



Original Contribution

Vitamin C treatment reduces elevated C-reactive protein

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ABSTRACT

Plasma C-reactive protein (CRP) is an inflammatory biomarker that predicts cardiovascular disease. Lowering elevated CRP with statins has reduced the incidence of cardiovascular disease. We investigated whether vitamin C or E could reduce CRP. Healthy nonsmokers ($N=396$) were randomized to three groups, 1000 mg/day vitamin C, 800 IU/day vitamin E, or placebo, for 2 months. Median baseline CRP was low, 0.85 mg/L. No treatment effect was seen when all participants were included. However, a significant interaction was found, indicating that treatment effect depends on baseline CRP concentration. Among participants with CRP indicative of elevated cardiovascular risk (≥ 1.0 mg/L), vitamin C reduced the median CRP by 25.3% vs placebo ($p=0.02$) (median reduction in the vitamin C group, 0.25 mg/L, 16.7%). These effects are similar to those of statins. The vitamin E effect was not significant. In summary, treatment with vitamin C but not vitamin E significantly reduced CRP among individuals with CRP ≥ 1.0 mg/L. Among the obese, 75% had CRP ≥ 1.0 mg/L. Research is needed to determine whether reducing this inflammatory biomarker with vitamin C could reduce diseases associated with obesity. But research on clinical benefits of antioxidants should limit participants to persons with elevations in the target biomarkers.

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Multiple avenues of research have led to the recognition that cardiovascular disease involves a systemic inflammatory process [1]. Prospective studies and primary prevention trials have demonstrated that high-sensitivity C-reactive protein (CRP), an acute-phase protein and marker of inflammation, predicts overall cardiovascular disease risk in those without prior disease and predicts multiple types of cardiovascular disease including myocardial infarction, stroke, and peripheral arterial disease [2]. Further evidence of a link between inflammation, CRP, and atherosclerosis comes from studies of aspirin and statins. Such anti-inflammatory therapies have been shown to lower CRP while also reducing cardiovascular events and slowing the progression of atherosclerosis. Moreover, the magnitude of the clinical benefit of these agents seems to be greatest in individuals with elevated CRP [3–8].

CRP may contribute directly to the atherosclerotic process. It has been found to play a role in early monocyte recruitment [9], adhesion molecule expression [10], and reduced production of the endothelial dilator nitric oxide [11], among other activities. However, the use of purified preparations [12,13], and transgenic mouse models [14–16], has yielded mixed results. Thus, although the extent of the direct role

of CRP in atherosclerosis has not yet been clearly elucidated, at the least CRP represents a surrogate marker by which the anti-inflammatory effectiveness of therapies can be evaluated, and it may itself be a potential target for reducing cardiovascular disease risk.

Based on prospective studies and primary prevention trials, the American Heart Association Consensus Panel *Guide to Comprehensive Risk Reduction for Adult Patients without Coronary or Other Atherosclerotic Vascular Diseases* and the Centers for Disease Control and Prevention (CDC/AHA) have designated a CRP concentration of <1.0 mg/L as representing low risk for cardiovascular disease, whereas concentrations of 1.0 to 3.0 mg/L and >3.0 mg/L have been designated as representing average risk and high risk, respectively [17]. Recent research has confirmed that concentrations in the intermediate range of 1.0 to 3.0 mg/L do in fact confer excess risk of cardiovascular disease [18]. The CDC/AHA also note that CRP values >10 mg/L represent acute infection or inflammatory disease and should not be used in the evaluation of cardiovascular disease risk.

We have previously shown CRP to be significantly associated with the biomarkers of oxidative stress malondialdehyde and F₂-isoprostanes [19]. F₂-isoprostanes seem to have a direct effect on inflammation, inducing the expression of interleukin-8 and intracellular inflammatory signals [20]. We recently demonstrated that treatment with vitamin C significantly lowers F₂-isoprostanes [21]. Our earlier

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research found a significant reduction in CRP as a result of treatment with 500 mg/day vitamin C among active and passive smokers [22]. The present study was designed to follow up on these 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 for 2 months on plasma CRP among healthy nonsmokers. Based on our previous work, we hypothesized a greater CRP-lowering effect of vitamin C compared to vitamin E. In addition, we sought to confirm an earlier observation [23] that the antioxidant treatment effect depended on baseline CRP level and was limited to persons with baseline CRP concentrations indicative of elevated cardiovascular risk, CRP ≥ 1.0 mg/L.

Materials and methods

Participants

Healthy nonsmokers were recruited between January 2005 and March 2006 from the communities of San Francisco, Berkeley, and Oakland, California, USA. Eligibility was not limited to persons with elevated CRP. Exclusion criteria included age <18 years, smoking, passive smoke exposure (exposed indoors ≥ 5 days/week), alcohol consumption (≥ 2 drinks/day), pregnancy or breast feeding, 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, statin, lipid-lowering, blood-thinning, hormone replacement therapy, or steroid medications), consumption of single iron supplements or vitamin E supplements in amounts greater than 400 IU/day, and body weight ≥ 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 the use of such agents for 30 days before their baseline visit and throughout the 60-day intervention.

Of 1535 subjects assessed for eligibility, 1139 were ineligible or declined to participate, 396 were enrolled, and 385 completed the study (Fig. 1). Of the 11 who terminated early, 3 withdrew due to inconvenience or unwillingness to refrain from taking supplements, 4 were lost to follow-up, 1 died in an automobile accident, and 3 were withdrawn administratively. The study design was approved by the institutional review boards of the University of California at Berkeley and Children's Hospital & Research Center of Oakland, California. 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 postmenopausal) for women or age (18–44, 45–59, or ≥ 60 years) for men. Lettered 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- α -tocopherol) and placebo tablet; and (3) placebo tablet and placebo capsule. Placebo tablets contained lactose,

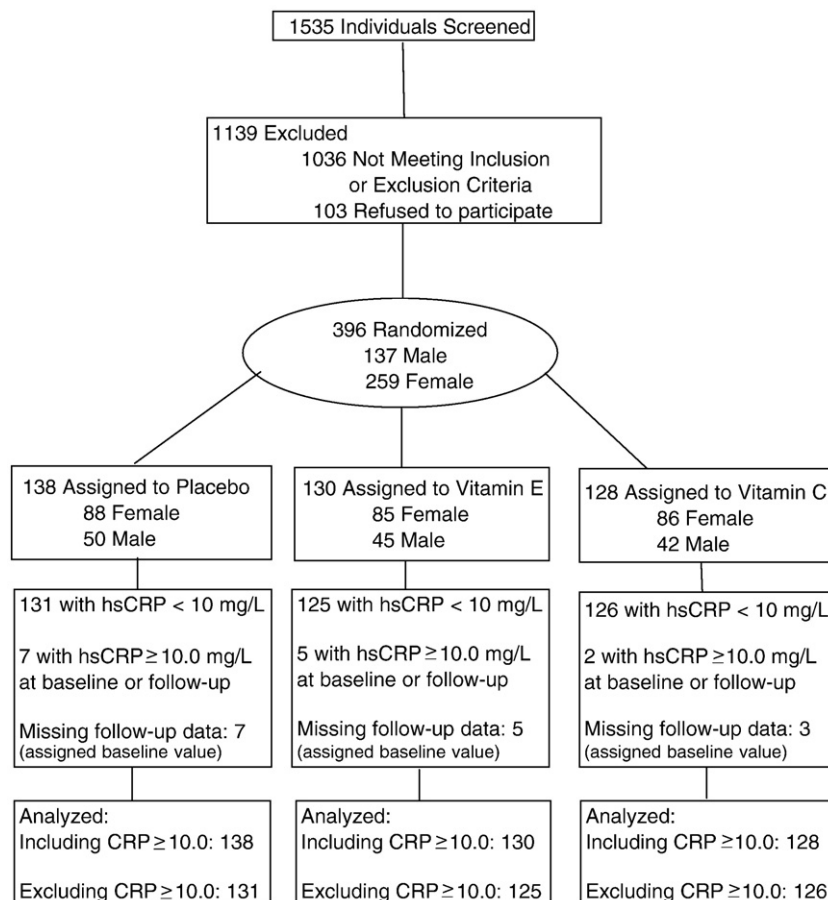


Fig. 1. Flow diagram of participant progress through the study.

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. Study supplements and placebo pills were provided by Pharmavite, LLC (Mission Hills, CA, USA). Investigators, clinic and laboratory staff, and participants were masked to treatment assignment. Separate study staff administered the intervention and analyzed data.

Procedures

Pre- and perimenopausal 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 midstudy 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 [24]. 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 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 (Hologic, Delphi A, Model QDR 4500A (S/N 4500); Serial No. 70580; using the QDR system software version 11.2).

Fasting venous blood was drawn into Vacutainer tubes (Becton–Dickenson, Rutherford, NJ, USA), protected from light, maintained at <15 °C, and processed within 6 h. Vacutainers were centrifuged at 5 °C for 10 min at 1200 g and aliquotted. 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. All sample batches included masked duplicates and replicated internal control samples.

Participants provided demographic information and completed self-administered diet and physical activity questionnaires (Nutrition-Quest, formerly Block Dietary Data Systems; www.nutritionquest.com). At both the baseline and the 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

Laboratory collaborators were masked with regard to sample treatment group and pre- vs. post-treatment status. High-sensitivity C-reactive protein concentration was measured by latex-enhanced nephelometry using a Hitachi 917 analyzer [25]. Serum lipids were measured using timed-endpoint, coupled enzymatic methodology [26]. Serum α -tocopherol and serum carotenoids were measured by reversed-phase HPLC with UV/Vis detection [27]. Serum ascorbic acid was measured spectrophotometrically using 2,4-dinitrophenylhydrazine as chromogen [28], a method that has been shown to correlate highly with HPLC analysis [28,29].

Statistical analysis

Differences in baseline measurements by treatment group were assessed using χ^2 tests for categorical variables and analysis of variance tests for continuous measures. Treatment effect was assessed using both nonparametric and parametric multiple regression techniques. In nonparametric analyses, median concentrations at baseline and at study completion were computed, and the significance of the changes within and between groups was assessed by the Wilcoxon signed rank test and two-sample test. In parametric analyses, change in CRP was the dependent variable and CRP was log-transformed to reduce skewness. For the intent-to-treat analysis,

Table 1
Participant characteristics

Variable	Total	Placebo	Vitamin C	Vitamin E	<i>p</i> ^a
<i>N</i>	396	138	128	130	
Male (<i>N</i> (%))	137 (34.6)	50 (36.2)	42 (32.8)	45 (34.6)	0.84
Ethnicity (<i>N</i> (%))					0.90
White	284 (71.7)	96 (69.6)	92 (71.9)	96 (73.9)	
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 (mean (SD))	44.0 (15.1)	43.8 (15.7)	44.2 (14.7)	44.0 (14.9)	0.98
BMI (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
BMI ≥ 25 (<i>N</i> (%))					
Total	223 (56.3)	72 (52.2)	74 (57.8)	77 (59.2)	0.47
Male	63 (46.0)	22 (44.0)	17 (40.5)	24 (53.3)	0.46
Female	160 (61.8)	50 (56.8)	57 (66.3)	53 (52.4)	0.43
C-reactive protein (mg/L)					
Including subjects with CRP ≥ 10	1.83 (3.07) ^b	1.96 (3.14)	1.43 (2.25)	2.07 (3.64)	0.21
Excluding subjects with CRP ≥ 10	0.87 [0.36, 1.73] ^c	0.81 [0.36, 1.92]	0.81 [0.36, 1.47]	0.95 [0.38, 2.02]	0.48
1.41 (1.64)	1.46 (1.68)	1.29 (1.51)	1.49 (1.72)		
0.84 [0.36, 1.61]	0.81 [0.37, 1.70]	0.72 [0.35, 1.47]	0.93 [0.36, 1.78]		
1.0 \leq CRP \leq 10.0 mg/L (<i>N</i> (%)) ^d	173 (44.7)	60 (44.8)	53 (41.7)	60 (47.6)	0.64
Serum vitamin C (reduced, μ M/L) (mean (SD))	57.77 (17.4)	57.32 (18.0)	56.06 (17.5)	59.93 (16.6)	0.19
Serum vitamin E (μ M/L) (mean (SD))	28.32 (6.9)	28.16 (7.2)	28.67 (6.2)	28.15 (7.4)	0.79
LDL cholesterol (mmol/L) (mean (SD))	3.16 (0.8)	3.10 (0.8)	3.31 (0.8)	3.07 (0.8)	0.047

^a Significance of the difference across treatment groups.

^b Mean (SD).

^c Median [25th, 75th percentile].

^d Among those without missing data.

participants who did not complete the study or were withdrawn administratively (7, 3, and 5 participants in the placebo, vitamin C, and vitamin E groups, respectively) were given a follow-up CRP value equal to their baseline value [AU1].

The examination of effect modification by baseline CRP level was begun by identifying three subgroups of baseline CRP based on previous data [23] and on preexisting recommendations by CDC/AHA [17]. Interaction was assessed by including the relevant cross-product term in the model. Age, sex, and body mass index (BMI), a variable strongly associated with CRP [30], were also examined for interaction.

Potential covariates were examined, including demographic factors (education, income, and poverty index ratio) and plasma measures at baseline (total, low-density lipoprotein (LDL), and high-density lipoprotein cholesterol). Gender and BMI (associated with change in CRP) and LDL-cholesterol (associated with both exposure and outcome) were included in further models. All models included adjustment for baseline CRP concentration. Significance was defined as $p < 0.05$ (two-tailed) for treatment effect and $p < 0.2$ for interaction terms.

Results

Participants had a mean age of 44 years, and 34.6% were male (Table 1). The treatment groups did not differ with respect to gender, ethnicity, age, BMI, or plasma vitamin C or E at baseline, but did differ with respect to LDL-cholesterol ($p = 0.047$). Plasma vitamin C changes were 3.8, -1.7, and 49.3% in placebo, vitamin E, and vitamin C groups, respectively, and changes in plasma vitamin E were 11.9, 103.3, and 5.2% in the respective groups. Adherence, defined as having taken 90% of prescribed pills, was 87% overall and did not differ by treatment group ($p = 0.41$).

The mean and median CRP in the sample were low, 1.83 and 0.87 mg/L, respectively, overall (1.43 and 0.85 mg/L among those who did not have evidence of acute inflammation) (Table 1). Among persons with CRP < 1.0 mg/L, the mean and median levels were 0.46 and 0.39 mg/L, whereas in those with CRP ≥ 1.0 and < 10.0 mg/L, mean and median levels were 2.70 and 1.94 mg/L, respectively (data not shown).

In the nonparametric and parametric strict intent-to-treat analysis including all 396 participants, there was no overall treatment effect ($p = 0.11$ and 0.42 for vitamin C and vitamin E, respectively, in parametric comparison with change in placebo) (Table 2).

We tested for the presence of interaction between treatment effect and baseline CRP concentration, based on values specified by the CDC/AHA. The first such value was CRP > 10 mg/L, found in nine persons at the baseline visit. CDC/AHA specifies that CRP concentrations > 10 mg/L are not informative regarding cardiovascular risk and should not be used: "If a level of > 10 mg/L is identified, there should be

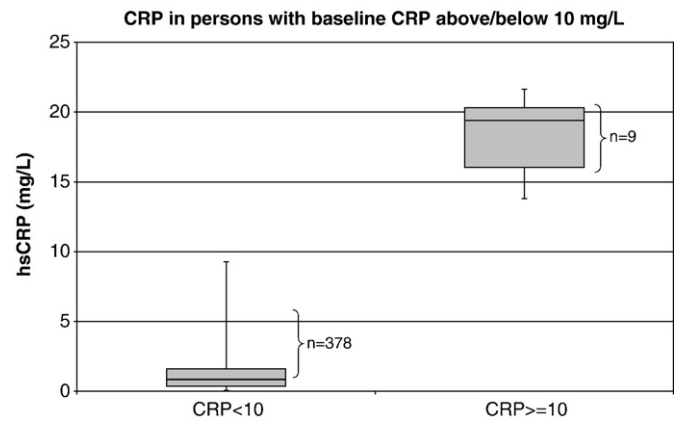


Fig. 2. CRP in persons with baseline CRP above and below 10 mg/L. Two plots are shown, CRP distribution in those with CRP < 10 mg/L and in those with CRP ≥ 10 mg/L. The boxes indicate 25th, 50th, and 75th percentiles, and the tails on the boxes represent minimum and maximum.

a search initiated for an obvious source of infection or inflammation, which could obscure any prediction of coronary risk that might be attributed to the elevated level. That result of > 10 mg/L should then be discarded ...” [17]. The interaction between treatment group and having CRP > 10 mg/L was highly significant, $p < 0.0001$. As can be seen in Fig. 2, persons with CRP > 10 mg/L represent a distinctly different population, with no overlap with those with lower CRP values. There was no evidence of an effect of either antioxidant on persons with acute elevations ($N = 14$ at either baseline or follow-up) (data not shown). Because of this interaction, and the fact that the study was intended to examine the potential benefit of antioxidants on chronic cardiovascular-relevant inflammation, further analysis focused on participants with CRP < 10.0 mg/L.

Among the 382 subjects with CRP concentrations < 10 mg/L, indicative of chronic inflammation, a significant interaction between baseline CRP level (above/below 1.0 mg/L) and treatment group remained. The a priori stratification was again based on the CDC/AHA criteria. The interaction between treatment group and CVD risk category was significant at $p = 0.03$, indicating that treatment effect differed depending on baseline CRP level.

Among those with CRP < 1.0 mg/L, there was no significant treatment effect ($p = 0.91$ for the comparison of change in either active treatment group with change in placebo group). Indeed, both placebo and active treatment groups had increases in CRP (Table 3, top). Among those with increased cardiovascular risk as represented by CRP ≥ 1.0 mg/L, treatment with vitamin C reduced CRP significantly in both nonparametric and parametric analyses (Table 3, bottom). In

Table 2

Change in CRP, no exclusions or stratification

	Placebo (N=138)	Vitamin C (N=128)	Vitamin E (N=130)
Pretreatment, unadjusted (median, mg/L) ^a	0.81	0.81	0.95
Posttreatment, unadjusted (median, mg/L) ^a	0.97	0.84	0.99
Unadjusted median change, mg/L (IQR) ^b	+0.05 (-0.17, +0.65)	-0.01 (-0.28, +0.38)	0.00 (-0.20, +0.39)
Unadjusted median % change (IQR) ^b	+8.33 (-25.00, +58.33)	-1.10 (-33.02, +40.00)	0.00 (-20.00, +40.77)
p (Is within-group median change different from 0?) ^c	0.02	0.75	0.38
p (Is change different from change in placebo group?) ^d	-	0.16	0.28
Parametric: adjusted change, % (mean, CL) ^e	+19 (+2, +37)	-1 (-14, +16)	+9 (-7, +27)
Parametric: p (significance of contrast with change in placebo group) ^e	-	0.11	0.42

N=396. Includes subjects with CRP > 10 mg/L.

^a Unadjusted median values, subjects without missing values.

^b IQR, 25th and 75th percentiles.

^c Wilcoxon signed rank test, nonparametric, significance of within-group changes.

^d Wilcoxon 2-sample test, nonparametric, significance of contrast with change in placebo group.

^e Parametric analysis, intention to treat, all 396 subjects, including those with missing data (assigned zero change) or with CRP > 10 mg/L. Based on least squares mean and standard error, from model with the dependent variable, change in CRP, on the log scale. Adjusted for baseline CRP, gender, BMI, and LDL. CL, 95% confidence limits.

Table 3
Change in CRP, by baseline CRP (excludes subjects with CRP>10 mg/L)

	CRP <1.0 mg/L at baseline		
	Placebo (N=76)	Vitamin C (N=75)	Vitamin E (N=69)
Pretreatment, unadjusted (median, mg/L) ^a	0.46	0.39	0.38
Posttreatment, unadjusted (median, mg/L) ^a	0.54	0.47	0.38
Unadjusted median change, mg/L (IQR) ^b	+0.03 (−0.08, +0.31)	+0.01 (−0.08, +0.43)	+0.02 (−0.04, +0.29)
Unadjusted median % change (IQR) ^b	+6.06 (−21.28, +62.86)	+5.88 (−25.00, +105.56)	+8.33 (−11.36, +62.50)
<i>p</i> (Is within-group median change different from 0?) ^c	0.01	0.02	0.01
<i>p</i> (Is change different from change in placebo group?) ^d	–	0.83	0.92
Parametric: adjusted change, % (mean, CL) ^e	+31.0 (+9, +57)	+29 (+8, +54)	+29 (+7, +56)
Parametric: <i>p</i> (significance of contrast with change in placebo group) ^e	–	0.91	0.91
	CRP ≥1.0 mg/L at baseline		
	Placebo (N=55)	Vitamin C (N=51)	Vitamin E (N=56)
Pretreatment, unadjusted (median, mg/L) ^a	1.92	1.68	2.02
Posttreatment, unadjusted (median, mg/L) ^a	2.19	1.34	1.83
Unadjusted median change, mg/L (IQR) ^b	+0.12 (−0.56, +1.09)	−0.25 (−0.123, +0.14)	−0.10 (−0.78, +0.40)
Unadjusted median % change (IQR) ^b	+8.57 (−21.82, +40.12)	−16.70 (−48.49, +11.57)	−6.80 (−47.83, +24.84)
<i>p</i> (Is within-group median change different from 0?) ^c	0.30	0.02	0.24
<i>p</i> (Is change different from change in placebo group?) ^d	–	0.02	0.15
Parametric: adjusted change, % (mean, CL) ^e	−7 (−23, +12)	−33 (−55, −19)	−20 (−34, +4)
Parametric: <i>p</i> (significance of contrast with change in placebo group) ^e	–	0.01	0.24

N=382. Excludes subjects with CRP >10 mg/L.

^a Unadjusted median values, subjects without missing values.

^b IQR, 25th and 75th percentiles.

^c Wilcoxon signed rank test, nonparametric, significance of within-group changes.

^d Wilcoxon 2-sample test, nonparametric, significance of contrast with change in placebo group.

^e Parametric analysis, intention to treat, all 382 subjects with CRP <10 mg/L, including those with missing data (assigned zero change). Based on least squares mean and standard error, from model with the dependent variable, change in CRP, on the log scale. Adjusted for baseline CRP, gender, BMI, and LDL. CL, 95% confidence limits.

the vitamin C group with baseline CRP >1 mg/L, the unadjusted median change was −0.25 mg/L, a 16.70% reduction. This within-group change was significant by Wilcoxon two-tailed signed rank test, $p=0.049$. The placebo group had an 8.57% increase in CRP. Thus, compared with subjects assigned to placebo, allocation to the vitamin C treatment group was associated with a 25.27% reduction in median CRP levels ($p=0.02$ by Wilcoxon 2-sample test). Vitamin E treatment effects were lower and were not significant. In parametric intention-to-treat analyses, the change in CRP in the vitamin C group was significantly different from change in placebo, $p=0.01$, adjusted for baseline CRP level, gender, BMI, and LDL. Again, vitamin E effects were weaker and not significant.

Other analyses

There was no evidence of interaction between ethnic group and treatment group. Tests of interaction between gender and treatment group, and between BMI and treatment group, were not statistically significant. However, the point estimates suggested a considerably stronger treatment effect among women and among persons with elevated BMI. This is likely owing to the substantially higher mean baseline CRP in women than in men, and in those with higher BMI.

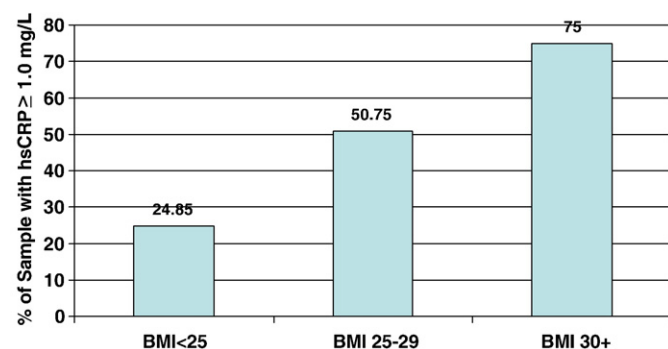


Fig. 3. Percentage with CRP ≥1.0 mg/L at baseline, by BMI status. $p=0.0001$ for difference in groups.

In the small subgroup with baseline CRP >3 mg/L, there was a suggestion of a substantial effect of vitamin E, but neither treatment group effect reached statistical significance.

We examined the proportion with baseline CRP above 1.0 mg/L in BMI groups and found a strong association of elevated CRP with BMI category (Fig. 3). The percentage with CRP ≥1.0 mg/L increased from 24.9 to 50.8 to 75% as BMI categories increased from normal to overweight to obese.

Baseline CRP was weakly associated with LDL-cholesterol (Spearman $r=0.23$). The changes in these two variables were not associated (Spearman $r=0.02$), and control for the change in LDL-cholesterol did not alter the conclusions. In addition, there were no significant differences between treatment groups in the proportion who reported taking new over-the-counter or prescription medications during the study.

Safety, adverse events, and adequacy of masking

One death, in the vitamin E group, occurred because of an automobile accident. No other serious adverse events were reported. Other adverse event reports were obtained by checklist for the following symptoms: health problems, upset stomach, diarrhea, nausea or vomiting, constipation, bleeding gums, poor night vision, nosebleeds, fatigue, tiredness, headache, irritability, dizziness, skin rash, bruising easily, any other symptoms. Postintervention, the vitamin E group reported significantly more dizziness than the placebo group, $p=0.008$ (data not shown), whereas the vitamin C group reported a significantly lower prevalence of fatigue ($p=0.005$). There were no other significant differences across treatment groups.

A postintervention questionnaire revealed that approximately 15, 20, and 17% of those in the vitamin E, vitamin C, and placebo groups, respectively, guessed their treatment correctly.

Discussion

This analysis has found that antioxidants have no effect on inflammation marker C-reactive protein when all randomized subjects are included, but that one of the antioxidants, vitamin C,

does have a statistically significant effect when analysis is restricted to those deemed by the CDC/AHA [17] to be at elevated cardiovascular risk. Among persons with baseline CRP ≥ 1.0 mg/L, treatment with 1000 mg/day vitamin C for 2 months was associated with a 16.7% within-group change, $p < 0.05$. Compared to subjects in the placebo group, allocation to vitamin C was associated with a 25.27% reduction in median CRP concentrations, $p = 0.02$. Significant differences between the vitamin C and the placebo group were also seen in parametric analysis, despite the very conservative approach of assuming that those with missing data had zero change. Treatment with 800 IU/day vitamin E was also associated with some reduction in CRP, but the treatment effect did not achieve statistical significance.

We have also reported a similar influence of baseline level on the treatment effect of antioxidants on the oxidative stress biomarker F₂-isoprostanes [21]. In that analysis, treatment with vitamin C reduced F₂-isoprostanes by 22%, $p = 0.01$, only among those with baseline concentrations ≥ 50 $\mu\text{g/mL}$, a subgroup representing 45% of the sample. The effect of vitamin E in that analysis was weaker and not significant. It is also perhaps worth noting that there was a significant ($p = 0.008$) association between having F₂-isoprostanes ≥ 50 $\mu\text{g/mL}$ and having CRP ≥ 1.0 mg/L.

Common sense suggests that biomarkers are likely to be reduced only if they are not already low. Indeed, in this study, initially low concentrations tended to increase, even in the active intervention groups, reflecting the effect of regression to the mean. Studies investigating the effect of antioxidant treatment should attempt to limit intervention to subjects who are susceptible to having a reduction in the baseline values. Often, such a cut-point is not known a priori. Inflammation research is fortunate in having such values defined and in having their prognostic value confirmed [17,18]. We have suggested such a cut-point for studies of F₂-isoprostanes [21].

These observations may shed light on the mixed results found in large “antioxidant” trials with clinical endpoints. No such trials have limited participants to persons with elevated CRP or oxidative stress, and most have not characterized participants with respect to those factors [31]. Clinical trial methodology demands that the primary result represents analysis that includes *all* randomized participants. Such an analysis protects against unexpected biases. However, such an analysis also risks obscuring a potentially important result. As Meagher et al. have noted [32], inclusion of subjects without evidence of an increase in the target biomarker in trials of antioxidants would tend to dilute the population susceptible to benefit and undermine the sample size and power calculations, leaving such studies open to type II error and outcomes reflecting random variation. If one mechanism of potential antioxidant treatment is reduction in CRP, inclusion of subjects without elevations in CRP would tend to have weakened such studies, particularly those involving healthy volunteers. In the present study of healthy volunteers, 55% of participants had CRP values < 1.0 mg/L.

We found, as others have [30], that CRP is strongly associated with BMI. We also found that a BMI reflective of obesity ($\text{BMI} \geq 30$) is a strong marker of having CRP ≥ 1.0 mg/L, the level indicative of increased risk of cardiovascular events. In our study, 75% of the obese had CRP ≥ 1.0 mg/L (Fig. 3). This at-risk group is an appropriate target for efforts to reduce inflammation, although elevations in CRP are found in all BMI categories.

It is notable that our sample was quite well nourished with respect to vitamin C, with a baseline mean plasma reduced ascorbic acid level of 57.8 $\mu\text{mol/L}$, whereas the average plasma vitamin C level in the U.S. adult population is 42.6 $\mu\text{mol/L}$ (G. Block, unpublished data from the Third National Health and Nutrition Examination Survey). Only 1.6% of our participants had ascorbic acid levels < 22.7 $\mu\text{mol/L}$ (0.4 mg/dl), whereas in national data 25.7% have levels below 22.7 $\mu\text{mol/L}$. It is likely that the treatment effect would be more substantial in a population more representative of the United States with respect to plasma ascorbic acid status. Vitamin E status was comparable to national levels. In our sample, mean α -tocopherol was 28.33 $\mu\text{mol/L}$,

and 0.79% had deficient levels. In national data among adults, mean serum vitamin E was 29.23 $\mu\text{mol/L}$, and 1.02% had deficient plasma levels (G. Block, unpublished data from the National Health and Nutrition Examination Survey 1999–2000).

The impact of antioxidants on plasma CRP may be mediated by effects on upstream cytokines, in particular interleukin-1 (IL-1), tumor necrosis factor- α (TNF α), and interleukin-6 (IL-6), which are the main inducers of the acute phase response [33]. Hartel et al. found that vitamin C inhibited the lipopolysaccharide-induced IL-6 and TNF α production, as well as IL-2 production after phorbol 12-myristate 13-acetate/ionomycin stimulation [34]. Those authors suggest several potential mechanisms, including both oxidative and nonoxidative processes [34]. Oxidative damage leads to an inappropriate activation of the transcription factor nuclear factor κB (NF- κB) and subsequently to an overexpression of inflammatory proteins [35]. Vitamin C has been shown to inhibit NF- κB activation [36–39]. This potential oxidative mechanism is consistent with our observation in the present dataset that plasma F₂-isoprostane, a biomarker of oxidative stress, was significantly reduced by vitamin C treatment [21]. We have also reported in earlier research that treatment with 500 mg/day vitamin C significantly reduced plasma F₂-isoprostanes among active and passive smokers [40].

Five studies have examined the effects of vitamin E on CRP [41–45], of which two found statistically significant treatment effects. All studies were small, $N = 5$ to 25 per treatment group, and several were in diabetics or hemodialysis patients. The factor that seems to distinguish studies that found or failed to find an effect of vitamin E is the presence of elevated CRP at baseline. The two that found significant CRP reductions with vitamin E treatment had subjects with very high initial CRP, > 5 mg/L in both cases [41,42]. Both also had subjects with high mean BMI, 27.5 and 31.5 kg/m^2 , respectively. In contrast, two of the three finding no effect had mean baseline CRP of 1.0 mg/L and mean BMI of only 24 kg/m^2 . This is consistent with our results, in which vitamin E had no effect among persons with baseline CRP < 1.0 mg/L, but in the group with CRP > 1.0 mg/L (mean CRP 2.7 mg/L) there was a 6.8% reduction in the vitamin E group, although this did not reach significance. These studies suggest that vitamin E may be effective in reducing CRP in persons with substantial CRP elevations, higher than what we observed in our healthy volunteers.

Three small earlier studies ($N = 12$ to 17 per treatment group) found no effect of vitamin C on CRP [41,46,47]. The two studies that did find an effect had adequate sample size ($N \geq 49$ [22] and 43 [23] in active treatment group) and were the only studies of either vitamin C or E to exclude persons with CRP > 10 mg/L and to adjust for baseline CRP. Both studies utilized nondiseased participants with BMI approximately 27 kg/m^2 , similar to that of the U.S. population. Both had a mean baseline CRP ≥ 2.4 mg/L. These two studies are consistent with our finding of a significant CRP-lowering effect of vitamin C in persons with elevated baseline CRP concentrations.

Church et al. [23] also conducted an analysis stratified by baseline CRP above or below 1.0 mg/L. Their results were identical to ours: there was no treatment effect among persons with initial CRP levels < 1.0 mg/L, but a significant treatment effect in persons initially above that concentration.

Two studies examined a combination of vitamins C and E with adequate sample size ($N \geq 49$ in the active treatment group), and neither found a significant reduction in CRP [22,48]. One had a sample with low initial CRP (median 1.0 mg/L) [48]. Both used a fairly low dose, 182 mg α -tocopherol and 500 mg vitamin C [48] or 371 mg α -tocopherol and 515 mg vitamin C [22]. The failure of the combination dosage in the latter study is unexplained and warrants further study.

A number of studies have shown that statins reduce CRP concentrations [49–51]. In an analysis of myocardial infarct patients enrolled in the Cholesterol and Recurrent Events Trial, median percentage change in CRP in the pravastatin group was 17.4% ($p = 0.004$) after 5 years of treatment [49]. In the Air Force/Texas

Coronary Atherosclerosis Prevention Study, median change in CRP in the lovastatin group after 1 year was 0.2 mg/L, 14.8% ($p < 0.001$) [50]. In the Pravastatin Inflammation/CRP Evaluation Trial, median CRP concentration in the pravastatin group was reduced by 0.2 mg/L, 14.2% ($p < 0.001$) [51]. In that study the baseline median CRP levels in the placebo and pravastatin groups were 2.1 and 2.0 mg/L, respectively, similar to the 1.9 mg/L found in our sample among participants with CRP ≥ 1.0 mg/L. Thus, the 0.25 mg/L, 16.7%, within-group reduction in mean CRP in the vitamin C group in the present study, in a group of healthy persons with baseline CRP levels above 1.0 mg/L, suggests an effect of vitamin C on CRP that is similar to that observed with statins.

The recent JUPITER trial (Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin) provided strong evidence of the benefit of lowering CRP. JUPITER enrolled over 15,000 healthy persons with elevations in CRP (> 2.0 mg/L) but with low or normal LDL-cholesterol, to test the hypothesis that long-term statin treatment could prevent cardiovascular disease in persons without cholesterol elevations [52]. The authors noted, "This issue is of exceptional clinical importance, as half of all vascular events occur among those with normal or even low levels of LDL-C" [52]. They went on to say that "a strong positive finding from JUPITER will dramatically affect public health and prevention and would provide a clear rationale for much broader use of statin therapy for the primary prevention of cardiovascular events than currently endorsed." JUPITER was stopped early because of unequivocal evidence of reduction in cardiovascular mortality and morbidity (URL: <http://www.cardiosource.com/rapid-newssummaries/summary.asp?SumID=318>. Accessed 30 September 2008. Archived by WebCite at <http://www.webcitation.org/5bDxL9su8>). This positive finding provides strong evidence that lowering CRP has a major cardiovascular benefit. Therefore, the CRP-lowering effects of vitamin C, to an extent virtually identical to that of statins, are of potential clinical importance and warrant further investigation.

Given the continuing epidemics of obesity and diabetes in the United States [53], and the demonstrated difficulty in achieving population-wide weight loss, strategies are needed to reduce the sequelae of obesity. Thus, future studies to determine whether vitamin C can reduce some of the inflammation-related adverse consequences of obesity should be considered. Such trials should focus on individuals with elevations (≥ 1.0 mg/L) in CRP, because studies with low-risk persons are less likely to show an effect, resulting in misleading outcomes. If persons with lower CRP levels must be included, separate randomization of those with CRP ≥ 1.0 mg/L would justify separate examination of this subgroup, assuming adequate power in this stratum. In addition, if the potential independent effect of vitamin C is to be determined, it would be necessary to exclude persons who are taking other anti-inflammatory drugs (except low-dose aspirin for heart disease prevention) and to exclude users of multiple vitamins (something which has not been done in most large antioxidant trials), because multiple vitamins alone can raise plasma ascorbic acid levels substantially and make the control group insufficiently different from the active treatment group. Finally, it may be prudent to evaluate vitamin C alone, unpaired with vitamin E, as we found a weaker CRP-lowering effect with the combination than with vitamin C alone in our previous trial [22].

In conclusion, this study has found a significant effect of treatment for 2 months with 1000 mg/day vitamin C on plasma CRP, in nondiseased moderately overweight nonsmokers with baseline CRP ≥ 1.0 mg/L. The magnitude of the effect was similar to that of statins. There was no significant effect of vitamin E. These data represent the largest study to date on the effects of vitamins C and E on CRP and extend our previous findings in overweight active and passive smokers. They indicate that vitamin C should be further investigated for its potential for reducing chronic inflammation and its consequences. And they identify a threshold concentration above which there is a potential for reduction in CRP.

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