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# Effects of antioxidant supplementation on insulin sensitivity, endothelial adhesion molecules, and oxidative stress in normal-weight and overweight young adults

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**Abstract**

The objective of the study was to determine whether short-term antioxidant (AOX) supplementation affects insulin sensitivity, endothelial adhesion molecule levels, and oxidative stress in overweight young adults. A randomized, double-blind, controlled study tested the effects of AOXs on measures of insulin sensitivity (homeostasis model assessment [HOMA]) and quantitative insulin sensitivity check index), endothelial adhesion molecules (soluble intercellular adhesion molecule–1, vascular adhesion molecule, and endothelial-leukocyte adhesion molecule–1), adiponectin, and oxidative stress (lipid hydroperoxides) in overweight and normal-weight individuals (N = 48, 18-30 years). Participants received either AOX (vitamin E, 800 IU; vitamin C, 500 mg; $\beta$-carotene, 10 mg) or placebo for 8 weeks. The HOMA values were initially higher in the overweight subjects and were lowered with AOX by week 8 (15% reduction, $P = .02$). Adiponectin increased in both AOX groups. Soluble intercellular adhesion molecule–1 and endothelial-leukocyte adhesion molecule–1 decreased in overweight AOX-treated groups by 6% and 13%, respectively ($P < .05$). Plasma lipid hydroperoxides were reduced by 0.31 and 0.70 nmol/mL in the normal-weight and overweight AOX-treated groups, respectively, by week 8 ($P < .05$). Antioxidant supplementation moderately lowers HOMA and endothelial adhesion molecule levels in overweight young adults. A potential mechanism to explain this finding is the reduction in oxidative stress by AOX. Long-term studies are needed to determine whether AOXs are effective in suppressing diabetes or vascular activation over time.


## 1. Introduction

Insulin resistance and endothelial dysfunction are 2 reciprocally related processes that precede metabolic and cardiovascular disease [1]. Although insulin has important metabolic actions, it also regulates vascular reactivity, activation, and endothelial cellular adhesion molecule (CAM) expression [2]. Both insulin resistance and endothelial dysfunction are features of obesity [3]. Oxidative stress is a common mechanism underlying insulin resistance and endothelial dysfunction, and is elevated in obesity [4]. Adiponectin is an adipocyte-derived, antiatherogenic insulin-regulating protein [5] that might in part be regulated by oxidative stress–related endothelial dysfunction [6]. Thus, correcting oxidative stress may be a good strategy to combat endothelial dysfunction, insulin resistance, and low adiponectin in obese individuals.

Obesity is associated with a low antioxidant (AOX) defense compared with normal weight [4]. Tissue AOX levels are lower in obese than normal-weight adults [7,8]. Furthermore, total AOX status is lower in obesity; and this AOX imbalance favors systemic oxidative stress [4]. Dietary AOXs

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may attenuate oxidative stress. However, a controversy exists in the literature whether AOX supplementation actually protects against the onset of cardiovascular disease or diabetes. Small studies have shown that supplementation with individual nutrients such as vitamins C, E, or A lowers oxidative stress, CAM expression, and insulin resistance and improves glucose disposal [9-16]. Other studies, however, show enhanced oxidative stress and increased insulin resistance with vitamin E alone [17]. Most of the major AOX trials in large cardiovascular-diseased cohorts have yielded an overall lack of protection against disease in humans [18-22]. The discrepancy among these studies may in part be explained by the fact that the major dietary AOXs work together within intracellular AOX defense systems [23] and may be most effective when administered together instead of 1 AOX alone [24]. In addition, the trial populations were generally older (≥40 years) [18] and/or have advanced stages of disease [19,20,25], or had major confounding variables that negated any potential positive supplementation effect [21]. Finally, the timing, type, and dosage of AOX supplementation may not have been optimal for the specific cardiovascular populations studied [22]. One possibility is that AOXs may not have been used early enough to offer protection before disease fulminates. Therefore, several questions remain unanswered regarding the efficacy of AOXs on early disease processes in the vulnerable overweight adult.

Available experimental evidence in diabetic animals and humans showed that short-term supplementation with vitamins C and/or E decreased insulin levels [15,26] and reduced CAM concentrations and oxidative stress [27,28]. To our knowledge, it is unknown whether the combination of vitamins E, C, and β-carotene can attenuate predisease processes of insulin insensitivity, elevated endothelial adhesion molecule levels, and oxidative stress levels in a younger, overweight population who is at risk for disease. To address this issue, this study examined the effects of short-term combined AOX supplementation on insulin sensitivity, CAM levels, and oxidative stress in overweight young adults compared with normal-weight controls.

## 2. Methods and procedures

A randomized, double-blinded, controlled study design was used. Forty-eight apparently healthy young men and women (18-30 years) volunteered for this study. Participants were recruited by flyers, newspapers, institutional Internet sites, and clinic advertisements from the Central Virginia region. All participants had to meet the following criteria before enrollment in the study: no participation in regular physical activity (vigorous exercise 2 times or more per week); no chronic health problems or current smoking; no history of cardiovascular, metabolic, or respiratory disease; no consumption of AOX supplements within the past 6 months; and no current use of any form of hormonal forms of contraceptives. All participants read and signed a written informed consent statement consistent with university policy on protection of human subjects. The protocol of the study was approved by the Institutional Review Board for Studies Involving Human Subjects at the University of Virginia (UVA).

### 2.1. Participants and study groups

After the investigators had determined body mass index (BMI) values, participants were stratified into normal-weight or overweight groups (normal weight, BMI <25 kg/m$^2$; overweight and obese, BMI ≥25 kg/m$^2$). Within each group, participants were randomized by the UVA investigational pharmacist to receive either AOX treatment or placebo. Hence, there were a total of 4 participant groupings: normal weight, AOX treated (N-AOX); normal weight, placebo (N-PL); overweight, AOX-treated (O-AOX); and overweight, placebo (O-PL). Adiposity status is represented by normal weight (N) and overweight (O). A total of 135 individuals responded to the advertisements and were screened for eligibility. Those who were not enrolled did not meet all inclusion criteria, were too old, or were beginning a new weight loss program at the same time. A total of 65 participants enrolled into the study; 11 were withdrawn because of overcommitted schedules, failure to comply with the study visits, failure to respond to investigator contact, and child care issues.

### 2.2 AOX intervention and testing schedule

Groups assigned to the AOX treatment were administered vitamin E (800 IU/d), vitamin C (500 mg/d), and β-carotene (10 mg/d). The supplements or placebos were administered by the UVA investigational pharmacist in opaque bottles and were taken once a day. Each supplement was provided in an individual capsule form; the vitamin C and β-carotene were provided in yellow and green polysaccharide capsules mixed with inert microcrystalline cellulose. The vitamin E was supplied in an opaque gel capsule containing a mixture of the 8 tocopherols and tocotrienols found naturally in food. The content of each AOX was independently verified using an external quality control laboratory contracted by the institution. The placebos were provided in identical opaque bottles with color-matched (yellow, green, and opaque), odorless capsules similar to those of the AOX. Placebos consisted of microcrystalline cellulose (90%) contained within a natural polysaccharide capsule (capsule derived from water, gelatin, and titanium dioxide). Vitamin E softgel capsule placebos contained cornstarch. The dosages of AOXs were chosen based on previous work indicating a 27% reduction in lipid peroxidation with this range of combined supplements [29]. Treatment was administered for 8 weeks, a time by which plasma concentrations of vitamin E stabilize with supplementation [30].

All participants visited the testing area 3 times (2 preintervention, 1 postintervention). During visit 1, participants were acclimated to the UVA Exercise Physiology Laboratory at the General Clinical Research Center (GCRC); body composition and vital signs were measured; and 3-day dietary record

forms were provided to participants. During visit 2, aerobic fitness levels (peak oxygen consumption [$VO_2$peak]) were measured using a load-incremented cycle ergometer protocol. Visit 3 was a repeat of visits of 1 and 2 combined that occurred after the supplementation period. Visits 1 and 2 were conducted within 1 week.

### 2.3. Anthropometric measures

Height and weight were measured using a standard medical-grade scale. For classification of obesity, waist and hip girths were measured using a soft, cloth measuring tape at anatomical landmarks described by the American College of Sports Medicine [31]. Body mass index values were determined by the following: BMI = weight (in kilograms)/height (in meters)$^2$. Body volume was estimated using air displacement plethysmography in a BodPod device (BodPod; Life Measurement Instruments, Concord, CA) corrected for thoracic gas volume; body density was calculated and used to predict body fat using the Siri [32] equation.

### 2.4. Dietary analysis

Three-day dietary record forms were provided to each participant with standard instructions on how to complete the record. Participants were instructed to estimate servings of foods using household measurements (volume) as described in national dietary guidance documents as previously described [33]. Each participant received individual training sessions with the same investigator with regard to measuring technique and volume estimation. Picture books of portion sizes were also provided after the dietary estimation training session [33]. Diet records were assessed by the same investigator using Nutritionist Pro Software (version 2.1.13; First DataBank, San Bruno, CA) and were analyzed for macronutrient, AOX, and caloric intake. To ensure the stability of the habitual diet of the subjects, all subjects completed a second 3-day dietary record 8 weeks later [33]. To further improve dietary stability, we performed 2 steps: (1) during screening and the initial days of the study, we indicated to each participant the importance of maintaining normal dietary patterns for the 2 months of the supplementation period; and (2) during the bimonthly periodic check-in visits during the study, we discussed with each participant whether any unusual dietary deviations occurred or whether there had been any systematic changes in food intake (volume or type of food) since the previous visit.

### 2.5. Peak oxygen consumption/lactate threshold test

A $VO_2$peak test was conducted to measure the aerobic fitness level of each participant. After a 12-hour overnight fast, participants arrived at the GCRC. A venous catheter was inserted into a forearm vein. Participants completed a $VO_2$peak/lactate threshold test on an electronically braked cycle ergometer (Ergo Metrics 800S; Sensor Medics, Yorba Linda, CA). The initial power output was set at 20 W, and the power output was increased by 15 W every 3 minutes until volitional fatigue. Blood samples were taken at rest and during the last 15 seconds of each exercise stage for the measurement of blood lactate concentration (model 2700; YSI Instruments, Yellow Springs, OH). Heart rates, blood pressures, and rating of perceived exertion were collected at every exercise stage [31]. Metabolic data were collected during both the $VO_2$peak test using standard open-circuit spirometric techniques (Vmax 229, Sensor Medics). Heart rate was determined electrocardiographically (Marquette Max-1 electrocardiograph, Marquette, WI).

### 2.6. Blood sampling

Fasting blood samples were collected from a catheter from an antecubital vein into heparinized Vacutainer tubes (Becton Dickinson, San Jose, CA) during visits 1 and 3. Blood samples were analyzed for glucose, insulin, lipid hydroperoxides (PEROX), glycated hemoglobin ($HbA_{1c}$) levels, and inflammatory cytokines and adiponectin. All samples were batched within each subject and run in the same assay. A portion of the blood was immediately centrifuged at 1500*g* for 5 minutes to separate plasma from red blood cell pellets. Plasma samples were immediately frozen and stored at −70°C until analysis. Insulin sensitivity biomarkers, vascular endothelial adhesion molecules, and adiponectin were kindly performed by the GCRC at UVA. Cholesterol, glucose, and $HbA_{1c}$ samples were collected in the UVA GCRC and sent to the UVA Health System Clinical and Toxicology Laboratories in the same medical building for processing. The PEROX assays were performed by a member of the investigational team (HKV).

### 2.7. Insulin sensitivity estimates

The homeostasis model assessment (HOMA) calculation was calculated from fasting glucose ($G_0$) and insulin ($I_0$) concentrations using the following formula: $(G_0 \times I_0)/22.5$ [34,35]. The quantitative insulin sensitivity check index (QUICKI) was determined by the following calculation: 1/[log (fasting glucose) + log (fasting insulin)] [36].

### 2.8. Lipid peroxidation measurements

Lipid hydroperoxides were quantified using the colorimetric ferrous oxidation/xylenol orange spectrophotometric technique previously described, where cumene hydroperoxide was used as the standard for this assay. Hydroperoxide formation was reflected by the accumulation of a purple chromatophore within the sample that was read at 580 nm [37]. All samples were performed in triplicate in a single batch analysis. The coefficient of variation for this assay was 4%.

### 2.9. Vascular endothelial adhesion molecules and adiponectin

Soluble intercellular adhesion molecule–1 (sICAM), vascular adhesion molecule (sVCAM-1), and endothelial-leukocyte adhesion molecule–1 (sE-selectin) were assessed by the GCRC Core Laboratory using enzyme-linked immunosorbent assays. R&D Quantikine ELISA kits

(Minneapolis, MN; catalog nos. BBE 2B, BBE 1B, BB3) were used to measure adhesion molecules. Monoclonal antibodies specific for sVCAM-1, sICAM, and sE-selectin were precoated onto microplates; controls, standards, and samples were pipetted into each microplate; and the biomarkers were sandwiched between the plate bound antibody and the enzyme-linked monoclonal specific antibody for each biomarker. A substrate solution specific for each assay was added and generated color in the well in proportion to the amount of biomarker bound to the plate. All samples were performed in duplicate.

### 2.10. Cholesterol, glucose, and $HbA_{1c}$

To document levels of lipid substrates in the blood available for oxidation, plasma cholesterol subfractions (total cholesterol, high-density lipoproteins [HDL-C], and triglycerides) were analyzed using standard automated spectrophotometric laboratory procedures (Olympus AU640 [Olympus Diagnostic Systems, Southall, Middlesex, UK], Olympus calibrator catalog no. DR0040, and Genzyme [Genzyme Corp, Cambridge, MA] HDL-C calibrator catalog no. 80-4529-00). Low-density lipoproteins (LDL-C) were estimated from the following equation: LDL-C = total cholesterol − HDL-C − (triglycerides/5). Blood glucose was assessed using an Olympus AU640 procedure, in which glucose was phosphorylated by hexokinase in the presence of adenosine triphosphate and magnesium. The resultant glucose-6-phosphonate dehydrogenase oxidized glucose-6-phosphonate to 6-phosphogluconate and reduced nicotinamide adenine dinucleotide to NADH. The change in absorbance at 340/380 was proportional to the amount of glucose in the sample (Olympus Glucose Reagent, calibrator catalog no. DR0040). Glycated hemoglobin was analyzed using automated high-performance liquid chromatography (Tosoh G7 Automated HPLC Analyzer [Tosoh Bioscience, Minato-Ku, Japan], using TSKgel G and HSi elution columns). All samples were performed in duplicate.

Table 2
Average dietary intakes of major macronutrient of normal-weight and overweight groups by AOX treatment

| | Normal weight | | Overweight | |
|---|---|---|---|---|
| | AOX (n = 12) | PL (n = 13) | AOX (n = 12) | PL (n = 11) |
| Energy intake (kcal) | 2357 ± 171 | 1940 ± 210 | 2104 ± 213 | 2422 ± 223 |
| Protein (g/d) | 83 ± 11 | 72 ± 7 | 77 ± 9 | 98 ± 13 |
| Carbohydrate (g/d) | 286 ± 17 | 262 ± 26 | 288 ± 28 | 282 ± 32 |
| Fat (g/d) | 96 ± 3 | 65 ± 12 | 74 ± 10 | 101 ± 13 |
| Saturated fat (g/d) | 31 ± 5 | 20 ± 3 | 24 ± 4 | 36 ± 5* |
| Vitamin C (mg) | 124 ± 34 | 113 ± 29 | 103 ± 28 | 98 ± 26 |
| Vitamin E (mg) | 11 ± 2 | 5 ± 1 | 7 ± 2 | 6 ± 1 |
| α-Tocopherol (mg) | 4 ± 1 | 2 ± 1 | 3 ± 1 | 3 ± 1 |
| β-Carotene (mg) | 2847 ±1193 | 743 ± 312 | 807 ± 201 | 1267 ± 387 |
| Zinc (mg) | 10 ± 1 | 7.0 ± 1 | 9 ± 1 | 11 ± 2 |
| Selenium (μg) | 86 ± 15 | 57 ± 9 | 67 ± 13 | 95 ± 18 |

Values are means ± SE.
* Different from N-PL at $P < .05$.

### 2.11. Statistics

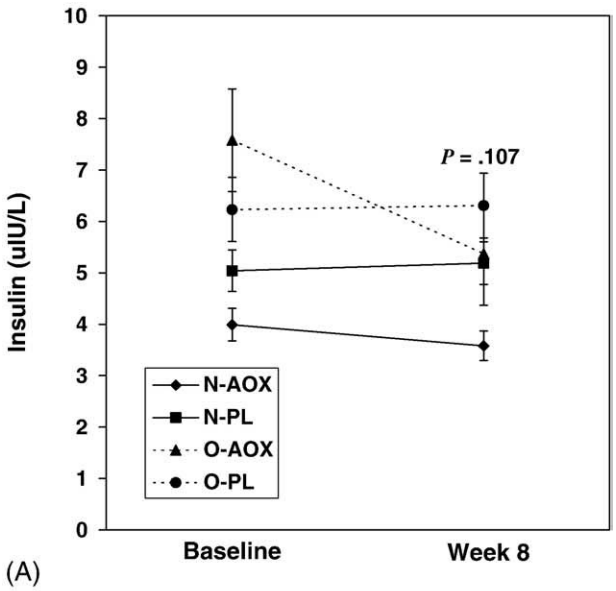
All data are expressed in mean ± standard error (SE). Data were analyzed using the Statistical Package for the Social Sciences (version 12.0; SPSS, Chicago, IL). Percentage of change (from baseline) was calculated for several blood measures. Descriptive variables were analyzed using a 2-way analysis of variance. If differences did not exist between groups at baseline, repeated-measures analyses of variance

Table 1
Subject characteristics at baseline

| | Normal weight | | Overweight | |
|---|---|---|---|---|
| | AOX (n = 12) | PL (n = 13) | AOX (n = 12) | PL (n = 11) |
| Sex (M/F) | 7/5 | 3/10 | 3/9 | 4/7 |
| Age (y) | 22 ± 1 | 22 ± 1 | 23 ± 1 | 27 ± 1 |
| Height (cm) | 174 ± 3 | 170 ± 2 | 171 ± 3 | 171 ± 2 |
| Weight (kg) | 69 ± 3 | 62 ± 2 | 94 ± 6* | 100 ± 7* |
| Fat mass (kg) | 14 ± 1 | 14 ± 1 | 46 ± 5* | 48 ± 6* |
| Fat-free mass (kg) | 55 ± 23 | 48 ± 2 | 57 ± 4 | 55 ± 4 |
| BMI ($kg/m^2$) | 23 ± 1 | 21 ± 1 | 32 ± 2 | 34 ± 2 |
| Waist circumference (cm) | 76 ± 2 | 70 ± 2 | 96 ± 4* | 100 ± 4* |
| WHR | 0.76 ± 0.1 | 0.74 ± 0.1 | 0.83 ± 0.1* | 0.85 ± 0.1* |
| $VO_2$peak (mL/[kg min]) | 33 ± 2 | 31 ± 2 | 21 ± 2* | 19 ± 1* |
| Glucose (mmol/L) | 4.4 ± 0.1 | 4.4 ± 0.1 | 4.9 ± 0.3 | 5.0 ± 0.3 |
| $HbA_{1c}$ (%) | 5.0 ± 0.1 | 5.0 ± 0.1 | 4.9 ± 0.1 | 5.0 ± 0.1 |
| Insulin (μIU/mL) | 5.6 ± 0.7 | 5.9 ± 0.4 | 11.7 ± 1.8* | 11.6 ± 4.0* |
| HOMA | 1.1 ± 0.1 | 1.2 ± 0.1 | 2.6 ± 0.5* | 2.6 ± 0.8* |
| QUICKI | 0.39 ± 0.1 | 0.38 ± 0.1 | 0.35 ± 0.1* | 0.33 ± 0.2* |
| Total cholesterol (mmol/L) | 4.3 ± 0.1 | 3.8 ± 0.2 | 4.0 ± 0.3 | 4.3 ± 0.3 |
| HDL-C (mmol/L) | 1.5 ± 0.2 | 1.4 ± 0.3 | 1.2 ± 0.3 | 1.2 ± 0.4 |
| LDL-C (mmol/L) | 2.4 ± 0.4 | 2.0 ± 0.6 | 2.3 ± 0.7* | 2.7 ± 0.8* |
| Triglycerides (mmol/L) | 0.8 ± 0.4 | 0.9 ± 0.5 | 1.3 ± 0.8 | 1.0 ± 0.6 |

Values are means ± SE. WHR indicates waist to hip ratio.
* Different from normal-weight group at $P < .05$.

were performed for the change scores (Δ values from baseline to 8 weeks) for the inflammatory cytokines, blood lipids, and PEROX. The between-group factors were adiposity status (nonobese, overweight) and treatment (AOXs, placebo), and the within-group factor was time (pre- and postexercise, baseline and 8 weeks). When baseline differences existed for blood measures, 2-way analyses of covariance were performed using the baseline value as the covariate. The between-group factors were adiposity status (nonobese, overweight) and treatment (AOXs, placebo). The level of significance was set at .05 for all statistical tests.



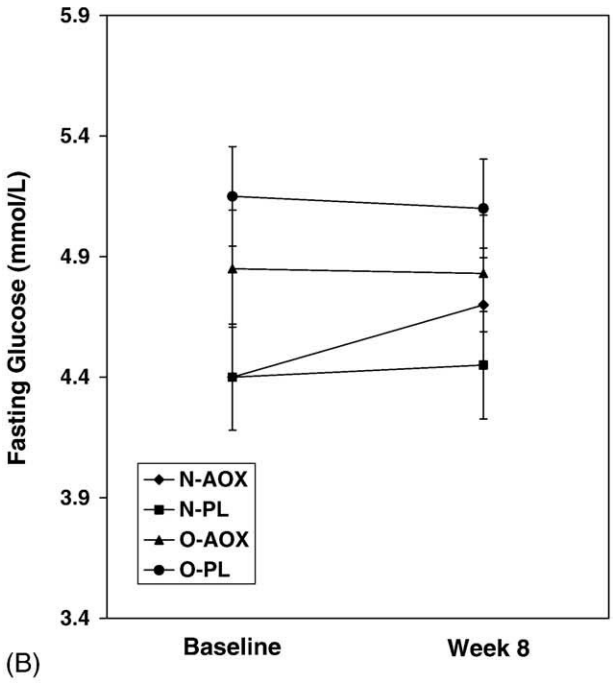




Fig. 1. A, Fasting insulin values for normal-weight and overweight young adults treated with AOXs (vitamins E, C, and β-carotene) or placebo at baseline and 8 weeks. Values are means ± SE. B, The HOMA values for normal-weight and overweight young adults treated with AOXs (vitamins E, C and β-carotene) or placebo at baseline and 8 weeks. Values are means ± SE. C, Fasting plasma glucose concentrations for normal-weight and overweight young adults treated with AOXs (vitamins E, C, and β-carotene) or placebo at baseline and 8 weeks. Values are means ± SE.

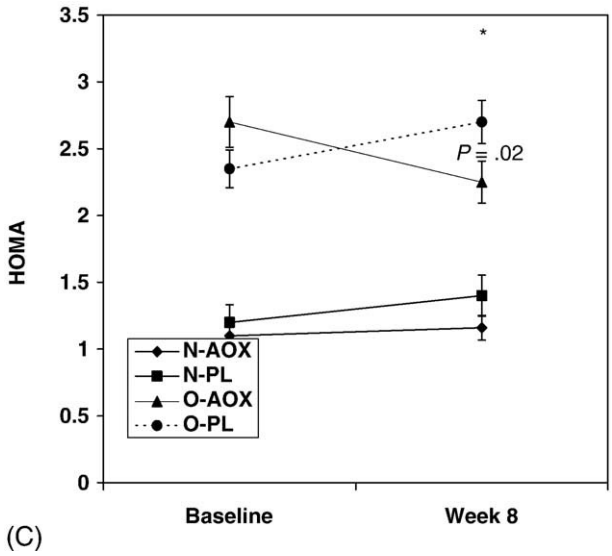



Fig. 1. (*continued*)

## 3. Results

### 3.1. Subject characteristics at baseline

As expected, several indices of body composition were significantly different between the normal-weight and overweight groups (all $P < .05$, Table 1). Aerobic fitness was lower in the overweight group ($P < .0001$). Fasting measures of blood glucose were not different between groups at baseline, but HOMA and QUICKI scores were different in the overweight group than the normal-weight group ($P < .024$). The LDL-C concentrations were higher in the overweight group than the normal-weight group ($P < .05$). Soluble intercellular adhesion molecule−1 and sE-selectin tended to be higher in the overweight group than the normal-weight group ($P = .077$).

### 3.2. Dietary intake

Correlation values ($r$) between specific macro- and micronutrient intake between the first and second dietary records ranged from 0.7 to 0.93, indicating a stable intrasubject dietary pattern. The only significant differences with regard to dietary intakes were found to be with saturated fat (Table 2) and copper. The O-PL had higher saturated fat intake than the N-PL group ($P < .05$). Copper intakes were (in milligrams) 1.1 ± 0.1, 0.8 ± 0.1, 0.7 ± 0.1, and 1.0 ± 0.1 in the N-AOX, N-PL, O-AOX, and O-PL groups, respectively (O-AOX were different, $P < .05$). Manganese intakes were (in milligrams) 2.9 ± 0.6, 2.4 ± 0.5, 1.5 ± 0.3, and 1.8 ± 0.4 in the N-AOX, N-PL, O-AOX, and O-PL groups, respectively. We presented the raw values for the calculated micronutrient

intakes here for the reader; however, we acknowledge that the level of precision reported for these values is likely much lower given the subject reporting of dietary intake.

### 3.3. Post-AOX supplementation

There were no changes in body weight, fat or fat-free mass, and waist to hip ratio after the treatment period in either the placebo or AOX group. No significant changes occurred in exercise capacity ($VO_2$peak) or cholesterol profiles by week 8 of the intervention in any experimental group, indicating that these potential confounders remained stable from pre- to postintervention.

### 3.4. Insulin sensitivity–related variables

Although the fasting insulin levels increased in the placebo group by week 8, insulin levels decreased in the O-AOX group; but this decrease did not achieve statistical significance ($P$ = .107, Fig. 1A. Relative to participants treated with placebo, the HOMA scores in the AOX group were significantly lower in the overweight group at week 8 ($P$ = .02, Fig. 1C). The HOMA scores in the normal-weight group were not changed by AOX at 8 weeks. By week 8, the QUICKI scores were improved in the AOX overweight and normal-weight groups by 2.5% and 1%, respectively. The QUICKI scores were reduced in the PL-treated overweight and normal-weight groups by 3.0% and 2.5%, respectively, by week 8. These QUICKI changes, however, were not significant ($P$ = .407). Fasting glucose levels were not different between groups or over time ($P$ = .973, Fig. 1B).

### 3.5. Lipid peroxidation, adhesion molecules, and adiponectin

Data regarding lipid peroxidation, adhesion molecules, and adiponectin are shown in Table 3. Relative to participants receiving placebo, those treated with AOX (both N-AOX and O-AOX) showed significant reductions in PEROX ($P$ = .013). Endothelial-leukocyte adhesion molecule–1 and sICAM-1 concentrations also decreased over time in participants treated with AOX, although these reductions were significant only in the overweight group ($P$ = .023 and .14, respectively). Adiponectin increased in normal-weight and overweight participants treated with AOX, whereas the placebo-treated groups demonstrated decreases in adiponectin over time ($P$ = .05).

## 4. Discussion

The combination of vitamins C, E, and β-carotene during an 8-week supplementation period moderately reduced the HOMA values and the sICAM-1 and sE-selectin levels in overweight young adults. Oxidative stress was also reduced by week 8 and may be a potential mechanism underlying these favorable changes in cardiovascular disease and diabetes precursors. These preliminary findings suggest that obesity-induced early developmental stages of insulin resistance and endothelial dysfunction might be influenced by combined AOX administration.

Previous supplementation studies of individual AOXs C and E in humans and animals have shown inconsistent effects on insulin sensitivity, with some showing improvements [15,38,39], whereas others do not [39,40]. It is not known whether isolated β-carotene affects insulin sensitivity in obese young adults. A limitation to these and other studies is that AOXs do not work optimally in isolation, but rather serve as part of an AOX system where optimal protection against disease processes occurs with several AOXs together, as found in natural foods. In the present study, the combination of vitamins E, C, and β-carotene lowered the HOMA index in overweight but not in normal-weight young adults. This moderate change reflects an improvement in the responsiveness to insulin in the overweight participants. We propose that the AOXs work in concert to act at several sites in the insulin metabolism pathways. For example, vitamin C supplementation increases AOX defenses in tissue [41], and potentiates insulin action and

Table 3
Fasting measurements of oxidative stress, vascular inflammation, and adiponectin in normal-weight and overweight groups by AOX treatment at baseline

| | Normal weight | | | | Overweight | | | |
|---|---|---|---|---|---|---|---|---|
| | AOX (n = 12) | | PL (n = 13) | | AOX (n = 12) | | PL (n = 11) | |
| | Baseline | Wk 8 | Baseline | Wk 8 | Baseline | Wk 8 | Baseline | Wk 8 |
| PEROX | 2.7 ± 0.2 | 2.3 ± 0.2 | 2.6 ± 0.2 | 2.8 ± 0.2 | 2.5 ± 0.2 | 2.2 ± 0.2 | 2.6 ± 0.2 | 2.6 ± 0.2 |
| Change | | −0.3 ± 0.2* | | 0.6 ± 0.4 | | −0.7 ± 0.3* | | 0.1 ± 0.1 |
| sE-selectin | 37 ± 5 | 39 ± 5 | 48 ± 4 | 47 ± 4 | 52 ± 8 | 45 ± 5* | 49 ± 6 | 48 ± 6 |
| Change | | −1 ± 2 | | −1 ± 1 | | −9 ± 3* | | −2 ± 6 |
| sICAM-1 | 220 ± 13 | 209 ± 13 | 213 ± 14 | 214 ± 15 | 231 ± 15 | 216 ± 14* | 254 ± 18 | 254 ± 19 |
| Change | | −12 ± 8 | | 0.5 ± 0.8 | | −14 ± 5* | | −0.2 ± 6 |
| sVCAM-1 | 546 ± 45 | 524 ± 46 | 551 ± 42 | 556 ± 43 | 570 ± 42 | 537 ± 42 | 676 ± 45 | 643 ± 48 |
| Change | | −25 ± 12 | | 9 ± 32 | | −33 ± 26 | | −10 ± 24 |
| Adiponectin | 9730 ± 1242 | 11 339 ± 1286 | 9903 ± 1189 | 9152 ± 1231 | 9103 ± 1142 | 9534 ± 1182 | 9157 ± 1302 | 8662 ± 1348 |
| Change | | 1748 ± 629* | | −751 ± 938 | | 194 ± 446* | | −495 ± 583 |

Change values are the raw differences between baseline and week 8 values for each measure. Values are means ± SE. Lipid hydroperoxides are expressed in nanomoles per milliliter; sE-selectin, sICAM-1, sVCAM-1, and adiponectin are expressed in nanograms per milliliter.

* Different from PL groups at $P < .05$.

improves glucose uptake in humans [42]. Vitamin E protects against oxidative modification of insulin metabolism–mediating proteins such as glucose transporters and insulin kinases [41]. Inhibition of free radical production and restoration of cell redox status with vitamins E, C, and β-carotene might preserve insulin receptor structure and function, and improve insulin sensitivity in obesity [43]. The fact that the normal-weight participants did not demonstrate significant improvements is not surprising given that oxidative stress was not elevated in this group, and fasting insulin and glucose values were already within appropriate ranges and likely had little room for improvement with the AOX treatment.

The effects of combined AOX on early disease biomarkers such as endothelial adhesion molecules or adiponectin in overweight humans are not clear. We hypothesized that the AOX may work synergistically to combat the stimulus of obesity-induced oxidative damage to the endothelium and may indirectly suppress high levels of sVCAM-1, sICAM-1, and sE-selectin. Previous studies in healthy adults [11] have shown that vitamin E supplementation suppresses sVCAM-1, sICAM-1, and sE-selectin levels, whereas studies of uncomplicated type 1 diabetes mellitus patients treated with vitamin E did not show changes in sVCAM-1 [44]. Other data have shown that vitamins E (300 g/d) and C (250 g/d) and *n*-acetylcysteine can rapidly affect change by attenuating sVCAM-1 levels after a high-fat meal [45]. This finding may be especially relevant for obese individuals who might consume more dietary fat and fewer AOXs (eg, copper), as observed in some of the overweight participants in this study. Previous studies have shown reductions in lipid peroxidation (estimate of oxidative stress) and concurrent reductions in VCAM-1 [45], E-selectin [11], and ICAM-1 [46] with supplementation of individual or combined AOXs. Supplementation with AOXs such as vitamins E, C, or β-carotene is purported to squelch free radicals such as superoxide, directly within the endothelium; to disrupt tumor necrosis factor–α intracellular signaling cascades; or to reduce oxidative modification of LDL-C, all of which would otherwise initiate expression of endothelial adhesion molecules [47].

Antioxidants significantly lowered sICAM-1 and sE-selectin in the healthy young overweight adults in this study, but not normal-weight adults. Although sVCAM-1 levels decreased in the AOX group regardless of adiposity status, this change was not statistically significant. Eight weeks of treatment may not be sufficient to produce statistical reductions in sVCAM-1 such as those reductions observed in supplementation studies that were 6 months [48]. Our normal-weight participants demonstrated some improvement in these endothelial adhesion molecule levels, although these improvements were not significant. Given that these participants were healthy, there was likely a ceiling effect of the AOX treatment on adhesion molecule levels.

Data are sparse regarding the effects of AOX on circulating adiponectin levels. However, one recent study indicated that vitamin E alone (800-1200 IU/d) for 6 months did not significantly change adiponectin levels in middle-aged, overweight subjects [5]. One animal study that used a vitamin C–rich extract reduced oxidative stress and increased adiponectin levels in experimentally induced diabetic rats [49]. Here, combined AOX moderately increased adiponectin and decreased oxidative stress; these data suggest that combined AOXs may be required to appreciably increase adiponectin levels in the overweight human. The incorporation of multiple AOX into multiple physiologic mechanisms at the level of the endothelium likely suppresses oxidative stress pathways that inhibit adiponectin expression [5].

### 4.1. Limitations

Limitations to this study deserve comment. This was a small exploratory study, and the study should be expanded to include a larger sample. The dietary intake data have accuracy limitations and contain a wide variation of interindividual differences in macro- and micronutrient intake; the reported differences in copper and saturated fat might simply reflect individual dietary choices of calorically richer or less nutrient-rich foods (eg, fewer fresh foods) in the overweight groups during the time frame of the dietary record. Furthermore, the N-AOX group contained a few vegetarians, a participant pool who normally consumed higher amounts of vitamin and mineral-rich fresh foods. A longer dietary record period might have identified additional dietary differences between the normal-weight and overweight groups, or washed out the differences. The supplementation period was short, and longer term and larger studies in young overweight or obese adults would reveal whether AOX can consistently and permanently reduce endothelial adhesion molecule levels. Ultimately, this intervention should be tested to determine whether the onset of cardiovascular disease and diabetes is influenced in the overweight adult population.

### 4.2. Conclusion

Short-term AOX supplementation moderately lowers HOMA and endothelial adhesion molecule levels and increases adiponectin in overweight but not normal-weight young adults despite no change in body composition or body weight. A potential mechanism to explain this finding is the reduction in oxidative stress by AOX.

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## References


[1] Kim J, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction. Circulation 2006;113:1888-904.

[2] Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, et al. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. J Biol Chem 2002;277:1794-9.

[3] Couillard C, Ruel G, Archer WR, Pomerleau S, Bergeron J, Couture P, et al. Circulating levels of oxidative stress markers and endothelial adhesion molecules in men with abdominal obesity. J Clin Endocr Metab 2005;90:6454-9.

[4] Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int J Obes 2006;30: 400-18.

[5] Sutherland WH, Manning PJ, Walker RJ, de Jong SA, Ryalls AR, Berry EA. Vitamin E supplementation and plasma 8-isoprostane and adiponectin in overweight subjects. Obesity (Silver Spring) 2007;15: 191-386.

[6] Hattori S, Hattori Y, Kasai K. Hypoadiponectinemia is caused by chronic blockade of nitric oxide synthesis in rats. Metabolism 2005;54: 482-7.

[7] Facchini FS, Humphreys MH, DoNascimento CA, Abbasi F, Reaven GM. Relation between insulin resistance and plasma concentrations of lipid hydroperoxides, carotenoids, and tocopherols. Am J Clin Nutr 2000;72:776-9.

[8] Strauss RS. Comparison of serum concentrations of alpha-tocopherol and beta-carotene in a cross-sectional sample of obese and nonobese children (NHANES III). National Health and Nutrition Examination Survey. J Pediatr 1999;134:160-5.

[9] Hartmann A, Niess AM, Grunert-Fuchs M, Poch B, Speit G. Vitamin E prevents exercise-induced DNA damage. Mutat Res 1995;346: 195-202.

[10] Goldfarb AH, Patrick SW, Bryer S, You T. Vitamin C supplementation affects oxidative-stress blood markers in response to a 30-minute run at 75% $VO_2$max. Int J Sport Nutr Exerc Metab 2005;15:279-90.

[11] van Dam B, van Hinsbergh VW, Stehouwer CD, Versteilen A, Dekker H, Buytenhek R, et al. Vitamin E inhibits lipid peroxidation–induced adhesion molecule expression in endothelial cells and decreases soluble cell adhesion molecules in healthy subjects. Cardiovasc Res 2003;57:563-71.

[12] Rayment SJ, Shaw J, Woollard KJ, Lunec J, Griffiths HR. Vitamin C supplementation in normal subjects reduces constitutive ICAM-1 expression. Biochem Biophys Res Commun 2003;308:339-45.

[13] Metin G, Atukeren P, Gumustas MK, Belce A, Kayserilioglu A. The effect of vitamin E treatment on oxidative stress generated in trained rats. Tohoku J Exp Med 2002;198:47-53.

[14] Manning PJ, Sutherland WH, Walker RJ, Williams SM, De Jong SA, Ryalls AR, et al. Effect of high-dose vitamin E on insulin resistance and associated parameters in overweight subjects. Diabetes Care 2004; 27:2166-71.

[15] Abdel-Wahab YH, O'Harte FP, Mooney MH, Barnett CR, Flatt PR. Vitamin C supplementation decreases insulin glycation and improves glucose homeostasis in obese hyperglycemic (*ob*/*ob*) mice. Metabolism 2002;51:514-7.

[16] Facchini F, Coulston AM, Reaven GM. Relation between dietary vitamin intake and resistance to insulin-mediated glucose disposal in healthy volunteers. Am J Clin Nutr 1996;63:946-9.

[17] Skrha J, Sindelka G, Kvasnicka J, Hilgertova J. Insulin action and fibrinolysis influenced by vitamin E in obese type 2 diabetes mellitus. Diabetes Res Clin Pract 1999;44:27-33.

[18] Blot WJ, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J Natl Cancer Inst 1993; 85:1483-92.

[19] Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. Lancet 2003;361:2017-23.

[20] Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. New England J Med 2000;342:154-60.

[21] Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. New England J Med 1996; 334:1150-5.

[22] Kris-Etherton PM, Lichtenstein AH, Howard BV, Steinberg D, Witztum JL. Antioxidant vitamin supplements and cardiovascular disease. Circulation 2004;110:637-41.

[23] Tauler P, Aguilo A, Gimeno I, Fuentespina E, Tur JA, Pons A. Response of blood cell antioxidant enzyme defences to antioxidant diet supplementation and to intense exercise. European J Nutr 2005;22 [electronic publication ahead of print].

[24] Abudu N, Miller JJ, Attaelmannan M, Levinson SS. Vitamins in human arteriosclerosis with emphasis on vitamin C and vitamin E. Clin Chim Acta 2004;339:11-25.

[25] Boaz M, Smetana S, Weinstein T, Matas Z, Gafter U, Iaina A, et al. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. Lancet 2000;356:1213-8.

[26] Boshtam M, Rafiei M, Golshadi ID, Ani M, Shirani Z, Rostamshirazi M. Long term effects of oral vitamin E supplement in type II diabetic patients. Int J Vitam Nutr Res 2005;75:341-6.

[27] Tousoulis D, Antoniades C, Tentolouris C, Tsioufis C, Toutouza M, Toutouzas P, et al. Effects of combined administration of vitamins C and E on reactive hyperemia and inflammatory process in chronic smokers. Atherosclerosis 2003;170:261-7.

[28] Koo JR, Ni Z, Oviesi F, Vaziri ND. Antioxidant therapy potentiates antihypertensive action of insulin in diabetic rats. Clin Exp Hypertens 2002;24:333-44.

[29] Schroder H, Navarro E, Mora J, Galiano D, Tramullas A. Effects of alpha-tocopherol, beta-carotene and ascorbic acid on oxidative, hormonal and enzymatic exercise stress markers in habitual training activity of professional basketball players. European J Nutr 2001;40: 178-84.

[30] Fischer CP, Hiscock NJ, Penkowa M, Basu S, Vessby B, Kallner A, et al. Supplementation with vitamins C and E inhibits the release of interleukin-6 from contracting human skeletal muscle. J Physiol 2004;558(Pt 2):633-45.

[31] Medicine ACoS. ACSM's guidelines for exercise testing and prescription. 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2000.

[32] Siri WE. Body composition from fluid spaces and density: analysis of methods. In: Brožek J, Henschel A, editors. Techniques for measuring body composition. Washington, DC: National Academy of Sciences; 1961. p. 223-44.

[33] Freese R, Alfthan G, Jauhiainen M, Basu S, Erlund I, Salminen I, et al. High intakes of vegetables, berries, and apples combined with a high intake of linoleic or oleic acid only slightly affect markers of lipid peroxidation and lipoprotein metabolism in healthy subjects. Am J Clin Nutr 2002;76:950-60.

[34] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

[35] Straczkowski M, Stepien A, Kowalska I, Kinalska I. Comparison of simple indices of insulin sensitivity using the euglycemic hyperinsulinemic clamp technique. Med Sci Monit 2004;10:CR480-4.

[36] Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocr Metab 2000;85:2402-10.

[37] Hermes-Lima M, Willmore WG, Storey KB. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. Free Radic Biol Med 1995;19:271-80.

[38] Paolisso G, Balbi V, Volpe C, Varricchio G, Gambardella A, Saccomanno F, et al. Metabolic benefits deriving from chronic vitamin C supplementation in aged non–insulin dependent diabetics. J Am Coll Nutr 1995;14:387-92.

[39] Barbagallo M, Dominguez LJ, Tagliamonte MR, Resnick LM, Paolisso G. Effects of vitamin E and glutathione on glucose metabolism: role of magnesium. Hypertension 1999;34(4 Pt 2):1002-6.

[40] Chen H, Karne RJ, Hall G, Campia U, Panza JA, Cannon III RO, et al. High-dose oral vitamin C partially replenishes vitamin C levels in patients with type 2 diabetes and low vitamin C levels but does not improve endothelial dysfunction or insulin resistance. Am J Physiol Heart Circ Physiol 2006;290:H137-45.

[41] Vinayaga Moorthi R, Bobby Z, Selvaraj N, Sridhar MG. Vitamin E protects the insulin sensitivity and redox balance in rat L6 muscle cells exposed to oxidative stress. Clin Chim Acta 2006;367:132-6.

[42] Paolisso G, D'Amore A, Balbi V, Volpe C, Galzerano D, Giugliano D, et al. Plasma vitamin C affects glucose homeostasis in healthy subjects and in non–insulin-dependent diabetics. Am J Physiol 1994;266(2 Pt 1):E261-8.

[43] Maziere C, Morliere P, Santus R, Marcheux V, Louandre C, Conte MA, et al. Inhibition of insulin signaling by oxidized low density lipoprotein. Protective effect of the antioxidant Vitamin E. Atherosclerosis 2004;175:23-30.

[44] Skyrme-Jones RA, Meredith IT. Soluble adhesion molecules, endothelial function and vitamin E in type 1 diabetes. Coron Artery Dis 2001; 12:69-75.

[45] Neri S, Signorelli SS, Torrisi B, Pulvirenti D, Mauceri B, Abate G, et al. Effects of antioxidant supplementation on postprandial oxidative stress and endothelial dysfunction: a single-blind, 15-day clinical trial in patients with untreated type 2 diabetes, subjects with impaired glucose tolerance, and healthy controls. Clin Ther 2005;27:1764-73.

[46] Desideri G, Croce G, Tucci M, Passacquale G, Broccoletti S, Valeri L, et al. Effects of bezafibrate and simvastatin on endothelial activation and lipid peroxidation in hypercholesterolemia: evidence of different vascular protection by different lipid-lowering treatments. J Clin Endocrinol Met 2004;89:1978.

[47] Frei B. On the role of vitamin C and other antioxidants in atherogenesis and vascular dysfunction. Proc Soc Exp Biol Med 1999;222:196-204.

[48] Tahir M, Foley B, Pate G, Crean P, Moore D, McCarroll N, et al. Impact of vitamin E and C supplementation on serum adhesion molecules in chronic degenerative aortic stenosis: a randomized controlled trial. Am Heart J 2005;150:302-6.

[49] Rao TP, Sakaguchi N, Juneja LR, Wada E, Yokozawa T. Amla (Emblica officinalis Gaertn.) extracts reduce oxidative stress in streptozotocin-induced diabetic rats. J Med Foods 2005;8:362-8.