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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

Objective—To determine whether short-term antioxidant supplementation affects insulin sensitivity, endothelial adhesion molecule levels, and oxidative stress in overweight young adults.

Methods and Procedures—A randomized, double-blind, controlled study tested the effects of antioxidants (AOX) on measures of insulin sensitivity (homeostasis model assessment, HOMA and QUICKI), endothelial adhesion molecules (sICAM-1, sVCAM-1, sE-selectin), adiponectin and oxidative stress (lipid hydroperoxides, PEROX) in overweight and normal weight individuals (N=48, 18-30 years). Participants received either AOX (vitamin E 800IU, vitamin C 500mg, β -carotene 10mg) or placebo (PLC) for 8 weeks.

Results—HOMA values were initially higher in the overweight subjects and were lowered with AOX by week 8 (15% reduction, $p=0.02$). Adiponectin increased in both AOX groups. sICAM-1 and sE-selectin decreased in overweight AOX treated groups by 6% and 13%, respectively ($p<0.05$). Plasma PEROX were reduced by 0.31 and 0.70 nmol/ml in the normal weight and overweight AOX treated groups, respectively, by week 8 ($p<0.05$).

Discussion—AOX 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 AOX are effective in suppressing diabetes or vascular activation over time.

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Keywords

obesity; antioxidant; inflammation; adiponectin

Introduction

Insulin resistance and endothelial dysfunction are two reciprocally related processes that precede metabolic and cardiovascular disease.(1) While 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, anti-atherogenic 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 defense compared with normal weight.(4) Tissue antioxidant levels are lower in obese than normal weight adults.(7, 8) Also, total antioxidant status is lower in obesity, and this antioxidant imbalance favors systemic oxidative stress.(4) Dietary antioxidants may attenuate oxidative stress. However, a controversy exists in the literature whether antioxidant 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 against oxidative stress, CAM expression, 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 antioxidant 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 facts that the major dietary antioxidants work together within intracellular antioxidant defense systems(23) and may be most effective when administered together instead of one antioxidant 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 antioxidant supplementation may not have been optimal for the specific cardiovascular populations studied.(22) One possibility is that antioxidants may not have been used early enough to offer protection before disease fulminates. Therefore, several questions remain unanswered regarding the efficacy of antioxidants 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 vitamins E, C and β -carotene can attenuate pre-disease 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 antioxidant supplementation on insulin sensitivity, CAM levels and oxidative stress in overweight young adults compared to normal weight controls.

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, newspaper, institutional internet sites and clinic advertisements from the Central Virginia region. All participants had to meet the following criteria prior to 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 antioxidant supplements within the past 6 months, no current usage 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).

Participants and Study Groups

After the investigators had determined BMI values, participants were stratified into normal weight or overweight groups [normal weight, ($BMI < 25 \text{ kg/m}^2$) and overweight and obese ($BMI \geq 25 \text{ kg/m}^2$)]. Within each group, participants were randomized by the UVA investigational pharmacist to receive either antioxidant (AOX) treatment or placebo. Hence, there were a total of four participant groupings: normal weight, antioxidant-treated (N-AOX), normal weight, placebo (N-PL), overweight antioxidant-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 either did not meet all inclusion criteria, were too old, or who were beginning a new weight loss program at the same time. A total of 65 participants enrolled into the study; 11 were withdrawn due to overcommitted schedules, failure to comply with the study visits, failure to respond to investigator contact and child care issues.

Antioxidant 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 placebo were administered by the UVA investigational pharmacist in opaque bottles, and were taken once a day. Each supplement was provided in an individual capsule forms; 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 eight tocopherols and tocotrienols found naturally in food. The content of each antioxidant 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 antioxidants 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 three times (two pre-intervention, one post-intervention). During visit one, 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 two, aerobic fitness levels ($\text{VO}_{2\text{peak}}$) were measured using a load-incremented cycle ergometer protocol. Visit 3 was a repeat visit of 1 and 2 combined that occurred after the supplementation period. Visits 1 and 2 were conducted within one week.

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 ACSM.(31) Body mass index (BMI) values were determined by the following: $\text{BMI} = \text{weight}(\text{kg})/\text{height}(\text{m})^2$. Body volume was estimated using air displacement plethysmography in a BodPod® device (Bod-Pod, Life Measurement Instruments, Concord, CA) corrected for thoracic gas volume; body density was calculated and used to predict body fat using the Siri equation.(32)

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 (v 2.1.13, First DataBank, San Bruno CA) and were analyzed for macronutrient, antioxidant and caloric intake. To ensure the stability of the habitual diet of the subjects, all subjects completed a second three-day dietary record eight weeks later.(33) To further improve dietary stability, we performed two 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 two 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.

Peak Oxygen Consumption ($\text{VO}_{2\text{peak}}$)/Lactate Threshold (LT) test—A peak oxygen consumption 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 peak oxygen consumption ($\text{VO}_{2\text{peak}}$)/lactate threshold (LT) test on an electronically braked cycle ergometer (Ergo Metrics 800S; Sensor Medics, Yorba Linda, CA). The initial power output (PO) was set at 20 W, and the PO was increased 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 (HLA)(model 2700, YSI Instruments, Yellow Springs, OH). Heart rates (HR), blood pressures and rating of perceived exertion (RPE) were collected at every exercise stage.(31) Metabolic data were collected during both the $\text{VO}_{2\text{peak}}$ test using standard open-circuit spirometric techniques (Vmax 229; Sensor Medics, Yorba Linda, CA). HR was determined electrocardiographically (Marquette Max-1 electrocardiograph, Marquette, WI).

Blood Sampling—Fasting blood samples were collected from a catheter from an antecubital vein into heparinized vacutainer tubes during visits 1 and 3. Blood samples were analyzed for glucose, insulin, 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 $1,500 \times g$ 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 General

Clinical Research Center at UVa (UVa GCRC). 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. PEROX assays were performed by a member of the investigational team (HKV).

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: $1/(\log(\text{fasting glucose}) + \log(\text{fasting insulin}))$.⁽³⁶⁾

Lipid Peroxidation Measurements—PEROX 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 chromophore within the sample that was read at 580nm.⁽³⁷⁾ All samples were performed in triplicate in a single batch analysis. The coefficient of variation for this assay was 4%.

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

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), triglycerides) were analyzed using standard automated spectrophotometric laboratory procedures (Olympus AU640, Olympus calibrator Cat #DR0040 and Genzyme HDL-C calibrator Cat#80-4529-00). Low density lipoproteins (LDL-C) were estimated from the following equation: $LDL-C = \text{total cholesterol} - HDL-C - (\text{triglycerides}/5)$. Blood glucose was assessed using an Olympus AU640 procedure, in which glucose was phosphorylated by hexokinase in the presence of ATP and magnesium. The resultant glucose-6-phosphonate (G-6-P) dehydrogenase oxidized G-6-P to 6-phosphogluconate and reduced nicotinamide adenine dinucleotide (NAD⁺) to NADH. The change in absorbance at 340/380 was proportional to the amount of glucose in the sample (Olympus Glucose Reagent, Calibrator Cat# DR0040). HbA_{1c} was analyzed using automated high performance liquid chromatography (Tosoh G7 Automated HPLC Analyzer, using TSKgel G& HSi elution columns). All samples were performed in duplicate.

Statistics—All data are expressed in mean $\pm$ standard error (SE). Data were analyzed using Statistical Package for the Social Sciences (v. 12.0, Chicago, IL). Percent changes (percent change from baseline) were calculated for several blood measures. Descriptive variables were analyzed using a two way analysis of variance (ANOVA). If differences did not exist between groups at baseline, repeated measures ANOVAs were performed for the change scores (Δ values from pre-post CLPO exercise at baseline and 8 weeks) for the inflammatory cytokines, blood lipids and PEROX. The between group factors were

adiposity status (non-obese, overweight) and treatment (antioxidants, placebo) and the within group factor was time (pre and post-exercise, baseline and 8 weeks). When baseline differences existed for blood measures, two way analyses of covariance (ANCOVAs) were performed using the baseline value as the covariate. The between group factors were adiposity status (non-obese, overweight) and treatment (antioxidants, placebo). The level of significance was set at 0.05 for all statistical tests.

Results

Subject Characteristics at Baseline—As expected, several indices of body composition were significantly different between the normal weight and overweight group (all $p < 0.05$; Table 1). Aerobic fitness was lower in the overweight group ($p < 0.0001$). Fasting measures of blood glucose were not different between groups at baseline, but HOMA and QUICKI scores were different in the overweight group than in the normal weight group ($p < 0.024$). LDL-C concentrations were higher in the overweight group than the normal weight group ($p < 0.05$). sICAM-1 and sE-selectin tended to be higher in the overweight group than the normal weight group ($p = 0.077$).

Dietary Intake—Correlation values (r) between specific macro and micronutrient intake between the first and second dietary records ranged from 0.7-0.93, 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 ($p < 0.05$). Copper intakes were (in mg) 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; $p < 0.05$). Manganese intakes were (in mg) 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.

Post- AOX supplementation—There were no changes in body weight, fat or fat free mass, waist to hip ratio (WHR) after the treatment period in either the placebo or AOX group. No significant changes occurred in exercise capacity (VO_{2peak}) or cholesterol profiles by week 8 of the intervention in any experimental group, indicating that these potential confounders remained stable from pre- to post-intervention.

Insulin Sensitivity Related Variables—While 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 ($p = 0.107$; Figure 1a). Relative to participants treated with placebo, HOMA scores in the AOX group were significantly lower in the overweight group at week 8 ($p = 0.02$; Figure 1c). 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. 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 ($p = 0.407$). Fasting glucose levels were not different between groups or over time ($p = 0.973$; Figure 1b).

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 ($p = 0.013$). sE-selectin and sICAM-1 concentrations also decreased over time in participants treated with AOX, although these reductions were significant only in the overweight group ($p = 0.023$, 0.14 , respectively). Adiponectin

increased in normal weight and overweight participants treated with AOX, while the placebo treated groups demonstrated decreases in adiponectin over time ($p=0.05$).

Discussion

The combination of vitamins C, E and β -carotene during an 8 week supplementation period moderately reduced HOMA values, and 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 antioxidant administration.

Previous supplementation studies of individual antioxidants 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 antioxidants do not work optimally in isolation, but rather serve as part of an antioxidant system where optimal protection against disease processes occurs with several antioxidants together, as found in natural foods. In the present study, the combination of vitamins E, C and β -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 antioxidants work in concert to act at several sites in the insulin metabolism pathways. For example, vitamin C supplementation increases antioxidant defenses in tissue (41) and potentiates insulin action and 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 I diabetics 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 antioxidants (e.g., 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 antioxidants. Supplementation with antioxidants such as vitamins E, C or β -carotene are purported to either squelch free radicals such as superoxide, directly within the endothelium, to disrupt TNF- α intracellular signaling cascades, or to reduce oxidative modification of LDL-C, all of which would otherwise initiate expression of endothelial adhesion molecules.(47)

AOX significantly lowered sICAM-1 and sE-selectin in the healthy young overweight adults in this study, but not normal weight adults. While 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 (800-1200IU/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 antioxidants 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)

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 inter-individual 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 (e.g., fewer fresh foods) in the overweight groups during the time frame of the dietary record. Also, 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.

Conclusion

Short term AOX supplementation moderately lowers HOMA and endothelial adhesion molecule levels and increased 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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Abbreviations

HOMA	homeostasis model assessment
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AOX	antioxidant
sICAM	soluble intercellular adhesion molecule-1
sVCAM-1	vascular adhesion molecule
sE-selectin	endothelial-leukocyte adhesion molecule-1

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Figure 1a.

Fasting insulin values for normal weight and overweight young adults treated with antioxidants (AOX, vitamins E, C and β -carotene) or placebo (PL) at baseline and 8 weeks. Values are means $\pm$ SE.

Figure 1b.
HOMA values for normal weight and overweight young adults treated with antioxidants (AOX, vitamins E, C and β -carotene) or placebo (PL) at baseline and 8 weeks. Values are means $\pm$ SE.

Figure 1c.

Fasting plasma glucose concentrations for normal weight and overweight young adults treated with antioxidants (AOX, vitamins E, C and β -carotene) or placebo (PL) at baseline and 8 weeks. Values are means $\pm$ SE.

Table 1Subject characteristics at baseline. Values are means $\pm$ SE.

	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	22 $\pm$ 1	22 $\pm$ 1	23 $\pm$ 1	27 $\pm$ 1
Height (cm)	174 $\pm$ 3	170 $\pm$ 2	171 $\pm$ 3	171 $\pm$ 2
Weight (kg)	69 $\pm$ 3	62 $\pm$ 2	94 $\pm$ 6 *	100 $\pm$ 7 *
Fat mass (kg)	14 $\pm$ 1	14 $\pm$ 1	46 $\pm$ 5 *	48 $\pm$ 6 *
Fat free mass (kg)	55 $\pm$ 23	48 $\pm$ 2	57 $\pm$ 4	55 $\pm$ 4
BMI (kg/m ²)	23 $\pm$ 1	21 $\pm$ 1	32 $\pm$ 2	34 $\pm$ 2
Waist circumference (cm)	76 $\pm$ 2	70 $\pm$ 2	96 $\pm$ 4 *	100 $\pm$ 4 *
WHR	0.76 $\pm$ 0.1	0.74 $\pm$ 0.1	0.83 $\pm$ 0.1 *	0.85 $\pm$ 0.1 *
VO ₂ peak (ml/kg [*] min)	33 $\pm$ 2	31 $\pm$ 2	21 $\pm$ 2 *	19 $\pm$ 1 *
Glucose (mmol/L)	4.4 $\pm$ 0.1	4.4 $\pm$ 0.1	4.9 $\pm$ 0.3	5.0 $\pm$ 0.3
HbA _{1c} (%)	5.0 $\pm$ 0.1	5.0 $\pm$ 0.1	4.9 $\pm$ 0.1	5.0 $\pm$ 0.1
Insulin (μ U/ml)	5.6 $\pm$ 0.7	5.9 $\pm$ 0.4	11.7 $\pm$ 1.8 *	11.6 $\pm$ 4.0 *
HOMA	1.1 $\pm$ 0.1	1.2 $\pm$ 0.1	2.6 $\pm$ 0.5 *	2.6 $\pm$ 0.8 *
QUICKI	0.39 $\pm$ 0.1	0.38 $\pm$ 0.1	0.35 $\pm$ 0.1 *	0.33 $\pm$ 0.2 *
Total cholesterol (mmol/L)	4.3 $\pm$ 0.1	3.8 $\pm$ 0.2	4.0 $\pm$ 0.3	4.3 $\pm$ 0.3
HDL-C (mmol/L)	1.5 $\pm$ 0.2	1.4 $\pm$ 0.3	1.2 $\pm$ 0.3	1.2 $\pm$ 0.4
LDL-C (mmol/L)	2.4 $\pm$ 0.4	2.0 $\pm$ 0.6	2.3 $\pm$ 0.7 *	2.7 $\pm$ 0.8 *
Triglycerides (mmol/L)	0.8 $\pm$ 0.4	0.9 $\pm$ 0.5	1.3 $\pm$ 0.8	1.0 $\pm$ 0.6

* different from normal weight group at p<0.05; BMI = body mass index; WHR = waist to hip ratio; SBP; VO₂peak = peak rate of oxygen consumption; PL = placebo; AOX = antioxidant treated

Table 2

Average dietary intakes of major macronutrient normal weight and overweight groups by AOX treatment. Values are means $\pm$ SE.

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

* different from N-PL at $p < 0.05$

Table 3

Fasting measurements of oxidative stress, vascular inflammation and adiponectin in normal weight and overweight groups by AOX treatment at baseline. Change values are the raw differences between baseline and week 8 values for each measure. Values are means $\pm$ SE.

	Normal Weight			Overweight		
	AOX (n=12)		PL (n=13)	AOX (n=12)		PL (n=11)
	Baseline	Week 8	Baseline	Baseline	Week 8	Baseline
PEROX	2.7 $\pm$ 0.2	2.3 $\pm$ 0.2	2.6 $\pm$ 0.2	2.5 $\pm$ 0.2	2.2 $\pm$ 0.2	2.6 $\pm$ 0.2
change		-0.3 $\pm$ 0.2 *	0.6 $\pm$ 0.4		-0.7 $\pm$ 0.3 *	0.1 $\pm$ 0.1
sE-selectin	37 $\pm$ 5	39 $\pm$ 5	48 $\pm$ 4	52 $\pm$ 8	45 $\pm$ 5 *	49 $\pm$ 6
change		-1 $\pm$ 2	-1 $\pm$ 1		-9 $\pm$ 3 *	-2 $\pm$ 6
sICAM-1	220 $\pm$ 13	209 $\pm$ 13	213 $\pm$ 14	231 $\pm$ 15	216 $\pm$ 14 *	254 $\pm$ 18
change		-12 $\pm$ 8	0.5 $\pm$ 0.8		-14 $\pm$ 5 *	-0.2 $\pm$ 6
sVCAM-1	546 $\pm$ 45	524 $\pm$ 46	551 $\pm$ 42	570 $\pm$ 42	537 $\pm$ 42	676 $\pm$ 45
change		-25 $\pm$ 12	9 $\pm$ 32		-33 $\pm$ 26	-10 $\pm$ 24
Adiponectin	9,730 $\pm$ 1,242	11,339 $\pm$ 1,286	9,903 $\pm$ 1,189	9,103 $\pm$ 1,142	9,534 $\pm$ 1,182	9,157 $\pm$ 1,302
change		1,748 $\pm$ 629 *	-751 $\pm$ 938		194 $\pm$ 446 *	-495 $\pm$ 583

Lipid hydroperoxides (PEROX) expressed as nmol/ml; sE-selectin, sICAM-1, sVCAM-1 and adiponectin are expressed in ng/ml

* different from PL groups at $p < 0.05$