Reduction of C-Reactive Protein Levels Through Use of a Multivitamin

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PURPOSE: Elevated C-reactive protein levels are associated with the risk of cardiovascular disease and diabetes. We examined whether multivitamins reduce C-reactive protein levels.

METHODS: We performed a post hoc subgroup analysis of a 6-month, randomized, double-blind, placebo-controlled trial. Patients (n = 87; mean age, 53 years) for whom frozen plasma samples were available; who did not have an inflammatory condition at baseline; and who were not hospitalized, taking antibiotics, smoking, or starting statin therapy during the study were included. C-reactive protein and plasma vitamin levels were measured at baseline and 6 months.

RESULTS: At 6 months, C-reactive protein levels were significantly lower in the multivitamin group than in the placebo group (between-group difference = −0.91 mg/L; 95% confidence interval: −1.52 to −0.30; $P = 0.005$). The reduction in C-reactive protein levels was most evident in patients who had elevated levels (≥1.0 mg/L) at baseline. Of the six vitamins measured (C, E, B_6, B_{12}, folate, and beta carotene), only vitamin B_6 (baseline: $r = -0.31$, $P = 0.003$; 6 months: $r = -0.29$, $P = 0.006$) and vitamin C (baseline: $r = -0.25$, $P = 0.02$) were inversely associated with C-reactive protein level.

CONCLUSION: In a post hoc analysis of a randomized, double-blind, placebo-controlled study, multivitamin use was associated with lower C-reactive protein levels. Other similarly formulated multivitamins may yield comparable results. **Am J Med. 2003;115:702–707.** ©2003 by Excerpta Medica Inc.

Elevated C-reactive protein levels, a marker of systemic inflammation, is a risk factor for cardiovascular disease and diabetes (1–4). A recent small, uncontrolled study suggested that α-tocopherol (vitamin E) lowers C-reactive protein levels (5). Other studies have shown plasma concentrations of pyridoxal 5'-phosphate, a circulating form of vitamin B_6, to be inversely associated with C-reactive protein level, and ascorbate (vitamin C) to be negatively correlated with levels in patients with peripheral artery disease (6,7). Thus, we sought to determine if multivitamin supplementation reduces C-reactive protein levels.

METHODS

Study Design and Sample

We performed a post hoc analysis of frozen blood samples from the Cooper Complete Vitamin study, a randomized, double-blind, placebo-controlled trial that examined the effect of 6 months of multivitamin use on homocysteine levels and low-density lipoprotein oxidation. The multivitamin was a commercially available, 24-ingredient multivitamin/mineral formula (Cooper Complete, Dallas, Texas) (Table 1). Pill counts were performed to ensure compliance. All participants signed an informed written consent approved by The Cooper Institute Institutional Review Board.

Participants aged 30 to 70 years were recruited from the general public. Inclusion criteria in the parent study included good health, no vitamin or supplement use in the 6 weeks before study enrollment, and a homocysteine level >8 μmol/L for men and >7 μmol/L for women (8). Of the 192 patients who met these criteria, frozen plasma samples that were suitable for C-reactive protein analysis were available in 154 patients. Because there was no attempt to draw blood at a specific phase of the menstrual cycle and because C-reactive protein levels can fluctuate as much as 44% during the cycle (9), the present study was limited to men and postmenopausal women (n = 112). Postmenopause was defined as no natural menses or use of hormone replacement therapy for at least 1 year. Other exclusion criteria were hospitalization (n = 3), antibiotic treatment (n = 15), or new statin therapy (n = 4) during the study. We also excluded subjects with a baseline C-reactive protein level >10 mg/L, which was recently suggested to be related to acute inflammatory conditions (10), and because we did not have the opportunity to retest these subjects. As there was only 1 patient who smoked, and cigarette use has been associated with increased C-reactive protein levels (11), this patient was excluded.

Blood Collection and Analysis

Fasting venous blood samples were obtained at baseline and after 6 months. Samples were spun within 3 minutes of collection at 4°C and 1200 g, and stored at –70°C. Analyses were run in batches containing both pre- and postintervention samples. C-reactive protein levels were measured using a high-sensitivity assay on a Prospect

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Table 1. Composition of Multivitamin Supplement

Components	Daily Amount
Vitamin A (beta carotene)	5000 IU
Vitamin C	1000 mg
Vitamin D	400 IU
Vitamin E	800 IU
Vitamin K	25 μg
Thiamine	3 mg
Riboflavin	10 mg
Niacinamide	20 mg
Vitamin B_6	25 mg
Folic acid	800 μg
Vitamin B_{12}	400 μg
Biotin	300 μg
Pantothenic acid	10 mg
Iodine	150 μg
Magnesium	400 mg
Zinc	15 mg
Selenium	100 μg
Copper	2 mg
Chromium	100 μg
Potassium	400 mg
Choline	500 mg
Lycopene	10 mg
Lutein	6 mg
Coenzyme Q10	50 mg

nephelometer (Dade Division of Baxter Healthcare Corporation, Delaware, Maryland). The coefficient of variation for C-reactive protein in this analysis was 1.0. Plasma folate levels were measured by a microbial (*Lactobacillus casei*) assay in a 96-well plate (12,13). Plasma levels of vitamin B_6 were measured by the tyrosine decarboxylase apoenzyme method and vitamin B_{12} was measured by radioassay (Quantaphase II; Bio-Rad, Hercules, California) (14). Samples for plasma vitamin C were deproteinized with ice-cold 10% metaphosphoric acid before centrifugation and cold storage. The supernatant was purged with nitrogen and stored at –20°C in foil-covered tubes. Plasma vitamin C levels were determined using a spectrophotometer after derivatization with 2,4-dinitrophenylhydrazine. Vitamin E and beta carotene levels were measured in plasma and low-density lipoprotein following extraction by reverse-phase high-performance liquid chromatography (15). The plasma concentrations of vitamin E were standardized to total plasma lipids (cholesterol and triglycerides) (16).

Statistical Analysis

Spearman correlation was used to assess associations between C-reactive protein and vitamin levels. Changes in variables were normally distributed. The mean change in each variable was compared between treatment groups using analysis of covariance, with adjustment for age, body mass index, sex, hormone replacement therapy, statin use, and baseline value. Because only 1 participant reporting taking aspirin daily, we did not adjust for use of this medication. Results are reported as least squares adjusted means. For a more robust but unadjusted analysis, changes in C-reactive protein levels were computed and the differences between treatment groups were assessed with the Wilcoxon rank sum test.

Because 37% of the sample had baseline C-reactive protein levels <1.0 mg/L, which is considered a low-risk category for vascular events (10), changes in C-reactive protein level were also analyzed with stratification based on baseline level (< or ≥1.0 mg/L), adjusting for age, body mass index, sex, hormone replacement therapy, statin use, and baseline level. Further, we calculated the prevalence of a C-reactive protein level >3.0 mg/L, the cutpoint for high risk of cardiovascular disease, in both treatment groups at baseline and follow-up, and assessed between-group differences in the prevalence using the chi-squared test (10).

All statistical analyses were performed using SAS, version 8.1 (Cary, North Carolina). *P* values <0.05 (two-tailed) were considered statistically significant.

RESULTS

The post hoc study sample (n = 87) was predominantly male, middle-aged, and slightly overweight (Table 2), similar to the parent study sample in most characteristics except for sex distribution and hormone replacement therapy use. Eighty-five percent (n= 74) of participants were white, 9% (n = 8) were African American, and 6% (n = 5) were Hispanic. There were no significant differences in baseline C-reactive protein or plasma vitamin levels between the multivitamin and placebo groups (Table 2; Figure 1).

After 6 months of intervention, the median change in C-reactive protein level was –0.18 mg/L in the multivitamin group compared with 0.06 mg/L in the placebo group ($P = 0.01$; Figure 1). After adjusting for age, body mass index, sex, hormone replacement therapy, statin use, and baseline C-reactive protein level (Table 3), the mean change in C-reactive protein level and all measured plasma vitamin concentrations was significantly greater in the multivitamin group.

In the multivitamin group, the patient with the highest baseline C-reactive protein level (9.45 mg/L) also had the largest decrease at 6 months (–8.1 mg/L), which may have been due to an inflammatory process that resolved between baseline and follow-up. To ensure that this result did not affect the analysis, analyses were repeated with these data excluded. The adjusted mean change in the multivitamin group increased slightly from –0.70 mg/L to –0.56 mg/L, and the between-group comparison remained significant at $P = 0.007$. Because hormone replacement therapy increases C-reactive protein levels, we

Table 2. Baseline Characteristics and Select Plasma Vitamin Levels of Study Participants

Characteristic	Placebo (n = 44)	Multivitamin (n = 43)	Parent Study (n = 192)
	Mean ± SD or Number (%)		
Age (years)	54.3 ± 9.5	52.6 ± 9.9	50.5 ± 9.9
Male sex (%)	28 (64)	31 (72)	78 (42)
Body mass index (kg/m^2)	27.5 ± 3.8	26.9 ± 3.5	26.3 ± 4.0
Current hormone replacement therapy use in women	7/16 (44)	5/12 (42)	16/104 (15)
C-reactive protein (mg/L)	2.44 ± 2.57	2.19 ± 2.14	NA
Vitamin B_6 (nmol/L)*	67.3 ± 70.5	85.0 ± 75	71.7 ± 68.1
Vitamin C (μmol/L)†	0.56 ± 0.32	0.58 ± 0.33	0.60 ± 0.32
Vitamin E (μmol/mmol/lipid)‡	23.3 ± 7.0	25.4 ± 8.4	23.1 ± 7.8
Vitamin B_{12} (pmol/L)	381.3 ± 124.0	400.5 ± 141.0	396.4 ± 130.9
Beta carotene (μmol/mmol)§	0.27 ± 0.32	0.31 ± 0.24	0.29 ± 0.28
Folate (nmol/L)‖	12.8 ± 7.2	14.9 ± 6.8	14.4 ± 8.7
Homocysteine (nmol/L)	8.2 ± 3.0	9.3 ± 2.7¶	8.3 ± 2.94
Cholesterol (mg/L)	207.8 ± 39.1	208.1 ± 36.9	203.9 ± 38.7#
HDL (mg/L)**	53.4 ± 15.6	52.4 ± 12.3	57.7 ± 15.3
Hemoglobin A_{1C} (%)	4.8 ± 1.9	4.7 ± 1.9	4.8 ± 1.6

* Pyridoxal 5'-phosphate.
† Ns are 43 for the placebo group, 41 for the multivitamin group, and 171 in the parent study.
‡ Ns are 42 for the placebo group and 174 for the parent study.
§ Ns are 43 for the placebo group and 174 in the parent study.
‖ Ns are 43 for the placebo group, 42 for the multivitamin group, and 180 in the parent study.
¶ N = 42.
# N = 152.
** Ns are 39 for the placebo group, 30 for the multivitamin group, and 152 in the parent study.
HDL = high-density lipoprotein; NA = not available.

repeated the analysis excluding women who used hormone replacement therapy (n = 12). The adjusted mean change in the multivitamin group increased from –0.70 mg/L to –0.47 mg/L, but the between-group comparison remained significant at $P = 0.04$.

At baseline, vitamin B_6 ($r = -0.31$, $P = 0.003$) and vitamin C ($r = -0.25$, $P = 0.02$) were inversely associated with C-reactive protein levels (Table 4), whereas at 6 months, only vitamin B_6 was associated with C-reactive protein ($r = -0.29$, $P = 0.006$). There were no significant correlations between changes in any of the measured plasma vitamin and C-reactive protein levels. Further, adjustment for baseline values of and changes in vitamins B_6 and C levels did not significantly attenuate the change

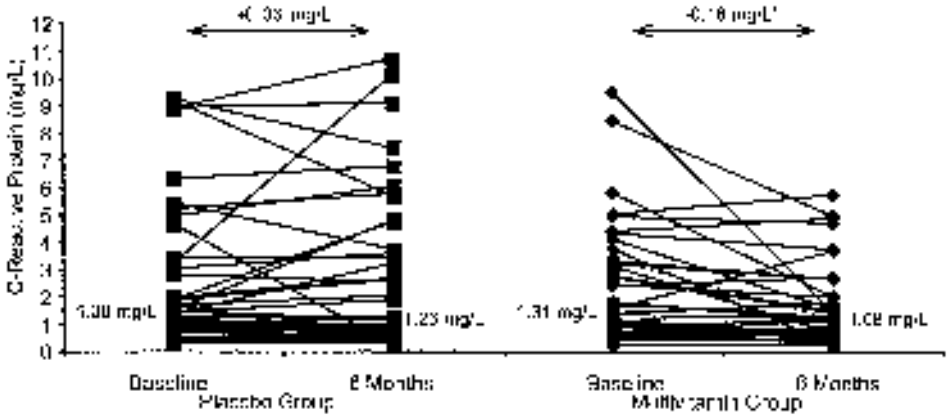

Figure 1. C-reactive protein levels at baseline and after 6 months of intervention in the placebo (n = 44) and multivitamin (n = 43) groups. Numbers adjacent to the scatter bars represent median values. Numbers above the scatter bars represent median change. The asterisk (*) indicates $P = 0.01$ for between-group comparisons of changes.

Table 3. Change in C-Reactive Protein and Plasma Vitamin Levels after 6 Months of Intervention, by Study Group

Variable	Placebo	Multivitamin	Mean Between-Group Difference* (95% Confidence Interval)
	Mean Change from Baseline to 6 Months* (95% Confidence Interval)		
C-reactive protein (mg/L)	0.21 (−0.22 to 0.64)	−0.70 (−1.11 to −0.26)	−0.91 (−1.52 to −0.30)†
Vitamin C (μmol/L)	0.12 (0.01 to 0.24)	0.31 (0.19 to 0.43)	0.19 (0.03 to 0.35)‡
Vitamin E (μmol/mmol/lipid)§	−2.7 (−5.8 to 0.5)	23.0 (19.9 to 26.1)	25.7 (21.3 to 30.1)
Vitamin B_6 (nmol/L)	16.1 (−28.0 to 60.2)	194.9 (150.3 to 239.5)	178.8 (116.6 to 241.0)
Vitamin B_{12} (pmol/L)	14.5 (−38.6 to 67.7)	175.9 (122.1 to 229.7)	161.4 (86.5 to 236.3)
Beta carotene (μmol/mmol)	−0.01 (−0.04 to 0.03)	0.11 (0.08 to 0.15)	0.12 (0.07 to 0.17)
Folate (nmol/L)	0.70 (−1.42 to 2.85)	5.41 (3.24 to 7.57)	4.69 (1.68 to 7.7)‡

* Adjusted for baseline level, age, sex, body mass index, statin use, and hormone replacement therapy.
† $P < 0.01$.
‡ $P < 0.05$.
§ Vitamin E adjusted for plasma cholesterol and triglyceride levels.

in C-reactive protein level in the multivitamin group (unadjusted, −0.70 mg/L; 95% confidence interval [CI]: −1.1 to −0.26 mg/L; vs. adjusted, −0.73 mg/L; 95% CI: −1.20 to −0.26 mg/L).

When results were stratified by baseline C-reactive protein level, we found that there was no change in C-reactive protein level in either the multivitamin or placebo group among subjects with a baseline level <1.0 mg/L (Figure 2). Among patients with baseline levels ≥1.0 mg/L, those who took multivitamins had a significant decrease in C-reactive protein level compared with those who took placebo. The prevalence of subjects with a high-risk C-reactive protein level (>3.0 mg/L) was similar at baseline between the multivitamin (30%) and placebo (27%) groups. After the intervention, the prevalence of a C-reactive protein level >3.0 mg/L decreased to 14% in the multivitamin group but increased to 32% in the placebo group ($P < 0.05$ for difference at 6 months).

DISCUSSION

In this post hoc analysis of a randomized, double-blind, placebo-controlled study, 6 months of multivitamin use was associated with a reduction in C-reactive protein levels. This finding is important because elevated levels of C-reactive protein are associated with an increased risk of cardiovascular disease and diabetes (1–4). However, despite recommendations for the widespread use of multivitamins (17,18), there have been no large, placebo-controlled, randomized studies to assess the effects of multivitamins on morbidity and mortality. Furthermore, the U.S. Preventive Services Task Force recently concluded that there is insufficient evidence to support the use of vitamin A, C, and E supplementation; multivitamins and folic acid; or antioxidant combinations in the prevention of cancer or cardiovascular disease (19).

Previous studies have found statin therapy to lower median C-reactive protein levels by 14% to 28% (20,21). Even though these studies reported higher baseline C-reactive protein levels (≥2.0 mg/L) than in our study, we found a similar decrease of 14% with multivitamin use. It is not known what this decrease means clinically; however, our finding that the percentage of patients in the multivitamin group with C-reactive protein levels >3.0 mg/L decreased from 30% to 14%, and that patients taking multivitamins with higher baseline levels of C-reac-

Table 4. Spearman Correlations for C-Reactive Protein and Plasma Vitamin Levels at Baseline and Follow-up, and for Change in C-Reactive Protein and Plasma Vitamin Levels

Variable	Baseline		Follow-up		Change in Vitamin vs. C-Reactive Protein Levels	
	r	*P* Value	*r*	*P* Value	*r*	*P* Value
Vitamin B_6	−0.31	0.003	−0.29	0.006	−0.13	0.21
Vitamin C	−0.25	0.02	−0.04	0.81	−0.17	0.11
Vitamin E*	0.16	0.13	0.04	0.73	−0.08	0.44
Vitamin B_{12}	−0.03	0.74	0.03	0.80	−0.30	0.76
Beta carotene	−0.12	0.26	−0.17	0.1	−0.18	0.10
Folate	0.06	0.55	−0.12	0.25	−0.10	0.34

* Vitamin E adjusted for plasma cholesterol and triglyceride levels.

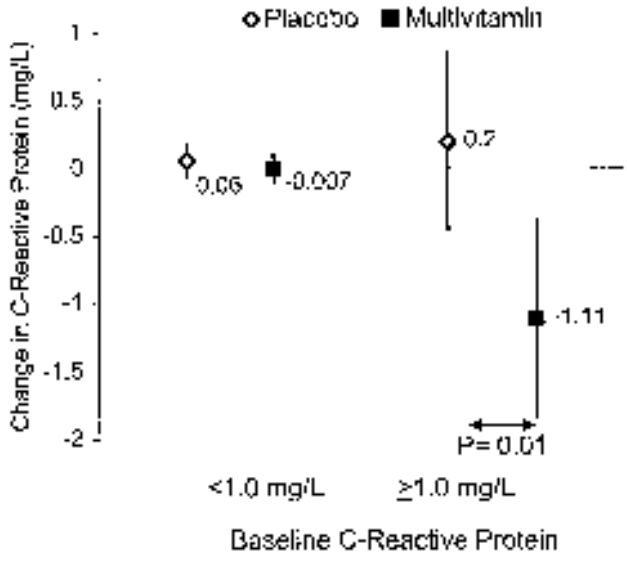

Figure 2. Mean change in C-reactive protein levels after 6 months of intervention in the placebo and multivitamin groups, by baseline C-reactive protein level, after adjustment for baseline C-reactive protein level, age, body mass index, sex, hormone replacement therapy, and statin use. Error bars represent 95% confidence intervals.

tive protein had the biggest reduction in levels, suggests that multivitamin supplementation may be most effective in patients with elevated baseline levels.

We examined the relation of six different vitamins to C-reactive protein level. We found a negative association between vitamin C and C-reactive protein levels, although the correlation was weaker than that reported by Langlois et al (7). However, that study included patients with peripheral artery disease who had much lower vitamin C but higher C-reactive protein levels. In addition, there was a greater variation in values in that study. Although we found vitamins B_6 and C to be inversely associated with C-reactive protein, we did not find an association between the change in C-reactive protein level and the change in any of the plasma vitamin concentrations measured. It may be that there was insufficient power to perform this analysis given the variability in C-reactive protein level. Furthermore, we only measured the plasma concentration of six vitamins, and the multivitamin contained 24 ingredients. The selection of these six vitamins was based on the main outcomes of the parent study that focused on homocysteine and low-density lipoprotein oxidation. Other vitamins in the formulation may be associated with C-reactive protein level. Additionally, the change in C-reactive protein level may have been the result of a combination of vitamins as opposed to just one component.

This study included very good data on monthly health status, including changes in medications or smoking habits, pill counts, and measures of plasma vitamin concentrations to ensure compliance. However, several limitations should be considered. We did not schedule the blood draws for the same phase of the menstrual cycle and consequently could not include premenopausal women in the analysis. Since we performed a post hoc analysis, there were a large number of exclusion criteria. However, the exclusion criteria were common to studies of C-reactive protein, such as recent illness or changes in medications that affect C-reactive protein levels, and all criteria were selected before the examination of C-reactive protein data. In addition, we tested only one type of multivitamin; however, there were no proprietary ingredients in this formulation.

In conclusion, in a post hoc subgroup analysis of a randomized, double-blind, placebo-controlled trial, use of a commercially available multivitamin was found to reduce C-reactive protein levels. Other similarly formulated multivitamins may yield comparable results.

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