

# Effects of long-term antioxidant supplementation and association of serum antioxidant concentrations with risk of metabolic syndrome in adults<sup>1–3</sup>

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## ABSTRACT

**Background:** Limited observational evidence suggests lower antioxidant concentrations in individuals with the metabolic syndrome (MetS); few randomized controlled trials have addressed the effect of multiple antioxidants on the risk of MetS.

**Objective:** The objective was to examine the effect of antioxidant supplementation for 7.5 y on the incidence of MetS and the epidemiologic association between baseline serum antioxidant concentrations and the prospective risk of MetS.

**Design:** Adults ( $n = 5220$ ) participating in the SUPplementation en Vitamines et Minéraux AntioXydants (SU.VI.MAX) primary prevention trial were randomly assigned to receive a supplement containing a combination of antioxidants (vitamins C and E,  $\beta$ -carotene, zinc, and selenium) at nutritional doses or a placebo. Subjects were free of MetS at baseline and were followed for 7.5 y.

**Results:** Antioxidant supplementation for 7.5 y did not affect the risk of MetS. Baseline serum antioxidant concentrations of  $\beta$ -carotene and vitamin C, however, were negatively associated with the risk of MetS; the adjusted odds ratios (and 95% CIs) for the highest compared with the lowest tertile were 0.34 (0.21, 0.53;  $P$  for trend = 0.0002) and 0.53 (0.35, 0.80;  $P$  for trend = 0.01), respectively. Baseline serum zinc concentrations were positively associated with the risk of developing MetS; the adjusted odds ratio (and 95% CI) for the highest compared with the lowest tertile was 1.81 (1.20, 2.72;  $P$  for trend = 0.01).

**Conclusions:** The experimental finding of no beneficial effects of antioxidant supplementation in a generally well-nourished population is consistent with recent reports of a lack of efficacy of antioxidant supplements. However, the relations observed between the risk of MetS and baseline serum antioxidant concentrations, which probably reflect associations with overall dietary patterns, do support the current recommendations to consume antioxidant-rich foods. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT00272428. *Am J Clin Nutr* 2009;90:329–35.

## INTRODUCTION

The metabolic syndrome (MetS) represents a clustering of abnormalities typically involving abdominal obesity, insulin resistance, dyslipidemia, and hypertension (1). MetS is common in adults (2, 3), and the risk of MetS increases with age (2). MetS has been related to an increased risk of development of type 2 diabetes and of subclinical and overt cardiovascular disease (CVD) morbidity and mortality (3–7). Several MetS components are characterized by an increased production of reactive oxygen

species and reactive nitrogen species and subsequent oxidative stress (8–12). Antioxidants, including vitamins such as  $\beta$ -carotene, vitamin C, and vitamin E, and the minerals selenium and zinc can diminish the oxidative process by inactivating free radicals and may protect the organism against MetS and its associated complications. Indeed, several case-control and prospective observational studies have suggested an inverse association between antioxidant intake and serum concentrations with individual components of MetS singly (13–20). However, few studies have examined the relation of serum antioxidants with MetS as an entity, which suggests reduced antioxidant concentrations in individuals with MetS (21, 22); furthermore, prospective studies that have examined this association are scarce. In addition, the effect of antioxidant supplementation on MetS incidence has not been examined extensively, particularly with randomized-controlled trials involving a relatively long period of follow-up, especially in the general population. This question is particularly important given that the prevalence of supplement use by adults is high [35–40% in the third National Health and Nutrition Examination Survey (NHANES III)] (23).

The randomized placebo-controlled SU.VI.MAX (SUPplementation en Vitamines et Minéraux AntioXydants) trial (24, 25) provides a unique opportunity for the assessment of the long-term effect of supplementation with a combination of antioxidant vitamins and minerals at nutritional doses in generally healthy

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Received February 13, 2009. Accepted for publication May 8, 2009.

First published online June 2, 2009; doi: 10.3945/ajcn.2009.27635.

middle-aged subjects on the incidence of MetS. Furthermore, because antioxidant concentrations were measured at baseline, the SU.VI.MAX trial allows the prospective examination of the association of MetS with baseline serum concentrations of these antioxidants. On the basis of SU.VI.MAX data, we reported that antioxidant supplementation had no effect on fasting plasma glucose or hypertension in this study population (19, 20), whereas adverse indications for blood lipids were noted (hypercholesterolemia in men and women and a trend for hypertriglyceridemia in women) in the supplement group (26). Interestingly, baseline concentrations of  $\beta$ -carotene and vitamin C were consistently negatively associated with plasma glucose and blood pressure in these analyses (19, 20). Because MetS represents a cluster of various risk factors for CVD, and differential effects with antioxidant supplementation were noted on MetS components in our previous analyses, our interest was to examine the effect of multinutrient antioxidant supplementation on MetS as an entity and to examine the prospective association of baseline serum antioxidant concentrations with the risk of MetS; to our knowledge, these questions have not been examined previously.

## SUBJECTS AND METHODS

### Subjects

The French SU.VI.MAX study is a randomized double-blind, placebo-controlled, primary prevention trial initially designed to test the effectiveness of supplementation with antioxidant vitamins and minerals at reducing the incidence of CVD and cancer (24, 25). From 1994, all 13,017 recruited participants underwent a yearly visit with alternating blood sampling (at baseline) or clinical examination (from 1995 to 1996) every other year until 2001–2002. This study was approved by the National Ethical Committee (CCPPRB number 706). Supplementation included either a combination of antioxidants at nutritional doses (120 mg vitamin C, 30 mg vitamin E, 6 mg  $\beta$ -carotene, and 100  $\mu$ g Se in the form of selenium-enriched yeast) and 20 mg zinc (as gluconate) or a matching placebo in a single daily capsule. The mean ( $\pm$  SD) follow-up time was  $7.5 \pm 0.3$  y.

We performed post hoc analyses in subjects with available data at baseline and after 7.5 y of follow-up on MetS-related variables ( $n = 6152$ ). For the current analysis, we excluded subjects with MetS at baseline ( $n = 348$ ) to determine incident MetS cases as the primary outcome variable. We further excluded subjects with missing values for confounders ( $n = 584$ ). Therefore, the final sample used to examine the intervention effect was 5220 men and women. From this sample, a subsample of 3336 subjects for whom serum antioxidants were measured at baseline was used to assess the associations between serum antioxidants concentrations and the risk of MetS.

### Biological and clinical variables

We used data collected at the baseline assessment, blood-based tests (1994–1995) and clinical examination (1995–1996) to ensure the absence of MetS at the onset of study and data collected at the last medical visit (both biochemical and clinical, from January 2001 to June 2002) to characterize MetS status at the end of follow-up. Blood samples were obtained after a 12-h fast; all

biochemical measurements were centralized in a single laboratory. Fasting blood glucose and total cholesterol were measured by using an enzymatic method (Advia 1650; Bayer Diagnostic, New York, NY) at baseline and at the end of follow-up.

For the measurement of vitamin C, whole venous blood was collected into heparin-containing tubes and immediately centrifuged, and 0.5 mL plasma was diluted 1/10 with 4.5 mL of an aqueous 5% metaphosphoric acid solution before freezing. Serum ascorbic acid was determined by using an automated method based on the principle of continuous flow (27). Serum concentrations of retinol,  $\beta$ -carotene, and tocopherol were measured by HPLC (Biotech-Kontron, Montigny-le-Bretonneux, France). Serum concentrations of zinc were measured by using flame atomic absorption spectrometry (model 3110; Perkin Elmer, Norwalk, CT) and of selenium by Zeeman background electrothermal atomic absorption spectrometry (4100 ZL; Perkin Elmer).

Waist circumference and blood pressure measurement protocols and the questionnaires used at baseline to assess education, smoking status, and physical activity were described elsewhere (24).

### Compliance and effectiveness of supplement

Compliance was monitored by asking the subjects to complete monthly questionnaires about treatment compliance and health events via Minitel (a French telephone based terminal), Internet, or mail (25). At the end of follow-up, 74% of participants reported having taken at least two-thirds of the capsules in the total sample. The corresponding percentage observed in the final sample of 5220 subjects used for these analyses was even higher (83%); no differences in capsule consumption were noted between the placebo and intervention groups. Compliance was confirmed by examining plasma concentrations of antioxidants at 2 and 7 y of follow-up in a subcohort selected from the baseline sample. Compared with baseline, the concentrations of all antioxidants examined increased significantly from baseline at both time points in the intervention group (25). Furthermore, the antioxidant concentrations were significantly higher in the intervention group than in the placebo group at both 2 and 7 y of follow-up (25).

Definition of MetS According to the third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (1), the subjects were considered to have MetS if they had  $\geq 3$  of the following 5 characteristics: 1) waist circumference  $>102$  cm in men and  $>88$  cm in women, 2) serum triglycerides  $\geq 150$  mg/dL (1.69 mmol/L); 3) HDL cholesterol  $<40$  mg/dL (1.04 mmol/L) in men and  $<50$  mg/dL (1.29 mmol/L) in women; 4) systolic blood pressure  $\geq 130$  and/or diastolic blood pressure  $\geq 85$  mm Hg, and 5) fasting glucose  $\geq 110$  mg/dL (6.1 mmol/L). Subjects using antidiabetic (oral agents or insulin) or antihypertensive or lipid-lowering medications were considered to have elevated fasting blood glucose concentrations or elevated blood pressure or dyslipidemia, respectively.

### Statistical methods

The results are expressed as percentages or means ( $\pm$ SD) for variables that were normally distributed. Serum concentrations



of triglycerides,  $\beta$ -carotene, and vitamin C were log transformed because of skewed distributions; geometric means and 95% CIs are presented for these variables. Baseline characteristics and antioxidant concentrations of the placebo and antioxidant intervention groups were compared by Student's *t* test, chi-square test, or Fisher's exact test as appropriate. Logistic regression models were used to estimate the odds ratios (ORs) for developing MetS after 7.5 y of follow-up according to the intervention group. Age and sex-adjusted incident risk of MetS was examined in the intervention as compared with the placebo group. Further multivariate models were run by including education, smoking, physical activity, and alcohol consumption. For fat-soluble vitamins, we reran these analyses by including serum triglycerides and total cholesterol concentrations as additional adjustment factors, as suggested by Ford et al (22). We also calculated tertiles of serum baseline antioxidant concentrations and computed the OR (95% CI) of incident MetS risk according to these tertiles using models adjusted for age, sex, and intervention group as well as multivariate-adjusted models as described above. The interactions of sex  $\times$  baseline antioxidant concentrations were first examined and found to not be significant. All statistical analyses were carried out by using SAS software (version 8.02; SAS Institute Inc, Cary, NC).  $P < 0.05$  was used as the level of significance.

## RESULTS

### Baseline characteristics and antioxidant concentrations

The placebo and intervention groups did not differ with respect to any sociodemographic or lifestyle variables examined, with the exception of physical activity (Table 1). No difference was observed between the groups with respect to baseline antioxidant concentrations (Table 1). Furthermore, none of the MetS-related characteristics at baseline differed between the placebo and intervention groups (data not shown).

### Effect of antioxidant supplementation on MetS incident risk

After a mean follow-up of 7.5 y, there were 263 cases of MetS with an approximately equal number of events in the placebo and intervention groups (Table 2). Antioxidant supplementation for 7.5 y had no effect on the age- and sex-adjusted incident risk of MetS compared with the placebo group (Table 2). Adjustment for other potential confounders, such as education level, smoking, physical activity, and alcohol consumption, did not alter these findings. These results were unchanged when analyses were performed in "compliant" subjects who reported having taken at least two-thirds of the capsules.

### Association between baseline serum antioxidant concentrations and MetS incident risk

No interaction between baseline antioxidant concentration and sex was observed (Table 3). For serum  $\beta$ -carotene and vitamin C concentrations, consistent significant protective associations were observed with incident MetS in all models (Table 3). Specifically, in the multivariate-adjusted model, ORs (95% CI) for developing MetS in the second and third tertiles were 0.55 (0.38, 0.80) and 0.34 (0.21, 0.53), respectively, for  $\beta$ -carotene

( $P$  for trend = 0.0002) and 0.76 (0.53, 1.09) and 0.53 (0.35, 0.80), respectively, for vitamin C ( $P$  for trend = 0.01). There was no association between tertiles of vitamin E and the risk of MetS in the age- and sex-adjusted model and in the multivariate model ( $P$  for trend = 0.75). Because concentrations of fat-soluble vitamins are closely correlated with blood lipid concentrations, we accounted for baseline triglyceride and cholesterol concentrations by including them in the multivariate model, as was done previously (22) (data not shown). There was a significant protective association between baseline vitamin E concentrations and the risk of MetS with this fully adjusted model; the OR (and 95% CI) for tertile 3 compared with tertile 1 was 0.61 (0.39, 0.96). Furthermore, the significant association between  $\beta$ -carotene and the risk of MetS noted in the multivariate model persisted after further adjustment for serum triglycerides and cholesterol. These results were similar when analyses were performed in compliant subjects only.

With regard to baseline antioxidant mineral concentrations, no association was observed for selenium concentrations and the risk of MetS and, interestingly, an increased risk was observed with increasing baseline zinc concentrations in the models adjusted for potential confounders (Table 3).

### Characteristics of subjects with MetS at the end of follow-up

Characteristics of the subjects according to the diagnosis of MetS at the end of the follow-up period are shown in Table 4.

**TABLE 1**

Characteristics of subjects and baseline antioxidant concentrations by group

| Characteristic                   | Placebo<br>( <i>n</i> = 2525)  | Intervention<br>( <i>n</i> = 2695) | <i>P</i> <sup>1</sup> |
|----------------------------------|--------------------------------|------------------------------------|-----------------------|
| Male sex (%)                     | 38.1                           | 37.9                               | 0.90                  |
| Age (y)                          | 49.0 $\pm$ 6.2 <sup>2</sup>    | 49.1 $\pm$ 6.2                     | 0.59                  |
| Postmenopausal women (%)         | 26.1                           | 27.2                               | 0.49                  |
| Educational level (%)            |                                |                                    | 0.47                  |
| Elementary school                | 19.6                           | 19.6                               |                       |
| Secondary school                 | 39.1                           | 37.6                               |                       |
| University or equivalent         | 41.3                           | 42.8                               |                       |
| Smokers (%)                      | 12.7                           | 13.4                               | 0.78                  |
| Physical activity (%)            |                                |                                    | 0.01                  |
| Irregularly active               | 23.3                           | 22.9                               |                       |
| <1 h/d                           | 29.5                           | 33.1                               |                       |
| $\geq$ 1 h/d                     | 47.2                           | 44.0                               |                       |
| Alcohol consumption (%)          |                                |                                    | 0.78                  |
| 0 g/d                            | 36.0                           | 35.4                               |                       |
| <15 g/d                          | 24.5                           | 25.3                               |                       |
| $\geq$ 15 g/d                    | 39.5                           | 39.4                               |                       |
| Serum antioxidants <sup>3</sup>  |                                |                                    |                       |
| $\beta$ -Carotene ( $\mu$ mol/L) | 0.52 (0.50, 0.53) <sup>4</sup> | 0.51 (0.49, 0.52)                  | 0.39                  |
| Vitamin E ( $\mu$ mol/L)         | 31.3 $\pm$ 7.7                 | 31.1 $\pm$ 7.7                     | 0.48                  |
| Vitamin C ( $\mu$ g/mL)          | 9.20 (9.01, 9.41)              | 9.05 (8.86, 9.24)                  | 0.27                  |
| Selenium ( $\mu$ mol/L)          | 1.11 $\pm$ 0.20                | 1.12 $\pm$ 0.19                    | 0.15                  |
| Zinc ( $\mu$ mol/L)              | 13.0 $\pm$ 1.8                 | 13.0 $\pm$ 1.9                     | 0.42                  |

<sup>1</sup> Student's *t* tests were used for continuous variables and chi-square tests for categorical variables.

<sup>2</sup> Mean  $\pm$  SD (all such values).

<sup>3</sup> Analyses were performed in 3336 subjects.

<sup>4</sup> Geometric means and 95% CIs in parentheses because of skewed distributions (all such values).

**TABLE 2**

Incident risk of the metabolic syndrome (MetS) after 7.5 y of follow-up by group

| Outcome                                  | Placebo<br>(n = 2525) | Intervention<br>(n = 2695)     | P    |
|--|-----------------------|--------------------------------|------|
| Incident MetS risk [n (%)]               | 130 (5.1)             | 133 (4.9)                      | 0.72 |
| Age- and sex-adjusted model              | 1                     | 0.95 (0.74, 1.22) <sup>1</sup> | 0.70 |
| Multivariate-adjusted model <sup>2</sup> | 1                     | 0.93 (0.73, 1.20)              | 0.60 |

<sup>1</sup> Odds ratio; 95% CI in parentheses (all such values).<sup>2</sup> Adjusted for age, sex, educational level, smoking status, physical activity, and alcohol consumption.

Compared with the subjects who did not develop MetS, the subjects who developed MetS during follow-up were more often men or postmenopausal women, were older, were current smokers, were less educated, were less physically active, and had a higher BMI. As expected, subjects with MetS were more likely to be taking antihypertensive medication than were those without MetS.

## DISCUSSION

This randomized trial involving long-term supplementation with multiple antioxidant vitamins and minerals at physiologic doses did not show an effect of combined antioxidant supplements on the incidence of MetS, in agreement with the findings from previous trials of antioxidants and cardiovascular disease risk. Of interest, however, was the finding that baseline concentrations of some of the antioxidants examined, particularly  $\beta$ -carotene and vitamin C and to some extent vitamin E, were negatively associated and zinc was positively associated with incident MetS risk.

**TABLE 3**

Incident risk of the metabolic syndrome after 7.5 y of follow-up according to baseline serum antioxidant concentrations (n = 3336)

|  | Tertile 1 | Tertile 2                      | Tertile 3         | P for trend | Sex $\times$ baseline antioxidant concentration interaction |
|--|-----------|--------------------------------|-------------------|-------------|---|
| <b><math>\beta</math>-Carotene</b>         |           |                                |                   |             |   |
| Median (mmol/L)                            | 0.29      | 0.52                           | 0.91              |             | 0.43  |
| Age-, sex-, and intervention-adjusted risk | 1         | 0.55 (0.38, 0.79) <sup>1</sup> | 0.32 (0.21, 0.50) | <0.0001     |   |
| Multivariate-adjusted risk <sup>2</sup>    | 1         | 0.55 (0.38, 0.80)              | 0.34 (0.21, 0.53) | 0.0002      |   |
| <b>Vitamin E</b>                           |           |                                |                   |             |   |
| Median (mmol/L)                            | 24.4      | 30.3                           | 37.6              |             | 0.29  |
| Age-, sex-, and intervention-adjusted risk | 1         | 0.95 (0.65, 1.39)              | 1.02 (0.70, 1.48) | 0.80        |   |
| Multivariate-adjusted risk <sup>2</sup>    | 1         | 0.94 (0.64, 1.38)              | 1.02 (0.70, 1.49) | 0.75        |   |
| <b>Vitamin C</b>                           |           |                                |                   |             |   |
| Median (mg/mL)                             | 6.47      | 9.93                           | 12.78             |             | 0.60  |
| Age-, sex-, and intervention-adjusted risk | 1         | 0.74 (0.52, 1.06)              | 0.50 (0.33, 0.76) | 0.005       |   |
| Multivariate-adjusted risk <sup>2</sup>    | 1         | 0.76 (0.53, 1.09)              | 0.53 (0.35, 0.80) | 0.01        |   |
| <b>Selenium</b>                            |           |                                |                   |             |   |
| Median (mmol/L)                            | 0.94      | 1.10                           | 1.28              |             | 0.83  |
| Age-, sex-, and intervention-adjusted risk | 1         | 1.07 (0.73, 1.55)              | 0.93 (0.63, 1.37) | 0.54        |   |
| Multivariate-adjusted risk <sup>2</sup>    | 1         | 1.09 (0.75, 1.59)              | 1.00 (0.68, 1.48) | 0.79        |   |
| <b>Zinc</b>                                |           |                                |                   |             |   |
| Median (mmol/L)                            | 11.3      | 12.9                           | 14.6              |             | 0.77  |
| Age-, sex-, and intervention-adjusted risk | 1         | 1.50 (0.99, 2.29)              | 1.82 (1.22, 2.74) | 0.01        |   |
| Multivariate-adjusted risk <sup>1</sup>    | 1         | 1.46 (0.96, 2.23)              | 1.81 (1.20, 2.72) | 0.01        |   |

<sup>1</sup> Odds ratio; 95% CI in parentheses (all such values).<sup>2</sup> Adjusted for age, sex, intervention group, educational level, smoking status, physical activity, and alcohol consumption.

The subjects with MetS at the end of follow-up had more unhealthy behaviors than did subjects without MetS; as expected, they were more often current smokers, were less physically active, had a higher BMI, and were more likely to be taking medications, particularly antihypertensive medications. MetS (8, 28) and several of its components (9–11) have been shown to be associated with increased oxidative stress. Increased oxidative stress has been implicated in the pathophysiology of diabetes, obesity, and CVD (29, 30). Serum antioxidant concentrations have been shown to be negatively associated with several components of MetS, such as obesity (31, 32), hypertension (19, 33), hyperglycemia, and/or type 2 diabetes (14, 20, 34) and dyslipidemia (35). However, the association with the clustering of abnormalities represented by MetS has been less widely investigated (22, 36).

In the current study, we examined the prospective associations of certain antioxidant vitamins and minerals (selenium and zinc), with the risk of MetS after 7.5 y of follow-up. Our findings of a negative association between baseline  $\beta$ -carotene and vitamin C concentrations and risk of incident MetS as well as no association between selenium concentrations and incident MetS are consistent with the findings of Ford et al (22) and in contrast with those reported by Obeid et al (21). In addition, on further adjustment for serum cholesterol and triglycerides, the association of baseline  $\beta$ -carotene with MetS was strengthened and that of baseline vitamin E concentrations with MetS became significant; the latter was also observed in the NHANES III data (22).

With respect to the positive association between zinc and the risk of MetS, the observed increased risk could be related to the involvement of zinc in numerous biological processes, including its possible negative association with HDL-cholesterol concentrations (37) and its role in physiopathological processes not

**TABLE 4**  
Characteristics of the population at the end of follow-up according to metabolic syndrome diagnosis

|  | Without metabolic syndrome<br>(n = 4957) | With metabolic syndrome<br>(n = 263) | P <sup>1</sup> |
|--|--|--------------------------------------|----------------|
| Male sex (%)   | 37.3                                     | 50.2                                 | <0.0001        |
| Age (y)  | 55.5 ± 6.2 <sup>2</sup>                  | 57.2 ± 5.8                           | <0.0001        |
| Postmenopausal women (%) (n = 3137)                  | 63                                       | 74                                   | 0.01           |
| Educational level (%)                                |  |                                      | <0.0001        |
| Elementary school                                    | 19.1                                     | 30                                   |                |
| Secondary school                                     | 38.4                                     | 35.7                                 |                |
| University or equivalent                             | 42.5                                     | 34.2                                 |                |
| Smokers (%) (n = 4930)                               | 11.3                                     | 17.3                                 | 0.003          |
| Physical activity (%)                                |  |                                      | 0.0002         |
| Irregularly active                                   | 33.7                                     | 40.3                                 |                |
| <1 h/d   | 36.4                                     | 39.2                                 |                |
| ≥1 h/d   | 29.9                                     | 20.5                                 |                |
| BMI (kg/m <sup>2</sup> ) (n = 5006)                  | 24.0 ± 3.3                               | 29.6 ± 4.3                           | <0.0001        |
| Waist circumference (cm) (n = 4925)                  | 80.9 ± 10.9                              | 97.7 ± 9.7                           | <0.0001        |
| Serum glucose (mmol/L) (n = 5142)                    | 4.97 ± 0.53                              | 5.71 ± 1.64                          | <0.0001        |
| Serum triglycerides (mmol/L) (n = 5143) <sup>3</sup> | 0.88 (0.87, 0.89)                        | 1.81 (1.72, 1.90)                    | <0.0001        |
| Serum HDL cholesterol (mmol/L) (n = 5216)            | 1.67 ± 0.39                              | 1.12 ± 0.23                          | <0.0001        |
| Systolic blood pressure (mm Hg) (n = 4951)           | 127.8 ± 14.5                             | 137.0 ± 13.0                         | <0.0001        |
| Diastolic blood pressure (mm Hg) (n = 4951)          | 81.2 ± 9.1                               | 86.1 ± 8.5                           | <0.0001        |
| Medication use (%)                                   |  |                                      | <0.0001        |
| Antihypertensive and lipid-lowering drugs            | 2.7                                      | 8                                    |                |
| Antidiabetic drugs only                              | 0.6                                      | 3                                    |                |
| Antihypertensive drugs only                          | 8.6                                      | 18.6                                 |                |
| Lipid-lowering drugs only                            | 7.4                                      | 10.3                                 |                |
| No drugs   | 80.7                                     | 60.1                                 |                |

<sup>1</sup> Student's *t* tests were used for continuous variables and chi-square tests for categorical variables.

<sup>2</sup> Mean ± SD (all such values).

<sup>3</sup> Geometric means and 95% CIs in parentheses because of skewed distributions.

directly related to MetS. Another hypothesis is that the epidemiologic associations noted between baseline concentrations of several antioxidant vitamins may reflect their strong association with intake of fruit, vegetables, and other plant foods, whereas the association of serum zinc and MetS could reflect less healthy eating habits such as high red meat consumption. This hypothesis is supported by evidence from epidemiologic data, which suggest benefits of fruit and vegetables, nuts, and other healthy dietary patterns, including the Mediterranean diet, on MetS and its components (38).

Importantly, no beneficial or adverse effects of long-term supplementation with multiple antioxidants were noted on the incidence of MetS in the current study. This finding is in line with the findings of most primary and secondary prevention trials, which found no beneficial effect of antioxidant supplementation on CVD risk factors or CVD (25, 39), except for the Cambridge Heart Antioxidant Study (40). The lack of an effect of antioxidant supplementation on the risk of MetS in the current study is consistent with the findings of large-scale prevention trials in persons with and without type 2 diabetes, which reported no beneficial effect of vitamin E and  $\beta$ -carotene supplementation at pharmacologic doses on diabetes incidence and cardiovascular outcomes and potential risks associated with high doses of antioxidants (33, 41, 42).

One of the main strengths of the current study was its large sample of subjects from the general population who were followed for a long period prospectively for the incidence of several health outcomes. In addition, in contrast with most of previous prevention trials, low-nutritional doses of antioxidants were used

as the intervention (25). This made the study attractive because this type of supplementation is more likely to reflect the supplementation practices of generally healthy individuals, and such intakes can be attained via healthy dietary choices. Furthermore, the analyses for several antioxidants and all other biochemical tests were centralized in the same laboratory and had adequate internal and external quality controls, which reduced the likelihood of measurement error. In addition, we examined the data as both an intervention trial and observational study to better understand the relation of antioxidants with MetS.

Potential limitations should also be considered. The participants in this study may have had healthier diets and lifestyles than the population at large, which could affect the generalizability of the study findings. Furthermore, all antioxidants were measured only once at each time point. We may not have adequately captured the intraindividual variability in the measures, which thereby may have resulted in the misclassification of the antioxidant status of participants. Another possible limitation was that of causal inference from post hoc analyses from a study that was not specifically designed to test the effect of antioxidants on MetS. Thus, the present findings should be considered exploratory and warrant further investigation.

Taken together, our results, which were obtained in a healthy population with no major risk of micronutrient deficiencies, suggest that low concentrations of  $\beta$ -carotene and vitamin C and a high concentration of zinc, in the upper part of the normal range, are associated with an increased risk of MetS. It is possible that the positive effect of  $\beta$ -carotene and vitamin C on the risk of MetS was counterbalanced by the negative effect of zinc,

which possibly accounted for the overall null finding noted with the intervention. Other possibilities that may explain the lack of an intervention effect of antioxidant supplementation on MetS incidence could be related to dose, duration, and the nature of the study participants. Because the study sample was recruited from a general healthy population, it is likely that the antioxidant status of the sample was not low and thereby additional antioxidant supplementation did not yield any beneficial effect. It is also possible that the events associated with the initiation of MetS were already present before the study began; it is likely that the study results may have been different if the younger participants and those at risk of impaired antioxidant status were included. These hypotheses remain to be verified in future studies.

In conclusion, no benefit or adverse effect of multiple antioxidant supplementation on MetS incidence was noted. Because nutrient supplementation including antioxidants is common in the general public (23), the lack of adverse effects was reassuring; however, the benefits associated with antioxidant supplementation are questionable.

The authors' responsibilities were as follows—SC, SH, PG, and NA: conceptualized the research; SC and NA: supervised the statistical analysis performed by A-CV; SC and NA: wrote the manuscript; and JA, AF, HF, and RH: revised the manuscript for important intellectual content. None of the authors reported a conflict of interest.

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