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Aerobic Exercise and Weight Loss Reduce Vascular Markers of Inflammation and Improve Insulin Sensitivity in Obese Women

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Abstract

Background/Objectives—To examine the relationships of plasma and tissue markers of systemic and vascular inflammation to obesity and insulin resistance and determine the effects of aerobic exercise training+weight loss (AEX+WL) and weight loss (WL) on these biomarkers.

Design—Prospective controlled study.

Participants—Seventy-seven overweight and obese sedentary postmenopausal women.

Interventions—Six months, 3d/wk AEX+WL (n=37) or WL (n=40).

Measurements—Total body dual-energy x-ray absorptiometry, abdominal computed tomography scans, hyperinsulinemic-euglycemic clamps, adipose tissue biopsies (n=28), and blood for Homeostasis model assessment-insulin resistance, and soluble forms of intracellular adhesion molecule (sICAM-1) and vascular CAM-1 (sVCAM-1), C-reactive protein (CRP), and serum amyloid A (SAA).

Results—Body weight, %fat, visceral fat, triglyceride levels and systolic blood pressure decreased comparably after WL and AEX+WL ($P<0.05$). VO_2 max increased 16% after AEX+WL ($P<0.001$). Insulin resistance decreased in both groups ($P<0.01$). Glucose utilization increased 10% ($P<0.05$) after AEX+WL and 8% with WL ($P=0.07$). AEX+WL and WL decreased CRP by 29% and 21%, ($P<0.05$). SAA levels decreased two-fold more after AEX+WL (–19%, $P<0.05$) than with WL (–9%, $P=0.08$). Plasma sICAM-1 and sVCAM-1 levels did not change; however, women with the greatest reduction in plasma sICAM-1 levels had the greatest reductions in fasting glucose, insulin and insulin resistance ($P<0.05$). Gluteal ICAM mRNA levels decreased 27% after AEX+WL ($P<0.05$) and did not change after WL.

Conclusion—Obesity and insulin resistance worsen markers of systemic and vascular inflammation. A reduction in plasma sICAM-1 is important to improve insulin sensitivity. CRP

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and SAA and tissue ICAM decrease with exercise and weight loss, suggesting that exercise training is a necessary component of lifestyle modification in obese postmenopausal women.

Keywords

Exercise; Diet; Inflammation; Insulin Sensitivity

INTRODUCTION

Vascular inflammation is involved in atherosclerotic processes associated with insulin resistance. The soluble forms of intracellular adhesion molecule (sICAM-1) and vascular CAM-1 (sVCAM-1) along with C-reactive protein (CRP) levels are vascular inflammatory markers for endothelial dysfunction associated with increased risk of diabetes and coronary heart disease^{1, 2}. These soluble adhesion molecules are also associated with insulin resistance in subjects with type 2 diabetes³. CRP, a biomarker of low-grade inflammation, and acute-phase serum amyloid A (SAA) which stimulates the production of inflammatory cytokines in coronary artery endothelial cells⁴, are elevated in obesity and can predict cardiovascular disease (CVD)^{1, 5}. Acute phase reactants, CRP and SAA, activate the endothelium and induce vascular adhesion molecules such as ICAM-1 and VCAM-1⁶. In addition, CRP levels are significantly associated with congestive heart failure events in elderly men and women in the Health ABC study⁷. Rates of CVD^{8, 9} and stroke^{10, 11} increase exponentially after age 55 and obesity increases relative risk of CVD by 64% in women, as opposed to 46% in men¹², emphasizing the need to identify risk factors early in postmenopausal women. At this age, interventions may reduce CVD and diabetes risk which may prolong the negative consequences of these diseases. Older women are less fit and less physically active than younger women emphasizing the need to intervene with exercise training to offset the decline in fitness with aging. Limited information is available on these markers and their relationship to central obesity and insulin action in postmenopausal women.

Lifestyle interventions of aerobic exercise and weight loss may alter vascular and systemic markers of inflammation to reduce CVD risk. In the LIFE trial, CRP levels decreased 32% after 12 months of physical activity in the elderly; however, there was no significant difference in CRP levels between treatment groups (physical activity versus health education) suggesting that exercise alone has a limited effect on CRP¹³. Weight loss, on the other hand, decreases CRP¹⁴ but may not alter sICAM-1 and sVCAM-1 concentrations^{15, 16}. Results are mixed as to the effects of exercise training with and without weight loss on the soluble adhesion molecules^{17–19, 20, 21} but there are no studies in middle-aged and older obese postmenopausal women.

It is our hypothesis that CRP and SAA, biomarkers of systemic inflammation, and sICAM-1 and sVCAM-1, biomarkers of vascular inflammation, are related to central obesity and insulin resistance in older, obese postmenopausal women and would be reduced by lifestyle modifications of exercise and weight loss. This investigation examined the relationships of plasma and tissue markers of systemic and vascular inflammation to obesity and insulin resistance and compared the effects of aerobic exercise training plus weight loss versus

weight loss alone on markers of inflammation in older, obese women. We also examined the response of ICAM-1 and VCAM-1 mRNA expression in abdominal and gluteal adipose tissue to these interventions as well as insulin sensitivity, lipid profiles, and blood pressure.

METHODS

Subjects were healthy, overweight and obese (body mass index, BMI ≥ 25 kg/m²; range of 25–46 kg/m²), postmenopausal women aged 50–76 years and had not menstruated for at least 1 year. Women were weight stable (<2.0 kg weight change in past year), sedentary (<20 min of aerobic exercise 2x/wk) and screened by medical history, physical examination, and fasting blood profile for overt and chronic disease which would preclude participation in exercise and weight loss programs. All subjects were nonsmokers and showed no evidence of cancer, liver, renal or hematological disease, or other medical disorders. Individuals with untreated hypertension (blood pressure $>160/90$ mmHg) or hyperlipidemia (triglycerides ≥ 4.5 mM, cholesterol > 6.2 mM, LDL-cholesterol >4.3 mM) were referred to their doctor for therapy and allowed to enter if treated with an antihypertensive or lipid-lowering drug that did not affect glucose metabolism. All subjects underwent a screening Bruce graded treadmill test to exclude those with asymptomatic coronary artery disease.

Women in this study were part of a larger cohort where body composition and glucose clamp results were previously published²², but not the lipid levels, blood pressure, inflammatory and vascular biomarkers. One-hundred and seventy-four women met study criteria and were assigned to weight loss (WL, n=86) or aerobic exercise+weight loss (AEX+WL, n=88). Sixty-three women dropped out of the program due to personal reasons, relocation, illness, and/or time constraints leaving 111 women. Sixteen women were excluded from the analysis either because they were not compliant to the weight loss or exercise guidelines described below (n=8) or they had type 2 diabetes (n=8) resulting in n=95 completed (WL, n=46 and AEX+WL, n=49). The baseline characteristics (age, race, BMI), within AEX+WL and WL for those who dropped out did not differ from those who completed (data not shown). Plasma samples were not available for 18 women so data are reported on 77 women in whom biomarkers were measured (WL:n=40 and AEX+WL:n=37). Women were 66% Caucasian and 34% African American. Sixteen WL women (40%) and 14 AEX+WL women (38%) were on an antihypertensive medication. Women were on lipid-lowering drugs in WL and AEX+WL (n=8, 20% and n=7, 19%, respectively). The Institutional Review Board of the University of Maryland approved all methods and procedures. Each participant provided written informed consent to participate.

Study Protocol

WL and AEX+WL Interventions—Prior to metabolic testing and initiation of the weight loss intervention(s), subjects received instruction in Therapeutic Lifestyle Changes (TLC) isocaloric diet²³ which emphasizes low dietary fat, high fiber and low sodium by a registered dietitian (RD) 1x/week for 6–8 weeks. Subjects were weight stable on the TLC program prior to baseline testing, and were instructed to maintain the same macronutrient guidelines for dietary intake throughout the study. After baseline testing, all subjects attended weekly weight loss classes for 6 months for diet instruction by a RD. Compliance

was monitored by 7-day food records using the American Diabetes Association exchange list system. Individuals were instructed to restrict their caloric intake by 300 to 500 kcal/d to achieve weight loss while maintaining the TLC diet for macronutrient composition. The AEX+WL group exercised at our facility 3x/week for 6 months using treadmills and elliptical trainers. Each exercise session included a 5–10 minute warm-up and a 5–10 minute cool-down. Women exercised at ~50–60% heart rate reserve (HRR) and gradually progressed in duration and intensity until women were able to exercise at >85% HRR for 45 minutes. The average compliance to exercise and weight loss classes was ~86%.

VO₂max, Body Composition, and Blood Pressure—VO₂max was measured using a continuous graded treadmill test²². Height, weight, BMI, and waist circumference was determined²². Fat mass, lean tissue mass and bone mineral content (BMC) (fat-free mass=lean+BMC) were determined by dual-energy X-ray absorptiometry (DXA) (Prodigy, LUNAR Radiation Corp., Madison, WI). A single computed tomography (CT) scan at L₄–L₅ region using a Siemens Somatom Sensation 64 Scanner (Fairfield, CT) was used to determine visceral adipose tissue (VAT) area, subcutaneous adipose tissue (SAT) area, and sagittal diameter and analyzed using MIPAV (Medical Image Processing, Analysis and Visualization, v.7.0.0). Sitting blood pressure was measured on three occasions and the average used in the analyses.

Metabolic Testing—All subjects were weight stable ($\pm 2\%$) for at least two weeks prior to metabolic testing before and after the interventions and were provided with a eucaloric diet for two days before the clamp to control nutrient intake. All testing was performed in the morning after a 12-hr overnight fast. Women in the AEX+WL group stabilized their weight and continued the aerobic training 3 d/wk during the final testing period, while the WL group was weight stabilized. The glucose clamps and blood draws were performed 36–48 hours after the last bout of exercise.

Oral glucose tolerance test (OGTT)—Blood samples were drawn before and at 30-min intervals for 2h after ingestion of 75g of glucose. Plasma glucose concentrations were measured in duplicate using the glucose oxidase method (2300-STAT Plus, YSI, Yellow Springs, OH). Subjects were classified as having normal glucose tolerance (NGT) and impaired glucose tolerance (IGT)²⁴. **Hyperinsulinemic-euglycemic Clamps:** Peripheral tissue sensitivity to exogenous insulin was measured using a 3-hour 240 pmol·m⁻²·min⁻¹ hyperinsulinemic-euglycemic clamp technique²⁵. Difficulty in obtaining venous access or scheduling conflicts occurred (n=19). Clamps were performed before and after WL (n=26) and AEX+WL (n=25). Plasma glucose and insulin levels (10–180 min) did not differ between interventions and before and after WL (4.96 $\pm$ 0.08 vs. 4.92 $\pm$ 0.06 mmol/l and 1084 $\pm$ 39 vs. 1100 $\pm$ 47 pmol/l, respectively) and AEX+WL (5.02 $\pm$ 0.07 vs. 4.99 $\pm$ 0.07 mmol/l and 1140 $\pm$ 49 vs. 1083 $\pm$ 34 pmol/l, respectively). This was 96 $\pm$ 0.3% of the desired goal with 6.5 $\pm$ 0.2% CV in all clamps (n=102). **Blood Samples** were collected in heparinized syringes, placed in prechilled test tubes containing 1.5mg EDTA/ml blood, centrifuged at 4°C and stored at –80°C until analysis. Plasma insulin was determined in duplicate by RIA²². Fasting plasma for CRP, SAA, sICAM-1, and sVCAM-1 were measured in duplicate with CV<10% by electrochemiluminescence using a multi-spot microplate by Meso Scale Discovery

(SECTOR Imager-6000). One woman didn't have blood drawn for the inflammatory markers. Blood for fasting lipid profiles were obtained three times with results averaged. Plasma TG and cholesterol were analyzed by enzymatic methods (Hitachi model-917 analyzer), and HDL-cholesterol measured in the supernatant after precipitation with dextran sulfate. $\text{LDL-cholesterol} = \text{total cholesterol} - (\text{TG}/5 + \text{HDL-C})^{26}$.

Adipose Tissue Aspirations—After a 12-h overnight fast, 28 women (n=14 AEX+WL and n=14 WL) had 4–6 g of subcutaneous adipose tissue obtained under local anesthesia (1% xylocaine) from abdominal (3–4 cm distal to the umbilicus) and gluteal (upper distal quadrant) regions by aspiration with a cannula. Aliquots of adipose samples were immediately frozen and stored at -80°C until analysis.

RNA Extraction, Reverse Transcription and Real-time RT-PCR—Total RNA was extracted from adipose tissue by the acid guanidinium isothiocyanate-phenol-chloroform method²⁷. One μg of total RNA for each sample was reverse transcribed into first strand cDNA using Transcriptor First Strand cDNA Synthesis Kit (Cat# 04896866001, Roche Applied Science). Quantitative Real-time PCR and data analysis were performed with LightCycler 480 Multiwell plate 384 (Cat# 04729748001), LightCycler 480 Probes Master kit (Cat# 04887301001,) and Taqman gene expression primer/probe set (Applied Biosystems, Foster City, CA, Human ICAM: Cat# Hs00164932_m1; Human VCAM: Cat# Hs01003372_m1). Each qPCR reaction was carried out in a final volume of $10\mu\text{l}$, consisting of $2\mu\text{l}$ 1:4 diluted template cDNA, $5\mu\text{l}$ LightCycler 480 Probes Master, $0.5\mu\text{l}$ Taqman gene expression primer and probe mix, and $2.5\mu\text{l}$ nuclease-free water. Gene expression data were analyzed by LightCycle 480 Software v.1.5 advanced relative quantification program. Data is presented as the $\text{mean} \pm \text{SEM}$ of three determinations for each sample and the normalized ratio of Target/Reference.

Statistical Analyses

Glucose utilization (M) for 30-min intervals was calculated²². Homeostasis model assessment-insulin resistance was computed $(\text{glucose in millimolar} \times \text{insulin in } \mu\text{ml})/22.5$. Univariate ANOVA tested for differences in changes between AEX+WL and WL. Within each intervention, differences between pre-intervention and post-intervention variables were determined using a paired t-test which were defined as secondary analyses. Regression analyses were used to determine correlations between blood and adipose tissue biomarkers, body composition, lipid levels, BP, and glucose metabolism. Statistical significance was set at $P < 0.05$. Data were analyzed by SPSS (PASW Statistics 18, SPSS Inc., Chicago) and expressed as $\text{mean} \pm \text{SEM}$.

RESULTS

Baseline Characteristics and Relationships of Vascular and Systemic Inflammation

Demographic characteristics: Women were living independently, 71% were married, 12% were divorced, 10% widowed, and 7% never married. Also, 52% worked full-time, an additional 12% worked part-time, and 37% of women were retired. In terms of education, 46% had a graduate degree, 12% had a bachelor's degree, 31% had vocational/some college/

associated degree, and 12% had high school degree. Individual incomes were the following: <\$11,000 (2%), \$11,000–20,000 (7%), \$21,000–30,000 (9%), \$31,000–40,000 (13%), \$41,000–50,000 (17%), >51,000 (52%).

The AEX+WL and WL groups were well matched with respect to physical and metabolic variables at baseline (Table 1). There was no difference in age (identical age range of 50–76 years in both groups), weight, BMI, waist circumference, percent body fat, fat mass, fat-free mass, visceral fat, subcutaneous abdominal fat, total abdominal fat, total abdominal/visceral fat ratio, sagittal diameter and DBP between the AEX+WL and WL groups at baseline. There were no differences in triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, fasting glucose, glucose@120 min, fasting insulin, HOMA-IR, M (mg/kg/min or $\mu\text{mol/kg}_{\text{FFM}}/\text{min}$), CRP, SAA, sICAM-1, sVCAM-1 concentrations between groups. The only difference between groups at baseline was that HDL₃-cholesterol was higher in WL whereas VO₂max and SBP were higher in AEX+WL ($P<0.05$). There was no difference by glucose tolerance between groups (60% had NGT and 40% had IGT).

Plasma sICAM-1, sVCAM-1, CRP and SAA levels correlated with each other ($r=0.53$ to 0.71 , $P<0.001$) except that sVCAM-1 was not related to CRP. sICAM-1 levels related to waist circumference, visceral fat, subcutaneous abdominal fat, sagittal diameter, glucose_{120min}, fasting insulin, HOMA-IR, and inversely to VO₂max and M ($P<0.05$ to $P<0.005$, Table 2). In contrast, sVCAM-1 was related to sagittal diameter ($P<0.05$) and tended to be related to VO₂max ($P=0.06$). Both CRP and SAA levels related to BMI, waist circumference, %fat, subcutaneous abdominal fat, and sagittal diameter ($P<0.05$ to $P<0.005$). CRP was associated with fasting insulin and HOMA-IR ($P<0.05$). SAA levels were associated with HOMA-IR ($P=0.05$) and inversely associated with M ($P<0.05$). In multivariate analysis with VAT, VO₂max, and M in the model, visceral fat remained the independent predictor of circulating concentrations of sICAM-1, CRP, and SAA levels (all $P<0.05$).

Effects of AEX+WL and WL—VO₂max (l/min) increased 16% after AEX+WL ($P<0.001$) but did not change with WL (group effect, $P<0.01$). There were no other significant differences in changes in either body composition or metabolic variables by intervention. Body weight decreased 8% after AEX+WL and WL ($P<0.001$) with comparable decreases in % fat, fat mass, visceral fat and subcutaneous abdominal fat ($P<0.01$) and FFM ($P<0.05$) (Table 1). SBP decreased ($P<0.01$) and DBP tended to decrease ($P=0.08$) after AEX+WL. Both SBP and DBP decreased after WL ($P<0.05$). Fasting TG decreased 14% after AEX+WL ($P<0.001$) and 10% after WL ($P<0.001$). Total cholesterol did not change significantly in either group, nor did LDL-C after WL ($P=0.11$) and AEX+WL ($P=0.08$). There was a 7% increase in both HDL-C ($P<0.01$) and HDL₃-C ($P<0.05$) after AEX+WL but neither HDL-C nor HDL₃-C changed after WL. Fasting plasma glucose ($P<0.05$) and insulin ($P<0.01$) decreased in both groups and HOMA-IR decreased after AEX+WL (–21%) and WL (–30%) ($P<0.01$). M ($\mu\text{mol/kg}_{\text{FFM}}/\text{min}$) increased 10% ($P<0.05$) after AEX+WL and tended to increase (8%, $P=0.07$) after WL.

CRP decreased 29% with AEX+WL ($P<0.001$) and 21% with WL ($P<0.05$) (Figure 1). Plasma SAA levels decreased 19% after AEX+WL ($P<0.05$) and tended to decrease after

WL ($P=0.08$). sICAM-1 tended to decrease after WL ($P=0.06$) and did not change after AEX+WL (Figure 2). sVCAM-1 levels did not significantly change after AEX+WL or WL. Although abdominal ICAM mRNA expression decreased 14% after AEX+WL (14.63 ± 2.24 vs. 12.54 ± 2.25 AU) and 19% after WL (12.18 ± 1.25 vs. 9.88 ± 1.09 AU), changes were not statistically significant. Gluteal ICAM mRNA levels decreased 27% after AEX+WL (14.05 ± 2.16 vs. 10.25 ± 1.64 AU, $P<0.05$). AEX+WL increased gluteal VCAM mRNA expression 42% (4.76 ± 0.66 vs. 6.77 ± 0.89 AU, $P<0.05$) and did not change abdominal VCAM mRNA levels (5.80 ± 1.04 vs. 5.61 ± 0.65 AU). There were no significant changes after WL in gluteal ICAM (9.97 ± 1.33 vs. 9.44 ± 1.56 AU), abdominal VCAM (5.42 ± 0.43 vs. 5.21 ± 0.52 AU) or gluteal VCAM (5.04 ± 0.74 vs. 4.62 ± 0.68 AU) mRNA expression.

When the effects of the interventions on the inflammatory and vascular markers were examined, absolute changes in CRP were related to changes in SAA ($r=0.45$, $P<0.001$) and changes in sICAM-1 were related to changes in sVCAM-1 ($r=0.63$, $P<0.001$). Changes in the inflammatory and vascular markers were not associated with changes in central obesity (waist circumference, visceral fat, subcutaneous abdominal fat). The change or decrease in sICAM-1 levels correlated with the decrease in fasting glucose ($r=0.27$, $P<0.05$), fasting insulin ($r=0.27$, $P<0.05$), and HOMA-IR ($r=0.33$, $P<0.01$). The change in gluteal ICAM mRNA correlated with VO_2 max change ($r=-0.52$, $P<0.05$). Changes in blood pressure and lipids did not correlate with changes in biomarkers.

DISCUSSION

This is the first report of the inverse relationships between sICAM-1 and SAA with insulin sensitivity measured by the glucose clamp in older, obese sedentary women. We found no differences in the response to the interventions in terms of the vascular markers. There was a reduction of plasma CRP and SAA and gluteal ICAM mRNA expression when aerobic exercise is combined with WL; however, plasma biomarkers of endothelial dysfunction do not appear to change with either lifestyle intervention in obese postmenopausal women.

In our subjects, CRP levels directly correlated with central obesity at baseline regardless of glucose tolerance status. This is concordant with findings that larger abdominal/waist circumference or WHR is directly associated with higher CRP levels^{28, 29}. CRP levels showed a stronger correlation with subcutaneous abdominal fat than visceral fat. Studies show correlations of CRP levels with both visceral and subcutaneous fat in both men and women, although the magnitude of these associations varies considerably^{30–33}. Generally, these studies do not indicate that one abdominal fat depot is more closely associated with CRP levels than another.

Previous WL intervention studies typically show a reduction in CRP levels, but there is variability among study results¹⁴. A recent systematic review by Selvin et al.¹⁴ indicates that WL reduces CRP regardless of stimulus (diet, exercise, surgical, etc.), with an mean reduction of 0.13 mg/L per kg WL. This rate approximates the reduction in CRP in our subjects who lost an average of 7–8 kg of body weight, as both AEX+WL and WL reduced CRP levels comparably in these postmenopausal women. While the reduction in CRP appeared greater after AEX+WL, there was no statistical difference between the responses

indicating that we could not discern an independent or additive effect of AEX. Studies of the effects of aerobic exercise training on CRP levels generally show mixed results^{30, 34–38} with some indicating that WL is a necessary component to reduce CRP levels^{39,40}. Given that reducing CRP levels independently predicts a reduced rate of atherosclerosis progression and coronary disease^{41,42}, our data suggests that either intervention would be expected to have similar salutary effects on atherosclerotic endpoints in postmenopausal women.

SAA is similar to CRP as both are major acute phase proteins mediated through pro-inflammatory and messenger cytokines⁴³ and are positively associated in this study and others⁴⁴. SAA levels are related to BMI, waist circumference, %fat, subcutaneous abdominal fat, and sagittal diameter but not visceral fat. In women followed through the menopause transition, the increase in visceral fat correlated with an increase in SAA⁴⁵. Work by our group⁴, and a recent meta-analysis⁴³, show that WL reduces SAA levels. Following 12 months moderate-intensity aerobic exercise, SAA decreased, but wasn't different from controls³⁵. In our study, AEX+WL reduced SAA but WL did not, despite similar reductions in body weight and fat mass suggesting that exercise has beneficial effects on SAA beyond WL.

We show for the first time that SAA is negatively associated with whole body (mg/kg/min) *in vivo* insulin sensitivity. In another study⁴⁴, subjects with IGT had elevated SAA compared to those with NGT. While the mechanism for these associations is unknown, elevated SAA could lead to pro-inflammatory production and infiltration of immune cells. This chronic activation of the innate immune system may disrupt whole-body insulin sensitivity and impair glucose homeostasis⁴⁶. In cell culture work, SAA inhibits genes involved in maintaining insulin sensitivity (i.e. adiponectin, *Glut4*, IRS-1)⁴⁷ and activates the JNK pathway⁴⁸. SAA may also reduce lipogenesis and increase production of lipolysis-promoting cytokines⁴⁹, resulting in increased circulating free-fatty acids and decrease in skeletal muscle and hepatic glucose uptake. More research is needed to investigate the mechanisms underlying the effects of these lifestyle interventions on SAA in older populations.

In these women, sICAM-1 levels were positively correlated with central obesity, glucose tolerance, and fasting insulin and inversely with M. In contrast, sVCAM-1 levels were only related to sagittal diameter. In previous studies, sICAM-1 and sVCAM-1 concentrations were higher in obese than nonobese by BMI²⁰, higher in diabetes⁵⁰, or not different between obese and type 2 diabetics¹⁷. Our findings are similar to those in children⁵¹ where greater insulin resistance was associated with higher sICAM-1 levels. Ours is the only report to use glucose clamps in older individuals to show the relationship of vascular markers to insulin resistance.

To our knowledge, there are no studies examining the effects of AEX+WL and WL on plasma sICAM-1 and sVCAM-1 levels or mRNA expression in postmenopausal women. Our results are consistent with reports that WL doesn't change sICAM-1 or sVCAM-1 in middle-aged obese men and women¹⁵ or hypercholesterolemic men¹⁶. There were no changes in these biomarkers either after 8-, 10- or 12-week aerobic exercise in obese subjects or those with type 2 diabetes^{17, 18, 50} or endurance+strength training in men with

the metabolic syndrome¹⁹ or in our AEX+WL. However, one-year of 3x/week exercise +10%WL, both sICAM-1 and sVCAM-1 levels decreased in obese premenopausal women²⁰. Estrogen therapy may significantly impact vascular inflammation and partially explain differences between pre- and post-menopausal women⁵². Likewise, sICAM-1 levels decreased in obese men after a 3-week low fat diet with daily treadmill exercise²¹. Although WL or exercise alone don't seem to reduce these circulating markers, the effects of a combined AEX+WL intervention aren't clear. Since acute phase reactants activate the endothelium and induce ICAM-1 and VCAM-1, it is possible that our interventions could not sufficiently induce a downstream effect to reduce the vascular markers. Alternative strategies may be necessary to reduce plasma intracellular adhesion molecules in postmenopausal women.

Expression of the adhesion molecules in adipose tissue hasn't been studied extensively and results are inconclusive. ICAM-1 and VCAM-1 mRNA expression in visceral adipose tissue is higher in obese than lean women but are not different between lean and obese in subcutaneous abdominal fat⁵³. In an earlier study, the same group showed conflicting evidence that the gene expression of adhesion molecules in subcutaneous abdominal adipose tissue was higher in obese than lean women⁵⁴. There were no differences in ICAM or VCAM expression in abdominal versus gluteal subcutaneous tissue in our study suggesting that there is not a depot difference in subcutaneous fat gene expression of these biomarkers. Even less is known about the effects of lifestyle interventions on mRNA expression of adhesion molecules. Although we find modest (14–19%) decreases in abdominal ICAM gene expression after AEX+WL and WL, only the decrease in gluteal ICAM expression after AEX+WL was significant. In contrast, a three-week diet-induced WL significantly increases ICAM-1 mRNA expression in subcutaneous fat⁵⁴ which may be due to an acute effect of a short-term dietary restriction program compared to our six-month intervention. Also, these subjects⁵⁴ may have been hypocaloric at time of study, thus confounding differentiation of the effects of WL from a hypocaloric state whereas our subjects were weight stable at the time of post-testing. We cannot explain the increase in VCAM-1 mRNA expression in gluteal adipose tissue after AEX+WL but this seems to support an increase in VCAM expression in subcutaneous adipose tissue after WL⁵⁴. Although we didn't find a relationship between ICAM-1 and VCAM-1 expression and glucose metabolism, one study showed a positive association between fasting insulin and VCAM-1 mRNA but not ICAM-1 expression in abdominal subcutaneous fat in lean and obese women⁵³. The correlation between ICAM-1 and VCAM-1 expression in VAT with BMI and CD68 (macrophage related marker) may explain the relationship between visceral obesity and macrophage fat infiltration and endothelial dysfunction⁵³.

These findings apply only to obese postmenopausal women. Another limitation is that there was no exercise alone or a non-intervention control group. In addition, we were unable to measure adipose tissue gene expression in all women. Future studies should examine these markers in men, as it appears that they have different pathophysiology involved in the early onset of CVD (e.g. men have higher degrees of atheroma and epicardial endothelial dysfunction, whereas women are more likely to have microvasculature diseases)⁵⁵. Notwithstanding these qualifications, our study utilized state of the art technique to measure insulin sensitivity, body composition, inflammatory biomarkers and vascular adhesion

molecules and carefully conducted weight loss and exercise regimes. This study provides evidence that in obese postmenopausal women, not only were markers of systemic and vascular inflammation associated with obesity and insulin resistance, but they decreased through combined weight loss and aerobic exercise. This suggests that multifaceted strategies may be needed to improve vascular inflammation, and thereby clinical outcomes in older obese women.

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Figure 1. Plasma C-reactive protein (CRP) and acute-phase serum amyloid A (SAA) levels pre and post aerobic exercise +weight loss (AEX+WL) (n=36) and weight loss (WL) (n=40) in postmenopausal women. *P<0.05; ‡P<0.001.

Figure 2.

Plasma intracellular adhesion molecule (sICAM-1) and vascular CAM-1 (sVCAM-1) levels pre and post aerobic exercise + weight loss (AEX+WL) (n=35) and weight loss (WL) (n=40) in postmenopausal women. *P<0.05

Table 1

Metabolic effects of AEX+WL and WL

	AEX+WL (n=37)		WL (n=40)		Intervention Comparison
	Pre	Post	Pre	Post	
Age (yr)	60 ± 1	---	61 ± 1	---	NS
Weight (kg)	85.2 ± 2.4	78.2 ± 2.2 [‡]	87.2 ± 2.1	80.0 ± 2.0 [‡]	NS
BMI (kg/m ²)	32 ± 1	30 ± 1 [‡]	33 ± 1	30 ± 1 [‡]	NS
Waist circumference (cm)	96.3 ± 2.4	91.8 ± 2.5 [‡]	96.7 ± 1.6	91.0 ± 1.7 [‡]	NS
Percent body fat	47.1 ± 0.9	43.5 ± 1.1 [‡]	47.8 ± 0.6	44.8 ± 0.8 [‡]	NS
Fat mass (kg)	40.6 ± 1.7	34.8 ± 1.7 [‡]	42.4 ± 1.5	36.7 ± 1.4 [‡]	NS
Fat-free mass (kg)	45.0 ± 1.1	44.1 ± 0.9 [*]	45.4 ± 0.8	43.9 ± 0.8 [‡]	NS
VO ₂ max (l/min)	1.78 ± 0.08 ^a	2.03 ± 0.10 [‡]	1.50 ± 0.06	1.48 ± 0.05	P<0.01
Visceral fat (cm ²)	145.8 ± 13.6	125.6 ± 11.7 [‡]	141.8 ± 8.8	120.2 ± 6.9 [‡]	NS
Subcutaneous abdominal fat (cm ²)	458.4 ± 24.4	390.4 ± 27.8 [‡]	432.0 ± 23.8	379.8 ± 21.4 [‡]	NS
Total abdominal fat (cm ²)	598.7 ± 34.2	505.9 ± 35.8 [‡]	567.6 ± 24.6	493.4 ± 23.3 [‡]	NS
Total abdominal/Visceral	4.97 ± 0.38	5.08 ± 0.40	4.64 ± 0.34	4.73 ± 0.35	NS
Sagittal Diameter (mm)	25.8 ± 0.7	23.8 ± 0.7 [‡]	25.3 ± 0.5	23.4 ± 0.5 [‡]	NS
Systolic BP (mmHg)	123 ± 2 ^a	118 ± 2 [‡]	119 ± 1	116 ± 2 [*]	NS
Diastolic BP (mmHg)	69 ± 1	67 ± 1	68 ± 1	65 ± 1 [*]	NS
TG (mg/dl)	118 ± 7	101 ± 5 [‡]	122 ± 8	111 ± 8 [‡]	NS
Total Cholesterol (mg/dl)	193 ± 6	188 ± 5	200 ± 5	193 ± 5	NS
HDL-C (mg/dl)	51 ± 2	55 ± 2 [‡]	56 ± 2	58 ± 2	NS
HDL ₃ -C (mg/dl)	41 ± 1 ^a	44 ± 1 [*]	45 ± 1	44 ± 1	NS
LDL-C (mg/dl)	118 ± 5	113 ± 4	119 ± 5	114 ± 4	NS
Fasting glucose (mmol/l)	5.2 ± 0.1	5.1 ± 0.1 [*]	5.4 ± 0.1	5.0 ± 0.1 [‡]	NS
Glucose@120min (mmol/l)	7.3 ± 0.3	6.8 ± 0.2	6.9 ± 0.3	6.7 ± 0.3	NS
Fasting insulin (pmol/l)	84 ± 8	69 ± 5 [‡]	88 ± 6	66 ± 4 [‡]	NS
HOMA-IR	2.9 ± 0.3	2.3 ± 0.2 [‡]	3.0 ± 0.2	2.1 ± 0.1 [‡]	

	AEX+WL (n=37)		WL (n=40)		Intervention Comparison
	Pre	Post	Pre	Post	
M (mg/kg/min)	6.2 ± 0.3	7.4 ± 0.4 [‡]	6.0 ± 0.3	6.6 ± 0.3 [‡]	NS
M (μmol/kgFFM/min)	64.6 ± 3.3	71.1 ± 3.1 [*]	63.8 ± 3.1	68.1 ± 3.6	NS

VO₂max = maximal oxygen consumption, BP = blood pressure, TG= triglycerides, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, HOMA-IR = Homeostasis model assessment-insulin resistance, M = glucose utilization.NS, nonsignificant. Values are mean ± SEM. Baseline differences tested between groups;

^aP<0.05.

Significantly different pre vs. post intervention:

^{*}P<0.05;

[‡]P<0.01;

[‡]P<0.001.

Table 2

Bivariate associations of vascular and systemic markers of inflammation in postmenopausal women

		sICAM-1	sVCAM-1	CRP	SAA
BMI	N=76	0.24 ^a	Ns	0.39 [‡]	0.33 [‡]
Waist	N=71	0.24 ^a	Ns	0.38 [‡]	0.35 [‡]
% Fat	N=76	ns	Ns	0.42 [‡]	0.30 [‡]
Visceral Fat Area	N=72	0.38 [‡]	Ns	0.25 ^a	0.30 [*]
Subcutaneous Abdominal Fat	N=60	0.31 [*]	Ns	0.59 [‡]	0.46 [‡]
Sagittal Diameter	N=72	0.47 [‡]	0.30 [*]	0.51 [‡]	0.49 [‡]
VO ₂ max	N=74	-0.23 ^a	Ns	-0.22 ^a	ns
Fasting glucose	N=76	0.24 [*]	Ns	ns	0.20 ^a
G ₁₂₀	N=76	0.26 [*]	Ns	0.19	ns
Fasting insulin	N=76	0.28 [*]	Ns	0.29 [*]	ns
HOMA-IR	N=76	0.31 [‡]	Ns	0.29 [*]	0.22 ^b
M (mg kg ⁻¹ ·min ⁻¹)	N=59	-0.29 [*]	Ns	-0.21	-0.29 [*]

BMI= body mass index, VO₂max = maximal oxygen consumption, HOMA-IR = Homeostasis model assessment-insulin resistance, M = glucose utilization.

ns = non-significant

^aP<0.07,

^bP=0.05,

* P<0.05;

[‡] P<0.01;

[‡] P<0.005