG (r=0.29, P<0.0001; Table 2). PAI-1 significantly and strongly correlated with insulin (r=0.61, P<0.0001), and with PEDF, BMI, total fat mass, leptin, free testosterone and free estradiol; and negatively with SHBG. PEDF showed similar associations: strongly correlated with insulin (r=0.53, P<0.0001), and with BMI, fat mass, leptin, free estradiol and negatively with SHBG; unlike PAI-1 it did not correlate with free testosterone.

There were no significant differences between levels of VEGF, OPN or PAI-1 between arms, comparing baseline to 12-month levels (Table 3). Women randomized to the exercise intervention had a significantly greater reduction in PEDF levels at 12-months (−3.7%), compared to women in the control arm (+3.0%; P=0.009), adjusted for BMI and baseline levels of PEDF.

We next examined the influence of changes in fat loss and VO_{2max} levels on these analytes. Fat loss had no effect on VEGF levels (Table 4). Decreasing levels of fat mass were significantly associated with decreasing levels of PEDF with a change of −7.5% in the group that lost the most fat; −2.0% in those who lost the least; compared to a gain of 2.8% in controls (P_{trend} =0.002); or compared to an increase of 1.4% in the group randomized to exercise who gained any fat (P_{trend}=0.013). PAI-1 showed a similar pattern, with the greatest decrease (−14.5%) in the group that lost the most fat compared to the control group (P_{trend}=0.03) or to those in the exercise group who gained any fat (P_{trend}=0.02).

Next, we compared levels between controls, participants who decreased their VO_{2max}, and those who increased less or more than the median of the increase in VO_{2max} (Table 5). Changes in levels of VEGF, OPN and PAI-1 were not associated with changes in VO_{2max} comparing those who increased their VO_{2max} to either controls, or to those who decreased their VO_{2max}. However, participants who increased their VO_{2max} had significantly smaller decreases in PEDF (<median: −2.4%; >median increase: −3.4%) when compared to participants who decreased their VO_{2max} (−6.2%, P_{trend}=0.03), but not when compared to controls.

Discussion

This study compared the effects of an exercise intervention on biomarkers of angiogenesis in a sample of healthy overweight/obese postmenopausal women. We found that a 12-month moderate exercise intervention significantly reduced levels of PEDF, a serpin with anti-tumorigenic and anti-angiogenic effects.(24, 38) Increasing levels of fat-loss were significantly associated with increasing reductions in levels of PEDF. Interestingly, participants who decreased their VO_{2max} (i.e. became less fit) had larger reductions in PEDF levels, compared to participants who increased their VO_{2max} levels. This suggests the possibility that PEDF may be differentially regulated via adipokine-related pathways, compared to those related to changes in aerobic capacity. While the exercise intervention was not significantly associated with changes in PAI-1 levels, increased reductions in fat mass were significantly associated with reductions in PAI-1. There were no significant effects of the intervention, or changes in fat-mass or VO_{2max} on either VEGF or OPN.

Given that PEDF is a negative regulator of angiogenesis, ‘shrinkage’ of fat-mass in theory would require up-regulation of anti-angiogenic factors in response to reduced requirements for neo-vascularization.(13) However, PEDF is positively associated with obesity,(39) is increased in diabetic patients compared to controls,(40) and percent changes in serum levels

of PEDF in a 1-year observational study were positively correlated with those of BMI.(41) Further studies confirmed the association of PEDF with the metabolic syndrome including positive significant associations with insulin,(42) and HOMA-IR, a measure of insulin resistance.(43) A study in 36 severely obese adults found that bariatric surgery resulted in significant reductions in PEDF, and that relative change in PEDF levels between baseline and 18 months post-surgery, was significantly associated with change in weight, BMI, fat mass, visceral fat diameter, insulin, and HOMA-IR.(44) Weight loss (on average 5 kg/m^2) in 33 obese/overweight men led to significantly decreased PEDF concentrations from 34.8 ± 19.3 to 22.5 ± 14.2 μg/ml ($P < 0.0001$).(45) Of interest, a recent study classified PEDF as a contraction-regulated myokine which can be secreted by primary human myotubes,(46) although myotubes secrete PEDF at significantly lower concentrations compared to preadipocytes and adipocytes.(47) The study also reported a significant reduction in PEDF serum levels from 8 healthy young men who underwent a 60 minute bout of cycling at VO_{2max} of 70%.(46) We did observe a reduction in PEDF concentrations among those participants who increased their levels of fitness: this may be explained by replacement of adipose tissue by muscle-mass and a concomitant decrease in overall levels of PEDF. In an exploratory study using a combined proteomic and metabolomic approach, PEDF was identified as an exercise-dependent predictor of fat mass difference in 5 lean and 5 obese healthy young male volunteers who underwent a 1-hour acute exercise bout.(48) Our findings that decreases in VO_{2max} were associated with reductions in PEDF levels are unexpected. It is possible that exercise and fat-loss have different mechanisms of action on skeletal muscle- vs. adipose tissue-secreted PEDF. It appears that PEDF has unexpected patterns of expression: elevated in the obese state, and lowered in cancer patients and suggests the possibility of resistance to this factor as is seen with insulin and leptin. Estrogen is an important upstream regulator of PEDF in vitro: treatment of an ovarian cancer cell line with 17 β-estradiol (E2) inhibited expression of PEDF transcription and translation, and was reversed by an ER antagonist, indicating that the regulation was ER-mediated.(49) In our study we found significant associations between PEDF levels and free estradiol, and negative associations with SHBG.

The association between reductions in fat mass and decreasing levels of PAI-1 is expected. PAI-1 is produced by adipocytes, endothelial cells, and stromal cells in adipose tissue,(50–53) and is involved in adipocyte differentiation and insulin signaling.(54) Obese individuals have higher levels of PAI-1.(55) PAI-1 levels correlate with BMI irrespective of gender and age,(56) and with BMI, and waist-hip ratio in non-diabetic healthy postmenopausal women.(57) Elevated levels of PAI-1 are associated with individuals with metabolic syndrome and type 2 diabetes,(58) and predicts type 2 diabetes independently of other known risk factors for diabetes.(59) In spite of its role as an endogenous protease inhibitor, PAI-1 appears to promote tumor growth, invasion, metastasis, and angiogenesis, rather than inhibiting these processes, by interacting with vitronectin, integrins, and other components of the plasminogen activation system and by affecting the extracellular matrix.(18, 60, 61) It has been hypothesized that, as a consequence of metabolic syndrome, the up-regulation of PAI-1 expression predisposes breast cancer to more aggressive stages, partially by affecting angiogenesis.(19, 61, 62) In vitro studies demonstrated that PAI-1 acts as a positive switch for angiogenesis by promoting endothelial cell migration toward fibronectin-rich tumor tissue, and that PAI-1 inhibitors prevent angiogenesis.(19, 20) A study in PAI-1 null mice demonstrated that angiogenesis was reduced approximately 60% compared with wild-type mice, while in mice overexpressing PAI-1, angiogenesis was increased nearly 3-fold.(63) Additionally, overexpression of PAI-1 has been found in many obesity-related types of cancer, and high levels of PAI-1 are also significantly associated with poor prognosis in breast and other cancers.(64–68) Some small studies examined the effect of exercise on PAI-1: a cross-sectional study in 27 post-menopausal women observed a significantly higher level of PAI-1 in postmenopausal sedentary women, compared to physically active women.

(26) An RCT in 188 men comparing a diet, exercise, combined and control interventions, found no change in PAI-1 levels post-intervention in any group.(28) In contrast, in 1817 overweight or obese diabetic patients randomized to the Look AHEAD RCT investigating the effects of an intensive lifestyle behavioral intervention for weight-loss, improvements in fitness were associated with decreased PAI-1 independent of weight loss (P=0.03).(69)

Osteopontin is involved in mediating angiogenesis, and interacts with VEGF.(70, 71) Osteopontin plays an important role in neoplastic transformation, malignant cell attachment and migration,(72) and is associated with increased invasiveness in mammary tumor cell lines.(73–76) It is over-expressed in a number of human cancers including breast, and elevated levels have been associated with increased metastatic burden and poor prognosis in breast cancer patients.(76–78) Elevated osteopontin expression in adipose tissue in obese individuals was paralleled by macrophage infiltration: levels of both were reversed after weight loss in morbidly obese individuals.(79) However we did not observe any changes in levels of osteopontin in the intervention arm compared to controls, or either by changes in percentage body fat, or by changes in VO_{2max}. It may be that the degree of weight loss was insufficient in this exercise intervention study to observe significant changes in osteopontin levels. To our knowledge, there have been no other studies of exercise on levels of osteopontin. One small cross-sectional study compared 13 endurance-trained athletes and 12 sedentary older adults (age 60–78 years; 13 men and 12 women) and found no difference in OPN plasma levels.(27)

VEGF is a key mediator of angiogenesis.(14) As mentioned above, expansion of adipose tissue is linked to the development of its vasculature, and this process was almost completely abolished by VEGF inhibitors in severely obese patients.(80) Adipose tissue produces VEGF in response to IL-6,(81) insulin (82) and TNF-α by a p38 MAPK-dependent mechanism.(83) Levels of VEGF are significantly higher in obese patients than in lean controls,(84) and leptin synergistically stimulates angiogenesis with VEGF,(85) However, VEGF did not correlate with leptin in our study. VEGF expression has also been found to correlate with risk and outcomes in breast cancer. High levels of VEGF in breast tumors predict both shorter disease-free survival and overall survival, and poorer response to adjuvant therapy(86, 87) and higher serum levels of VEGF are found in primary breast cancer (88, 89) and metastatic disease(90) compared to women with benign breast disease or normal controls. A 12-week study of 79 obese males and females randomized to 12-weeks exercise without diet-restriction (−3.5 kg weight loss); a hypocaloric diet (−12.3 kg); and a combination of the two interventions (−12.3 kg), reported that VEGF was non-significantly reduced in all 3 arms.(91)

To our knowledge, this is the first randomized study to investigate the effects of physical activity change on levels of these biomarkers of angiogenesis in postmenopausal overweight/obese women, a group of women at elevated risk of several cancers. Strengths of this study include a relatively large sample size, a randomized controlled trial design, long duration of the intervention (12 months), high retention (97.7% of participants gave blood at 12-months), and high adherence to intervention prescriptions. A limitation is the relatively homogeneous sample of overweight sedentary postmenopausal women, which may limit the generalizability of this study. As angiogenesis is a process involved with neoplastic promotion rather than the initial phases of carcinogenesis, these results from adipose tissue in healthy women may not reflect changes in the profile of angiogenic markers during tumor expansion. Finally, we tested only one exercise program, and therefore cannot extend results to other exercise modalities.

In summary, PEDF was significantly decreased in response to a moderate-intensity exercise intervention, which has unclear ramifications for cancer risk because PEDF is a negative

regulator of angiogenesis. Increased fat loss was associated with increased reductions in PEDF and PAI-1, and changes in VO_{2max} appeared to have effects on PEDF. Examination of the associations between PEDF and exercise and fat-loss, warrants further study, including examining effects of different types and intensities of exercise; and the role of weight loss via dietary changes with and without exercise.

Acknowledgments

Funding: This work was supported by grants from the National Cancer Institute at the National Institutes of Health *NIH R01 CA 69334 and 1R03CA152847*

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Table 1

Baseline characteristics of study participants

	Exercisers **Mean ± SD**	**Controls** **Mean ± SD**
N	85	84
Age (yrs)	60.7 ± 6.7	60.6 ± 6.8
BMI (kg/m^2)	30.5 ± 4.1	30.5 ± 3.7
Percent body fat (DXA)	47.5 ± 4.8	47.4 ± 4.6
VO_2max (ml/kg.min)	20.0 ± 3.6	20.5 ± 3.0
Education* *	N (%)	N (%)
Some high school or High school	10 (11.5)	9 (10.5)
Some college or vocational training	36 (41.4)	35 (40.7)
College graduate	23 (26.4)	25 (29..1)
Graduate degrees	18 (20.7)	17 (19.8)
Ethnicity (%)		
Non-Hispanic white	74 (85.1)	75 (87.2)
VEGF (pg/mL)	390.1 ± 250.4	499.8 ± 301.0
Median [range]	304.6 [82.5, 1352.7]	457.6 [88.6, 1787.7]
PEDF (μg/mL)	11.8 +2.3	11.7 ± 2.9
Median [range]	11.6 [6.7, 19.0]	11.3 [6.6, 23.8]
PAI (ng/mL)	5.9 +3.8	6.3 ± 4.0
Median [range]	4.9 [1.5, 21.7]	5.4 [1.8, 24.1]
OPN (ng/mL)	60.8 +14.3	59.8 ± 15.7
Median [range]	58.6 [36.7, 124.3]	56.2 [33.5, 107.6]

* T-test for continuous variables; Chi-square test for categorical variables.

Table 2

Pearson Correlations between Osteopontin, PAI-1, PEDF, VEGF, and anthropometric and previously tested serum biomarkers, corrected for multiple testing.[a] Significant associations are indicated by superscripts.

Covariates [c]	Osteopontin N=164	PAI-1 N=164	PEDF N=164	VEGF N=164
	r_s	r_s	r_s	r_s
Osteopontin	-	−0.04	0.02	−0.02
PAI-1	−0.04	-	**0.46** [b]	0.17
PEDF	0.02	**0.46**[b]	-	0.11
VEGF	−0.02	0.17	0.11	-
Age (years)	0.15	−0.10	−0.12	−0.02
Body Mass Index (kg/m^2)	−0.04	**0.36** [b]	**0.34** [b]	0.12
Total Bone Mineral Density (g/cm^2)	−0.08	0.23	0.15	0.07
Total fat mass (g)	−0.02	**0.31** [b]	**0.37** [b]	0.18
Testosterone (pg/ml)	−0.004	0.23	0.02	−0.01
Estrone (pg/ml)	−0.09	0.25	0.23	0.16
Estradiol (pg/ml)	−0.16	0.19	0.20	0.19
SHBG (nmol/L)	**0.29** [b]	**−0.34** [b]	**−0.31** [b]	−0.05
Free estradiol	−0.20	**0.30** [b]	**0.29** [b]	0.19
Free testosterone	−0.08	**0.45** [b]	0.18	0.01
Insulin (μu/ml)	−0.05	**0.61** [b]	**0.53** [b]	0.05
Leptin (ng/ml)	−0.03	**0.33** [b]	**0.38** [b]	0.10
Ghrelin	−0.04	−0.24	−0.23	0.07
IGF-1	−0.25	0.06	−0.00	−0.02

[a] Bonferroni correction. Significant at P=0.0003

[b] P<0.0001

Table 3

Geometric mean (95% CI) of angiogenesis biomarkers at baseline and 12 month, stratified by intervention arm, adjusted for BMI and baseline biomarker levels

	Stretching control							Exercise Intervention							
	BASELINE			12-MONTH			Change (%)	BASELINE			12-MONTH			Change (%)	P*
	N	Mean	95% CI	N	Mean	95% CI		N	Mean	95% CI	N	Mean	95% CI		
VEGF (pg/mL)	84	418.3	(367–479)	82	419	369–475	**−0.7 (−0.2)**	85	325	286–370	83	320.8	281–366	**−4.2 (−1.3)**	0.86
PEDF (ug/mL)	84	11.4	(10.9–12.0)	82	11.8	11.2–12.4	**0.3 (3.0)**	85	11.6	11.1–12.1	83	11.2	10.7–11.7	**−0.4 (−3.7)**	0.009
PAI-1 (ng/mL)	80	5.4	(4.8–6.1)	80	5.6	4.9–6.3	**0.2 (2.9)**	84	5.1	4.5–5.7	81	4.8	4.3–5.4	**−0.2 (−4.4)**	0.18
OPN (ng/mL)	80	57.9	(54.8–61.2)	80	57.2	54.4–60.2	**−0.7 (−1.2)**	84	59.3	56.5–62.2	81	57.5	54.8–60.3	**−1.8 (−3.0)**	0.47

* P value: GEE model, testing the difference in change from baseline to 12 month between Control group and Exercise group, adjusted for BMI and baseline biomarker level.

Table 4

Geometric mean (95% CI) of angiogenesis biomarkers at baseline and 12 month, stratified by change in percent body fat and adjusted for age.

		Baseline			12-Months			Difference		
	Fat changes	**N**	**Mean**	**95% CI**	**N**	**Mean**	**95% CI**	**Change (%)**	**†P**	**‡P**
VEGF (pg/mL)	Control	84	419	367–479	161	421	384–460	**1.1 (0.3)**	Ref.	-
	Gained any Fat	21	391	318–481	42	380	327–442	**−11.1 (−2.8)**	0.48	Ref.
	Lost <1.85%§	30	338	267–427	60	347	295–408	**9.2 (2.7)**	0.24	0.11
	Lost ≥1.85%	31	267	215–332	62	265	228–308	**−2.3 (−0.8)**	0.95	0.61
									(*P=0.63)	(**P=0.74)
PEDF (ug/mL)	Control	84	11.4	10.9–12.0	161	11.7	11.3–12.2	**0.3 (2.8)**	Ref.	-
	Gained any Fat	21	12.1	11.2–13.1	42	12.3	11.5–13.1	**0.2 (1.4)**	0.75	Ref.
	Lost <1.85%*	30	11.5	10.7–12.3	60	11.3	10.8–11.8	**−0.2 (−2.0)**	0.14	0.32
	Lost ≥1.85%	31	11.2	10.5–12.1	62	10.4	9.9–10.9	**−0.8 (−7.5)**	0.002	0.013
									(*P=0.002)	(**P=0.013)
PAI-1 (ng/mL)	Control	80	5.4	4.8–6.1	157	5.6	5.1–6.1	**0.2 (3.3)**	Ref.	-
	Gained any Fat	22	5.1	4.0–6.5	44	5.7	4.8–6.8	**0.6 (11.6)**	0.39	Ref.
	Lost <1.85%*	29	5.4	4.5–6.6	58	5.1	4.5–5.8	**−0.3 (−5.9)**	0.32	0.13
	Lost ≥1.85%	30	4.7	3.9–5.8	60	4.0	3.6–4.5	**−0.7 (−14.5)**	0.03	0.019
									(*P=0.03)	(**P=0.02)
OPN (ng/mL)	Control	80	57.9	54.8–61.2	157	57.1	55.1–59.2	**−0.8 (−1.4)**	Ref.	-
	Gained any Fat	22	63.1	56.7–70.3	44	56.2	52.4–60.2	**−6.9 (−11.0)**	0.02	Ref.
	Lost <1.85%*	29	59.0	54.8–63.4	58	59.4	56.7–62.3	**0.4 (0.7)**	0.54	0.011
	Lost ≥1.85%	30	57.4	53.1–62.2	60	56.7	53.4–60.2	**−0.7 (−1.3)**	0.98	0.03
									(*P=0.78)	(**P=0.05)

†Testing difference in change from baseline to 12 months in biomarkers compared to Controls.

‡Testing difference in change from baseline to 12 months in biomarkers compared to Gained any percent body fat, excluding Controls

*Testing for a trend in change from baseline to 12 months in s biomarkers from Controls through lost most percent body fat

**Testing for a trend in change from baseline to 12 months in biomarkers from Gained some body fat through lost most percent body fat

§1.85% corresponds to median levels of percentage of fat lost

Table 5

Geometric mean (95% CI) of levels of VEGF, PEDF, PAI-1 and OPN at baseline and 12 months, stratified by change in VO_{2max}

		Baseline			12-Months			Difference		
	VO_{2max} changes	**N**	**MEAN**	**95% CI**	**N**	**MEAN**	**95% CI**	**Change (%)**	**†P**	**‡P**
VEGF (pg/mL)	Control	84	419	367–479	161	421	384–460.3	**1.1 (0.3)**	Ref.	.
	Decreased VO_{2max}	22	410	318–529	22	384	312–470.7	**−26.7 (−6.5)**	0.46	Ref.
	Increased VO_{2max} by <13.4%	31	311	252–383	62	310	264–362.9	**−1.1 (−0.4)**	0.83	0.68
	Increased VO_{2max} by ≥13.4%	32	289	235–355	64	285	249–327.3	**−3.7 (−1.3)**	0.94	0.54
									(*P=0.99)	(**P=0.63)
PEDF (ug/mL)	Control	84	11.4	10.9–12.0	161	11.7	11.3–12.2	**0.3 (2.8)**	Ref.	.
	Decreased VO_{2max}	22	12.8	12.0–13.6	22	12.0	11.0–13.1	**−0.8 (−6.2)**	0.046	Ref.
	Increased VO_{2max} by <13.4%	31	11.6	10.7–12.6	62	11.3	10.7–12.0	**−0.3 (−2.4)**	0.14	0.43
	Increased VO_{2max} by ≥13.4%	32	10.8	10.3–11.4	64	10.4	10.0–10.9	**−0.4 (−3.4)**	0.049	0.56
									(*P=0.03)	(**P=0.71)
PAI-1 (ng/mL)	Control	80	5.4	4.8–6.1	157	5.6	5.1–6.1	**0.2 (3.3)**	Ref.	.
	Decreased VO_{2max}	22	5.6	4.5–6.9	22	5.6	4.3–7.2	**−0.0 (−0.0)**	0.87	Ref.
	Increased VO_{2max} by <13.4%	31	5.3	4.3–6.6	62	5.1	4.5–5.8	**−0.3 (−5.1)**	0.41	0.73
	Increased VO_{2max} by ≥13.4%	31	4.5	3.7–5.3	62	4.1	3.7–4.6	**−0.3 (−7.3)**	0.24	0.61
									(*P=0.20)	(**P=0.58)
OPN (ng/mL)	Control	80	57.9	54.8–61.2	157	57.1	55.1–59.2	**−0.8 (−1.4)**	Ref.	.
	Decreased VO_{2max}	22	56.9	50.8–63.7	22	57.8	52.9–63.1	**0.9 (1.6)**	0.45	Ref.
	Increased VO_{2max} by <13.4%	31	62.3	58.4–66.6	62	58.2	55.3–61.3	**−4.1 (−6.6)**	0.10	0.10
	Increased VO_{2max} x by ≥13.4%	31	58.1	53.7–62.9	62	56.1	53.0–59.4	**−2.0 (−3.5)**	0.47	0.26
									(*P=0.21)	(**P=0.44)

†Testing differences in change from baseline to 12 months biomarkers compared to Controls.

‡Testing difference in change from baseline to 12 months in biomarkers compared to theDecreasedVO_{2max} Group, excluding Controls

* Testing for a trend in change from baseline to 12 months in biomarkers from Controls through Increased most VO_2max Group

§ 13.4%% corresponds to median levels of percentage increase in VO_2max