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Effect of vitamins C and E on biomarkers of oxidative stress in nonsmokers

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Abstract

Background—Oxidative stress is elevated in obesity, and may be a major mechanism for obesity-related diseases.

Methods—Nonsmokers (n=396) were randomized to 1000 mg/day vitamin C, 800 IU/day vitamin E, or placebo, for two months. Treatment effect was examined in multiple regression analyses using an intention-to-treat approach.

Results—Vitamin C (p=0.001) and vitamin E (p=0.043) reduced plasma F₂-isoprostanes. In the overall sample, changes from baseline were +6.8%, −10.6%, and −3.9% for placebo, vitamin C, and vitamin E groups, respectively. However, a significant interaction with baseline F₂-isoprostane was found. When baseline F₂-isoprostane was > 50 µg/mL, vitamin C reduced F₂-isoprostane by 22% (p=0.01). Vitamin E reduced it by 9.8% (p=0.46). Below that cut-point, neither treatment produced further reductions. F₂-isoprostane > 50 µg/mL was strongly associated with obesity, and was present in 42% of the sample. Change in malondialdehyde concentration was minimal.

Discussion—These findings suggest a role for vitamin C in reducing lipid peroxidation. Future research on effects of vitamins C or E on plasma F₂-isoprostane should limit participants to those with baseline levels > 50 µg/mL. Further studies are needed to establish whether treatment with vitamins C or E in persons with concentrations above that cut-point could slow the development of cardiovascular disease.

Keywords

Antioxidants; biomarkers; F₂-isoprostane; obesity; oxidative stress; vitamin C; vitamin E; oxidative stress

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INTRODUCTION

Oxidative stress can lead to damage of biomolecules such as lipids, proteins, and DNA [1]. Elevated oxidative stress can be caused by exogenous exposures including ozone and cigarette smoke [2–5], but perhaps more important, is produced endogenously as a result of overweight and obesity [6]. Numerous studies have found elevated biomarkers of oxidative stress in obesity, in cross-sectional, longitudinal and animal studies [6,7]. Keaney et al., in an analysis of the Framingham data [8], found oxidative stress to be associated with body mass index and other body composition measures, even after controlling for several major risk factors including age, smoking and history of cardiovascular disease. A number of authors have suggested oxidative stress may be the unifying mechanism in the pathway from obesity to obesity-related diseases [9–11]. Oxidative stress also has increasingly been implicated as a causal factor in the pathophysiology of atherosclerosis [12,13], diabetes mellitus [12,13], chronic obstructive pulmonary disease [14], and Alzheimer's disease [15].

The oxidation of cellular lipids, typically referred to as lipid peroxidation, is an important consequence of oxidative stress. One biomarker of lipid peroxidation, F₂-isoprostanes, are prostaglandin-like compounds formed non-enzymatically as a result of the free-radical-mediated peroxidation of the polyunsaturated fatty acid, arachidonic acid [16,17]. They are formed *in situ*, esterified to phospholipids, and subsequently released by phospholipases into the plasma, where they can be measured [18]. Quantification of urinary or plasma F₂-isoprostanes, also referred to as 8-iso-prostaglandin F₂ α , has emerged as an accurate method to assess *in vivo* oxidant stress in humans and animals.

We previously reported a significant reduction in plasma F₂-isoprostanes among nonsmokers passively exposed to cigarette smoke following treatment with 500 mg/day vitamin C or an antioxidant mixture that included both vitamin C and vitamin E [19]. Among smokers we found that vitamin C reduced plasma F₂-isoprostanes, and that the effect was modified by body mass index (BMI) such that a greater magnitude reduction was seen in those with a BMI above the sample median of 26.6 kg/m² [20]. However, the antioxidant combination that included vitamins C and E had no significant impact on F₂-isoprostanes in smokers. Finally, we also observed a significant reduction in another marker of lipid peroxidation, plasma malondialdehyde (MDA), after treatment with vitamin C but not the antioxidant mixture, in active and passive smokers combined. (Block, unpublished data, 2002).

The present study was designed to follow up on those observations, in nonsmokers. In this randomized, placebo-controlled parallel-design study, we examined the separate effects of 1000 mg/day vitamin C and 800 IU/day vitamin E over two months on plasma F₂-isoprostanes and MDA among healthy nonsmokers. Based on our previous work, we hypothesized a greater effect of vitamin C than of vitamin E.

MATERIALS AND METHODS

Participants

Participants were recruited between January 2005 and March 2006 from the communities of San Francisco, Berkeley and Oakland, CA, via postings to internet sites, newspaper advertisements, flyers, health fairs, and by mailings to specific list-serves of the University of California at Berkeley (UCB) and Children's Hospital & Research Center of Oakland, CA (CHRCO). Exclusion criteria included age (<18 years), smoking, passive smoke exposure (exposed indoors $\geq$ 5 days/week), alcohol consumption ($\geq$ 2 drinks/day), pregnancy or breastfeeding, disease conditions (hemochromatosis, history of kidney stones or other kidney diseases, cancer, stroke, diabetes mellitus, human immunodeficiency virus infection), use of certain prescription medications (anti-inflammatory, blood-thinning, statin, lipid-lowering,

hormone replacement therapy, or steroid medications), consumption of single iron supplements or of vitamin E supplements in dosages > 400 IU/day, and body weight $\geq$ 300 pounds or height > 75 inches (due to equipment constraints). Those taking multivitamin supplements, vitamin C, lower-dose vitamin E supplements, or over-the-counter anti-inflammatory medications were given the option of participating if they discontinued use of such agents for 30 days prior to their baseline visit and throughout the two-month intervention.

Of 1535 subjects assessed for eligibility, 1139 were ineligible or declined to participate, 396 were enrolled, and 385 completed the study (Figure 1). Of the 11 who terminated early (7, 3, and 5 participants in the placebo, vitamin C, and vitamin E groups, respectively), three withdrew due to inconvenience or unwillingness to refrain from taking other dietary supplements, four were lost to follow-up, one died in an automobile accident, and three were withdrawn administratively. All 396 participants are included in the intention-to-treat analysis. The study design was approved by the institutional review boards of UCB and CHRCO. Signed informed consent was obtained from all participants.

Study supplements and allocation

Participants were assigned to one of three different treatment groups using blocked, stratified randomization, and a randomization sequence of letters of the alphabet representing treatment groups was generated by computer program. Randomization was stratified by gender, weight (men: < 68 kg, 68–82 kg, and > 82 kg; women: < 54 kg, 54–68 kg, > 68 kg), and either menopausal status (pre-, peri-, or post-menopausal) for women, or age (18–44 years, 45–59 years, or $\geq$ 60 years) for men. Coded bottles containing treatment components were provided to participants by clinical staff unaware of treatment allocation. The three treatment arms were 1) vitamin C tablet (1000 mg/day ascorbic acid) and placebo capsule; 2) vitamin E capsule (800 IU/day all-natural mixture of *RRR* d-alpha tocopherol) and placebo tablet; and 3) placebo tablet and placebo capsule. Placebo tablets contained lactose, microcrystalline cellulose, and small amounts of stearic acid, magnesium stearate, and croscarmellose sodium. Placebo capsules contained medium consisting of lecithin and soybean oil. Vitamin C tablets and vitamin E capsules were indistinguishable from placebo tablets and capsules. Participants were instructed to take the tablets/capsules twice daily, with meals. Study supplements and placebos were provided by Pharmavite, LLC (Mission Hills, CA). Investigators, clinic and laboratory staff, and participants were masked to treatment assignment. Separate study staff administered the intervention and analyzed data.

Procedures

Pre- and peri-menopausal women were tested for pregnancy and excluded if pregnant (Quidel QuickVue Semi-Q hCG-Combo Test). Anthropometric measurements were obtained by nurses and technicians who underwent extensive initial training and mid-study refreshers. Body weight (Health-O-Meter digital electronic) was measured without shoes, jackets, or heavy sweaters. Height was measured without shoes, using a wall-mounted stadiometer (Perspective Enterprise). Height and weight measurements were repeated until two measurements in succession were within 0.5 cm and 0.3 kg, respectively. Waist and hip circumference (Fiberglass, Gulick II) were measured using the National Heart, Lung, and Blood Institute's published guidelines [21]. Hip circumference was measured using fiberglass tape placed over the participant's underwear at the point yielding the maximum circumference over the buttocks. Sagittal abdominal diameter was measured with abdominal calipers (Holtain-Kahn) at the location of maximal abdominal diameter (directly above the iliac crest), with participants wearing sweatshirt or hospital gown and in the supine position. These latter three measurements were repeated until two consecutive measurements were within 1.0 cm. Body fat was assessed by a whole body scan, at baseline only, using dual energy x-ray absorptiometry (DXA)

(Hologic, Delphi A, Model QDR 4500A (S/N 4500); Serial No: 70580; Software: QDR system software version 11.2).

Fasting venous blood was drawn into Vacutainer tubes (Beckton Dickinson, Rutherford, NJ), protected from light, maintained at $<15^{\circ}\text{C}$, and processed within 6 hours. Studies have shown that F_2 -isoprostane levels are not elevated after several hours in whole blood on ice [22]. Vacutainers were centrifuged at 5°C for 10 minutes at $1,200 \times g$ and aliquoted. Plasma aliquots for ascorbic acid were mixed 1:1 with freshly-prepared 10% (w/v) meta-phosphoric acid to stabilize the ascorbic acid. All aliquots were protected from light and stored at -80°C for up to one year before assay for F_2 -isoprostane, MDA, α -tocopherol, ascorbic acid and other analytes. F_2 -isoprostane is stable at -80°C for up to 10 years [22]. All sample batches included masked duplicates and replicated internal control samples.

At both the baseline and follow-up visits, participants completed a questionnaire related to their health and symptoms. At the follow-up clinic visit, unused capsules were counted to assess adherence, and participants completed a questionnaire related to the adequacy of masking.

Laboratory methods

Pre- and post-treatment aliquots were assayed in the same batch to reduce laboratory artifact. F_2 -isoprostanes in plasma were quantitated in the laboratory of Dr. Jason Morrow, after purification and derivatization, by selected ion monitoring gas chromatography/negative ion chemical ionization-mass spectrometry (GC/NICI-MS) employing $[\text{}^2\text{H}_4]\text{8-iso-prostaglandin F}_{2\alpha}$ as an internal standard [22]. Plasma malondialdehyde was determined using lipid peroxidation analysis kits (Oxis International, Inc., Portland, Oregon). Briefly, MDA concentrations in plasma were obtained by 1) fitting 3rd degree polynomial regression lines to each sample's absorption spectra (530–610 nm), 2) deriving the 3rd derivative of each regression line with respect to x, 3) solving for MDA concentration using a linear equation that expressed the relationship between the 3rd derivative values for a set of standards as a function of concentration. This technique provided estimates for total MDA that are similar to levels obtained using HPLC [23–26].

Plasma alpha tocopherol was measured by reversed-phase HPLC with detection at 292 nm [27]. Plasma ascorbic acid was measured spectrophotometrically using 2,4-dinitrophenylhydrazine as chromogen [28], a method that has been shown to correlate highly with HPLC analysis [29–32]. Plasma lipids were measured using timed-endpoint, coupled enzymatic methodology [33].

Statistical analysis

Differences in baseline measurements by treatment group were assessed using chi-square tests for categorical variables and analysis of variance tests for continuous variables. Treatment effect was assessed using multiple regression techniques, with change in plasma F_2 -isoprostanes or MDA from baseline to 2 months as the dependent variable. Baseline values of the oxidative stress biomarkers were included in all models to control for regression to the mean. Interaction was assessed by including the relevant cross-product term in the model. Statistical models for change in the biomarkers were examined using both untransformed and log-transformed dependent (change) variables. Since conclusions were similar using either form of the dependent variable, only results for untransformed variables are presented, for ease in interpretation. In intention-to-treat analyses, participants with missing baseline or follow-up values due to laboratory or processing problems (F_2 -isoprostanes, $n=40$; MDA, $n=13$) were given a change score of zero. This is a stringent analysis, the effect of which is to bias results toward “no effect”, but also to protect against unexpected positive biases that could result from exclusion of some subjects.

RESULTS

The mean age of participants was 44 years, and 34.6% were male (Table 1). The treatment groups did not differ with respect to gender, ethnicity, age, BMI, plasma ascorbic acid, alpha-tocopherol, total or HDL cholesterol, triglycerides, or MDA concentrations at baseline, but did differ with respect to plasma F₂-isoprostane concentration and low-density lipoprotein cholesterol (LDL). Baseline F₂-isoprostane concentration was substantially lower in the vitamin E group compared to the vitamin C group. LDL and baseline F₂-isoprostane are included in subsequent models to control for potential confounding and regression to the mean.

Adherence, defined as having taken 90% of prescribed pills, was 87% overall and did not differ by treatment group ($p=0.41$). Plasma ascorbic acid changes were 3.8%, -1.7% and $+49.3\%$ in the placebo, vitamin E, and vitamin C treatment groups, respectively (data not shown). Changes in plasma alpha-tocopherol were 11.9%, 103.3% and 5.2% in the placebo, vitamin E, and vitamin C treatment groups, respectively. The percent who correctly identified their treatment group was 16.5%, 19.5% and 14.4% for placebo, vitamin C and vitamin E treatment groups respectively.

In unadjusted analyses, treatment with vitamin C but not vitamin E resulted in a statistically significant decrease in F₂-isoprostanes (table 2, top). Among all subjects combined, F₂-isoprostane concentration declined by 13.8% in the vitamin C group ($p=0.002$).

We examined the possibility of statistical interaction, that is, the possibility that treatment effect may differ depending on the level of a third variable. The treatment effect on F₂-isoprostanes was not modified by plasma lipid variables. For example, the p value for the interaction between LDL and treatment group was $p=0.77$. This indicates that treatment effects did not differ depending on baseline LDL. Interaction was found for BMI as a continuous variable (but not when categorized as Normal, Overweight, Obese) and baseline F₂-isoprostane. Further exploration suggested that the interaction with BMI was due to its strong relationship with baseline F₂-isoprostanes. For example, baseline F₂-isoprostanes were significantly higher in the obese ($69.2 \mu\text{g/mL}$) compared to normal or overweight ($51.9 \mu\text{g/mL}$), $p=0.001$ (data not shown). Further analysis focused on the interaction with baseline F₂-isoprostanes.

Figure 2 shows the varying treatment effect by baseline F₂-isoprostane concentration, over quintiles of baseline F₂-isoprostanes. The variation within the placebo group reflects the impact of regression to the mean: subjects with low baseline values have a tendency to increase, and those with high baseline values have a tendency to decrease, even in the absence of any treatment.

Figure 2 indicates that treatment was effective in the top two quintiles, or baseline F₂-isoprostane concentrations of $53 \mu\text{g/mL}$ or above, and especially in the fifth quintile, baseline F₂-isoprostane concentrations of $74 \mu\text{g/mL}$ or above. For greater generalizability to other samples, we suggest a cut-point of $50 \mu\text{g/mL}$ as indicating elevations potentially susceptible to reductions by vitamins C or E. Presence of this concentration F₂-isoprostanes indicating susceptibility to intervention was strongly associated with body mass index (Figure 3). Among the obese, 62.3% had F₂-isoprostane concentrations greater than $50 \mu\text{g/mL}$.

Further examination of unadjusted changes by this cut-point (table 2, bottom) indicated non-significant increases in F₂-isoprostanes in the vitamin C and vitamin E treatment group participants with baseline concentrations $\leq 50 \mu\text{g/mL}$. In those with baseline concentrations $> 50 \mu\text{g/mL}$, treatment with vitamin C resulted in more than a 25% reduction in F₂-isoprostanes. Treatment with vitamin E also reduced F₂-isoprostanes modestly but not significantly.

An intention-to-treat analysis in which participants with missing data were assigned a change score of zero was conducted, with adjustment for baseline F₂-isoprostanes and LDL. Both vitamin C and vitamin E were effective in reducing plasma F₂-isoprostane concentrations when all participants are included (Table 3, top). F₂-isoprostanes decreased 10.6% and 3.9% in the vitamin C and vitamin E groups, respectively (p=0.001 and 0.046 in vitamin C and E respectively for contrast with change in placebo).

Because of the significant interaction with baseline F₂-isoprostanes, treatment effects are also shown for the subgroups defined by the cut-point of 50 µg/mL (Table 3, bottom). In the subgroup with baseline concentration > 50 µg/mL, treatment with vitamin C decreased F₂-isoprostanes by 22.05%, p=0.01. The decrease of 9.81% in the vitamin E group was not significant (p=0.46). In contrast, in the subgroup with baseline concentrations ≤ 50 µg/mL, F₂-isoprostanes increased in all three treatment groups, and the effect of vitamin C and E was to minimize the rise in F₂-isoprostanes in this group with initially low concentrations.

We conducted several additional analyses to examine the possibility that the results seen in Table 3 were due to the differences in LDL across treatment groups seen in Table 1, or were due to treatment effects on LDL. First, we examined whether *change* in LDL differed across treatment groups; it did not (p=0.62). Second, correlations between change in F₂-isoprostanes and change in LDL was examined. Change in F₂-isoprostane was not correlated with change in LDL (r=0.02, 0.03, 0.11 in Vitamin C, Vitamin E and Placebo respectively.) Third, we repeated the intent-to-treat analysis in Table 3, adjusting for change in LDL instead of for baseline LDL. Adjusting for change in LDL instead of for baseline LDL did not change the results. For example, in the vitamin C group change in F₂-isoprostane was significant at p=0.001 in contrast with change in placebo, compared with the p=0.003 seen in Table 3 for all subjects. The significance of the effect in the vitamin E group was p=0.046 compared with the p=0.043 seen in Table 3 for all subjects. Finally, we repeated the Table 3 analyses, using a “lipid-adjusted” F₂-isoprostane variable (F₂-isoprostane divided by LDL). Again, results were unchanged. The significance of change in F₂-isoprostane compared with change in Placebo was p=0.001 and p=0.02 for vitamin C and vitamin E respectively, among all subjects. Conclusions were also unchanged when the group above > 50 µg/mL was examined. In that subgroup, using the ratio variable, change in F₂-isoprostane was significant in the vitamin C group (p=0.018) and not in the vitamin E group (p=0.38). These results are similar to the findings in Table 3, in which the p values were 0.01 and 0.46. Thus, these analyses indicate that the treatment effects on F₂-isoprostanes are not artifacts related to LDL.

In an intention-to-treat analysis for plasma MDA, adjusted for baseline MDA, change in MDA in the vitamin C group in comparison to placebo was statistically significant, p=0.047 (Table 4). However, this treatment effect was due to the contrast between the large increase in the placebo group and the small (<1%) decrease in the vitamin C group. As in the F₂-isoprostane analysis, the treatment effect differed depending on baseline MDA. In persons in the top quintile (MDA ≥ 1.3 µmol/L), reductions in the vitamin C, vitamin E and placebo groups were -0.70, -0.08 and -0.21 µmol/L respectively, but this did not reach significance (p=.10) (data not shown).

DISCUSSION

In this placebo-controlled trial of healthy, nonsmoking individuals, 1000 mg/day vitamin C for two months reduced *in vivo* lipid peroxidation as measured by plasma F₂-isoprostanes by 10.56% (p=0.001) in the total sample and by 22% (p=0.01) in the group with baseline F₂-isoprostane > 50µg/mL. Treatment with 800 IU/day vitamin E produced a relatively attenuated lowering effect on plasma F₂-isoprostanes, 3.94% in the total sample (p=0.04) and 9.81% in the group with elevated baseline F₂-isoprostanes which did not reach significance (p=0.46).

Although treatment with vitamin E produced a lesser effect, it is notable that there were substantially fewer participants with F₂-isoprostane > 50 µg/mL in the vitamin E group than in the Vitamin C group (tables 1 and 2). This could have weakened the ability to detect a treatment effect of vitamin E. On the other hand, if the lesser effectiveness of 800 IU/day vitamin E is real, it may be relevant to the inconsistent effects seen in vitamin E observational and intervention studies [34].

This analysis has shown the impact of regression to the mean. In any dataset, initially low values will tend to increase, and initially high values will tend to decrease. Use of a parallel placebo control group, and control for baseline values in multiple regression models, is essential to minimize the role of regression to the mean.

In addition, this analysis emphasizes the importance of baseline concentrations in studies of antioxidant treatment effects on oxidative stress biomarkers. Common sense indicates that values are only likely to decrease if they are not already low. Studies investigating the effect of antioxidant treatment should focus on groups that are susceptible to having a reduction in the baseline values. In our data, we suggest that values above 50 µg/mL are susceptible to having reductions in F₂-isoprostanes as a result of vitamin C treatments of 1000 mg/day for two months. Treatment effects were particularly notable where F₂-isoprostanes were ≥ 74 µg/mL. Other data [35] suggest that vitamin E at higher doses and/or with longer treatments could have an impact at lower initial F₂-isoprostane concentrations.

The effects of antioxidant supplements on lipid peroxidation have been reported in a number of studies, with and without a placebo control, and in a variety of subgroups. The discrepancies in patient populations, study designs, types and dosages of antioxidants, and their duration of use, makes the comparison and interpretation of these studies difficult. We summarize here only randomized placebo-controlled trials testing either vitamin C or vitamin E separately on plasma F₂-isoprostanes.

Seven research groups have examined the effect of vitamin E on F₂-isoprostanes [35–42], and four of the seven studies found statistically significant reductions in F₂-isoprostanes. (Reference 41 is an earlier report of the same study reported in reference 42.) Factors that appear to distinguish studies that found or failed to find an effect are sample size, obesity and/or the presence of elevated F₂-isoprostanes at baseline. The three studies finding no effect had sample sizes per treatment group of 5, 10, and 12. One of the three that found no effect had very lean participants (mean BMI=23) [36]; the other two had unreported BMI but relatively young participants (mean age <38) [37,38], who might be expected to have lower BMI. Weinberg et al. [38] tested an artificial situation in which 20% of calories came from polyunsaturated fat, shown to increase F₂-isoprostanes [38].

In contrast, the studies that found a significant effect of vitamin E had overweight participants and/or elevated baseline F₂-isoprostanes. The mean baseline F₂-isoprostanes in Sutherland et al. [39] was 87.5 µg/mL, considerably higher than our overall mean and even higher than the 79.4 µg/mL seen in our study among persons with baseline values above 50 µg/mL. The BMI in Sutherland et al. [39] and Huang et al. [40] was 34.4 and 28.7 respectively. The ASAP study [42], which found a significant effect in men but not in women, reported mean waist/hip ratios indicative of obesity, suggesting the likelihood of elevated baseline F₂-isoprostanes, although persons with BMI>32 were excluded. The study by Roberts et al. [35] also reported significant treatment effects of vitamin E. Although Roberts et al. do not report the BMI or baseline F₂-isoprostanes of their subjects, their inclusion criteria (cholesterol > 200 mg/dL and baseline F₂-isoprostanes > 2 SD above 'normal') forced participants to have F₂-isoprostanes of approximately ≥ 59 µg/mL at baseline. In our sample, participants meeting those criteria had a BMI of 28.7 and baseline F₂-isoprostanes of 86.5 µg/mL, and treatment with vitamin E and

vitamin C in that subgroup produced a 10% and 26% decrease respectively in F₂-isoprostanes in our data. Roberts et al. had sample sizes of only 5 per treatment group, and consequently a statistically significant effect was found only for doses of 1600 and 3200 IU/day. These results suggest that among subjects with substantial elevations in F₂-isoprostanes, vitamin E can be effective in reducing that oxidative stress biomarker, perhaps at higher doses and longer durations than we employed.

Five studies have examined the effect of vitamin C on plasma F₂-isoprostanes [19,20,42–44]. Three found no effect [42–44], and two by the present authors found significant effects. Dietrich et al. [19] found a significant reduction, in a sample of passive smokers with mean BMI of 29.1 kg/m². Among active smokers, Dietrich found a significant reduction, of approximately 19%, only among participants with BMI > 26.6 kg/m² [20]. Two of the studies that found no effect had sample sizes of 7 in the active treatment group [43,44], and in one of those [44], mean BMI was only 22.3 kg/m². In the one large study that found no effect of 500 mg/day vitamin C, waist/hip ratios were indicative of overweight participants but baseline F₂-isoprostane concentrations were not reported and participants with BMI>32 were excluded from participation [42]. These results suggest that further research on vitamin C should include adequate sample size and should include participants with F₂-isoprostane concentrations amenable to reduction. Inclusion of overweight, or especially, obese subjects would increase the proportion of such at-risk subjects, and randomization by baseline F₂-isoprostane concentrations above/below 50 µg/mL would justify separate examination of those subgroups.

In this and our previous research we have now shown that vitamin C in doses of 500 or 1000 mg/day over 2 months reduces *in vivo* lipid peroxidation, as measured by plasma F₂-isoprostanes, in overweight smokers, passively exposed nonsmokers (mean BMI of 29 kg/m²), and healthy nonsmoking individuals with overall BMI of 27 kg/m². The magnitude of the reductions has been approximately 10–11% when all subjects including those without elevated F₂-isoprostanes are included, and approximately 20% when only overweight persons or those with baseline F₂-isoprostane > 50 µg/mL are included.

The strong association between oxidative stress and obesity, seen by our group [7] and others [8], raises the possibility that recommended dietary allowances for antioxidants may need to be adjusted to reflect increased demand for antioxidant capacity among overweight or obese persons. Landmark studies by Levine et al. [44,45], which provided a pharmacokinetic basis for vitamin C recommendations, were conducted in seven male and 13 female young nonsmokers, with mean BMI among the women of 22.3 kg/M². In the United States population in 2003–2004, 73% of U.S. women had BMIs greater than 22.3 and almost 2/3 had BMIs of 25 or greater (Block, unpublished data). This suggests that further research among overweight and obese persons is needed.

Among the strengths of the present study are its randomized controlled design, use of a washout period for participants taking multivitamins or vitamins C and E and restriction of those supplements during the study, control for baseline levels of the biomarkers, and the sample size and measurements that permitted examination of effect modification and control for confounding. In future studies it would be useful to screen subjects for baseline F₂-isoprostanes, so as to either select only those with concentrations above 50 µg/mL or to randomize separately those above and below 50 µg/mL F₂-isoprostanes; and to ensure adequate power to detect an effect in those with baseline levels > 50 µg/mL. If prescreening is not feasible, then limiting subjects to persons who are overweight or obese would increase the proportion of subjects susceptible to having reductions in F₂-isoprostanes. Further studies are needed to establish the effect of higher doses or durations longer than eight weeks.

The relevance of our observation of a treatment effect on biomarkers of oxidative stress may be questioned, in view of the failure of recent large randomized trials to find a beneficial effect of “antioxidants” on cardiovascular disease. However, there may be several explanations for the failure of such trials other than ineffectiveness of the agents. Chief among such explanations is the fact that no trials have characterized the baseline plasma antioxidant concentrations nor limited participants to those with elevated oxidative stress biomarker concentrations [47]. As noted by Meagher et al. [37] “... the inclusion of patients without biochemical evidence of increased oxidative stress in clinical trials of antioxidants would be expected to dilute the population susceptible to benefit.... This might seriously undermine the sample size calculations used in such trials, leaving them open to a type II statistical error and outcomes reflecting random variation about the mean.” As the present study has shown, more than half of a healthy volunteer sample is likely to have plasma F₂-isoprostane concentrations too low to be susceptible to further reductions. In addition, all major trials of healthy subjects have permitted both intervention and control groups to take multivitamin supplements, which can raise plasma ascorbic acid levels substantially and make the control group insufficiently different from the active treatment group. In the recent Women’s Antioxidant Cardiovascular Study (WACS), which found no benefit of 500 mg/day vitamin C or 600 IU/day vitamin E on cardiovascular outcomes, approximately 27% of those in the control group took multivitamins during the study [46].

Several researchers have suggested that the effect of obesity on health outcomes may be mediated through obesity’s effect on oxidative stress [6,8–11]. In an analysis of the CARDIA study, Gross et al. suggested that “a general state of oxidative stress plays a role in the etiology of cardiovascular disease” [11, p 130]. In the Framingham study, Keaney et al. [8] concluded that their data suggest that “obesity is associated with a state of excess oxidative stress and suggest yet another contributing mechanism for excess CVD with obesity.” [8, p 439]. Higdon and Frei [10] and Vincent et al. [6] have suggested that “Oxidative stress ... may be a unifying mechanism in the development of major obesity-related comorbidities such as cardiovascular disease (CVD) and diabetes” [6]. If this is the case, it is possible that reductions in oxidative stress could reduce obesity-related comorbidities. It has been shown that weight loss, diet and exercise can produce significant reductions in biomarkers of oxidative stress [48,49], and improvement in obesity, diet and exercise is certainly an important societal goal. However, progress in achieving improvement in those factors has been limited. Our data suggest that supplementation with vitamin C, in obese persons or those with elevated F₂-isoprostanes, may merit further study in efforts to reduce the sequelae of obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

IsoP

F₂-isoprostanes

MDA

malondialdehyde

UCB

University of California at Berkeley

CHRCO

Children's Hospital Research Center of Oakland

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Figure 1.
Participant flow.

Figure 2.

Change in F₂-isoprostanes, by quintile of baseline F₂-isoprostanes. Boxes beneath quintile 5 in each treatment group show number of participants in the fifth quintile. Quintile cut-points were as follows, in ug/mL: Q1: 12.0–35.0; Q2: 36.0–43.0; Q3: 44.0–52.0; Q4: 53.0–73.0; Q5: 74.0–190.0.

Figure 3.
Percent of study participants with F₂-isoprostanes > 50 µg/mL, in BMI categories representing Normal (<25 kg/m²), Overweight (25–29.9 kg/m²), and Obese (≥ 30 kg/m²).

TABLE 1

Characteristics of study participants.

Variable	Total sample	Treatment groups			P*
		Placebo	Vitamin C	Vitamin E	
N	396	138	128	130	
Male, n (%)	137 (34.6)	50 (36.2)	42 (32.8)	45 (34.6)	0.84
Ethnicity, n (%)					
White	284 (71.7)	96 (69.6)	92 (71.9)	96 (73.9)	0.90
African American	38 (9.6)	15 (10.9)	13 (10.2)	10 (7.7)	
Other	74 (18.7)	27 (19.6)	23 (18.0)	24 (18.5)	
Age, years, mean (SD)	44.0 (15.1)	43.8 (15.7)	44.2 (14.7)	44.0 (14.9)	0.98
BMI, kg/m ² , mean (SD)					
Total	26.6 (5.5)	26.3 (5.4)	26.5 (5.1)	26.9 (5.9)	0.67
Male	25.1 (3.7)	24.8 (3.6)	24.6 (3.6)	25.7 (3.9)	0.32
Female	27.4 (6.1)	27.2 (6.2)	27.5 (5.5)	27.5 (6.6)	0.91
Malondialdehyde, $\mu\text{mol/L}$, mean (SD) #	1.02 (0.56)	1.07 (0.65)	0.96 (0.45)	1.04 (0.56)	0.31
F ₂ -isoprostanes, $\mu\text{g/mL}$, mean (SD) #	55.43 (27.42)	54.95 (26.59)	60.64 (30.79)	50.81 (23.89)	0.02
F ₂ -isoprostanes, % above 50 $\mu\text{g/mL}$ (n,%) #	152 (42.11)	50 (40.00)	56 (47.86)	46 (38.66)	0.30
Plasma ascorbic acid, $\mu\text{mol/L}$, mean (SD) #	57.77 (17.4)	57.32 (18.0)	56.06 (17.5)	59.93 (16.6)	0.19
Plasma alpha-tocopherol, $\mu\text{mol/L}$, mean (SD) #	28.32 (6.9)	28.16 (7.2)	28.67 (6.2)	28.15 (7.4)	0.79
Total cholesterol, mg/dL, mean (SD) #	196.28 (56.00)	192.46 (40.23)	198.94 (34.40)	190.97 (37.53)	0.21
LDL-cholesterol, mg/dL, mean (SD) #	124.32 (55.48)	119.75 (32.08)	127.72 (31.14)	118.46 (32.55)	0.05
HDL-cholesterol, mg/dL, mean (SD) #	54.77 (50.91)	53.07 (15.29)	50.74 (12.96)	52.91 (14.19)	0.35
Triglycerides, mg/dL, mean (SD) #	101.86 (73.80)	97.92 (64.63)	102.44 (53.70)	98.06 (52.91)	0.78

* Significance of the difference across treatment groups.

Among those without missing data.

To convert F₂-isoprostanes from $\mu\text{g/mL}$ to pmol/L , multiply by 2.86, i.e., 2.86 $\text{pmol per } \mu\text{g}$

Table 2Unadjusted treatment effect on F₂-isoprostanes*

All Subjects				
	Placebo (n=124)	Vitamin C (n=115)	Vitamin E (n=117)	
Before intervention, µg/mL, mean (SD)	54.84 (26.67)	60.67 (30.98)	51.04 (23.99)	
After intervention, µg/mL, mean (SD)	58.73 (32.17)	52.31 (24.94)	50.60 (28.99)	
Change (µg/mL)	+3.90 (26.11)	−8.37 (28.12)	−0.44 (23.38)	
Change (%)	+7.11	−13.80	−0.86	
P (change)	0.10	0.002	0.84	
Subjects with baseline F ₂ -isoprostanes ≤ 50 µg/mL				
	Placebo (n=75)	Vitamin C (n=60)	Vitamin E (n=71)	
Before intervention, µg/mL, mean (SD)	38.80 (8.12)	37.97 (8.17)	37.14 (8.30)	
After intervention, µg/mL, mean (SD)	47.68 (19.57)	41.82 (16.85)	40.11 (18.91)	
Change (µg/mL)	+8.88 (18.38)	+3.85 (17.25)	+2.97 (17.60)	
Change (%)	+22.89	+10.14	+8.00	
P (change)	<0.0001	0.09	0.16	
Subjects with baseline F ₂ -isoprostanes > 50 µg/mL				
	Placebo (n=49)	Vitamin C (n=55)	Vitamin E (n=46)	
Before intervention, µg/mL, mean (SD)	79.39 (26.52)	85.44 (27.49)	72.50 (24.51)	
After intervention, µg/mL, mean (SD)	75.65 (39.74)	63.76 (27.32)	66.78 (34.19)	
Change (µg/mL)	−3.73 (33.59)	−21.67 (31.59)	−5.72 (29.67)	
Change (%)	−4.70	−25.36	−7.89	
P (change)	0.44	<0.0001	0.20	

* Includes only those without missing data

Table 3Intention-to-treat analysis of treatment effect on F₂-isoprostanes*

All Subjects			
	Placebo (n=138)	Vitamin C (n=128)	Vitamin E (n=130)
Change (µg/mL)	+3.76 (2.08)	-5.85 (2.08)	-2.18 (2.11)
Change (%) [#]	+6.79	-10.56	-3.94
P (difference from change in placebo)	--	0.001	0.043
Subjects with baseline F₂-isoprostanes ≤ 50 µg/mL			
	Placebo (n=75)	Vitamin C (n=62)	Vitamin E (n=75)
Change (µg/mL)	+9.57 (2.09)	+3.69 (2.24)	+2.54 (2.10)
Change (%) [#]	+25.22	+9.72	+6.69
P (difference from change in placebo)	--	0.06	.02
Subjects with baseline F₂-isoprostanes > 50 µg/mL			
	Placebo (n=63)	Vitamin C (n=66)	Vitamin E (n=55)
Change (µg/mL)	-3.35 (3.69)	-16.65 (3.65)	-7.41 (4.08)
Change (%) [#]	-4.44	-22.05	-9.81
P (difference from change in placebo)	--	0.01	0.46

* Intention to treat analysis, 40 subjects with missing baseline or follow-up F₂-isoprostane concentration assigned zero change. Adjusted for baseline F₂-isoprostane concentration and LDL cholesterol.

[#] Change based on mean baseline F₂-isoprostanes: For overall group, mean = 55.40 µg/mL. For group with baseline F₂-isoprostanes ≤ 50 µg/mL, mean = 37.95 µg/mL. For group with baseline F₂-isoprostanes > 50 µg/mL, mean = 75.51 µg/mL.

Table 4

Intention to treat analysis of treatment effect on malondialdehyde*

All Subjects	Placebo (n=138)	Vitamin C (n=128)	Vitamin E (n=130)
Change ($\mu\text{mol/L}$)	+0.145 (0.054)	-0.010 (0.055)	+0.123 (0.056)
Change (%) [#]	+14.27	-1.00	+12.08
P (difference from change in placebo)	--	0.047	0.79

* Intention to treat analysis, 13 subjects with missing baseline or follow-up MDA concentration assigned zero change. Adjusted for baseline MDA concentration and LDL cholesterol. Change represents adjusted (least squares) mean change.

[#] Change based on mean baseline MDA, 1.016 $\mu\text{mol/L}$.