

## Antioxidant status and risk of cancer in the SU.VI.MAX study: is the effect of supplementation dependent on baseline levels?

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The SUPplementation en Vitamines et Minéraux AntioXydants (SU.VI.MAX) study, a randomised double-blind, primary-prevention trial showed that after 7.5 years, low-dose antioxidant supplementation lowered the total cancer incidence in men, but not in women. To explain this difference in the impact of antioxidant supplementation in men and women, we hypothesised that the effect of supplementation is dependent on initial antioxidant status; 12 741 French adults (7713 females aged 35–60 years; 5028 males aged 45–60 years) received daily antioxidant supplementation (120 mg vitamin C, 30 mg vitamin E, 6 mg  $\beta$ -carotene, 100  $\mu$ g Se, 20 mg Zn daily) or a matching placebo. Cut-off limits for baseline serum concentrations of the different antioxidant vitamins and minerals were defined as follows for both men and women: 0.3  $\mu$ mol/l for  $\beta$ -carotene, 11.4  $\mu$ mol/l for vitamin C, 15  $\mu$ mol/l for vitamin E, 0.75  $\mu$ mol/l for Se and 10.7  $\mu$ mol/l for Zn. The percentage of men with serum concentrations under cut-off limits was higher for vitamins C and E and  $\beta$ -carotene in those who developed a cancer than in those who did not. The risk of cancer was higher in men with baseline concentrations of serum vitamin C or vitamin E under cut-off limits, but not in women. The effect of supplementation was greater in men with baseline serum concentrations of vitamin C, vitamin E and  $\beta$ -carotene below the cut-off limits compared with those above it. This effect was maintained only for vitamin E after adjustment for age, tobacco, and alcohol consumption and BMI. No effect of supplementation could be seen in women. Baseline antioxidant status is related to the risk of cancer in men but not in women and therefore does not entirely explain the differences observed in the effect of antioxidant supplementation on cancer risk between sexes in the SU.VI.MAX study.

### Antioxidants: Cancers: Primary-prevention trial: Minerals: Vitamins: Supplementation

The SUPplementation en Vitamines et Minéraux AntioXydants (SU.VI.MAX) study, a randomised double-blind, placebo-controlled primary-prevention trial, was designed to test whether a combination of antioxidant nutrients, at nutritional doses (reachable by a healthy diet with high consumption of fruits and vegetables) can reduce the incidence of cancers and ischaemic CVD in a middle-aged general French population (Hercberg *et al.* 1998b). Intention-to-treat analysis showed that after 7.5 years, low-dose antioxidant supplementation lowered the total cancer incidence in men, but not in women (Hercberg *et al.* 2004). The same pattern was observed for all-cause mortality. Our results differ from those observed in previous intervention trials on this topic, which have not been able to show a beneficial effect (Physicians' Health Study (Hennekens *et al.* 1996); Women's Health Study (Lee *et al.* 1999)) or which even found a deleterious effect (Alpha-Tocopherol, Beta Carotene Cancer

Prevention Study (Anonymous, 1994); Beta Carotene and Retinol Efficacy Trial (Omenn *et al.* 1996)). However, those trials used pharmacological doses of antioxidant nutrients, mainly alone or in pairs, and included subjects at high cancer risk (heavy smokers and occupationally exposed subjects). Only the Linxian Trial (Blot *et al.* 1993), which included nutritional doses of a combination of several antioxidant vitamins and minerals, also showed a beneficial effect on total mortality and cancer incidence. Furthermore, in contrast to the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study and the Beta Carotene and Retinol Efficacy Trial, the Linxian trial was conducted in a general Chinese population with very low baseline micronutrient status, due to poor life conditions in this region.

An explanation for some of the differences in the impact of antioxidant supplementation on cancer incidence in all published randomised trials might lie in the differences in pre-inclusion

antioxidant status of the studied populations. Similarly, the fact that supplementation was effective in men but not in women in the SU.VI.MAX study (Hercberg *et al.* 2004) may be due to a lower nutritional intake and thus lower baseline concentrations of  $\beta$ -carotene and vitamin C in men than in women.

We hypothesise that the effect of antioxidant supplementation depends on baseline antioxidant status; only when the baseline status is low, can antioxidant supplementation have a beneficial effect on cancer incidence. We tested this hypothesis in the SU.VI.MAX study using cut-off limits for serum concentrations of vitamins and minerals previously published as corresponding to risk of biological deficiencies without obvious clinical manifestation (Pilch & Senti, 1985; van Dael & Deelstra, 1993; Hercberg *et al.* 1994).

## Materials and methods

### Study design

Details concerning study rationale, design, methods and participant characteristics have been reported elsewhere (Hercberg *et al.* 1998*a,b*, 2004). In March–July 1994, information on the objectives and outline of the study was presented in various media types, along with a call for volunteers (women, aged 35–60 years, or men, aged 45–60 years, living in France). Candidates responded by phone or mail. Comprehensive information on the scientific and practical features of the study was sent to those subjects that met the above criteria. They were expected to return a signed informed consent and a completed questionnaire to screen for eligibility. Further eligibility criteria were: lack of disease likely to hinder active participation or threaten 5-year survival, acceptance of the idea of a placebo and of the implications of participation, lack of regular supplementation with any of the tested vitamins or minerals and absence of extreme beliefs or behaviour regarding diet. Finally, the study cohort involved 12 741 French adults (7713 females aged 35–60 years and 5028 males aged 45–60 years). They were randomly allocated to receive either a combination of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg  $\beta$ -carotene (as synthetic forms), 100  $\mu$ g Se (as Se-enriched yeast) and 20 mg Zn (as gluconate)) or a matching placebo, in a single daily capsule. The study agents were provided in size 1, hard gelatin capsules dispensed in weekly strips. A special box containing fifty-two weekly strips was given to the subjects at the time of the inclusion visit and at each following yearly check-up visit. The median follow-up time was 7.54 years.

The protocol was approved by a medical ethics committee and the national committee for the protection of privacy and civil liberties (Hercberg *et al.* 1998*b*).

Data on medical status, alcohol consumption, smoking, etc. were obtained at study entry, along with height and weight. Age was defined in four classes (<45 years, 45–50 years, 50–55 years and >55 years, the first class including only women). The classes for smoking were defined as: smokers; former smokers; current smokers. Alcohol consumers were defined as those subjects who reported consuming more than 4 g ethanol/d in the baseline questionnaire. BMI was calculated as weight (kg) divided by height squared ( $m^2$ ) and was divided into three classes (<25, 25–30 and >30 kg/ $m^2$ ). Obesity was defined as a BMI >30 kg/ $m^2$ .

Venous blood samples were obtained at inclusion from 12 h fasting subjects to measure serum cholesterol and antioxidant concentrations in all subjects. All biochemical measurements were performed in only one laboratory for each nutrient being analysed.

Vitamin C status was evaluated using an automated method based on continuous flow analysis (Bourgeois *et al.* 1989). Serum  $\beta$ -carotene and  $\alpha$ -tocopherol were measured by HPLC (Vuilleumier *et al.* 1983). Serum Zn was determined by flame atomic absorption spectrometry (Arnaud *et al.* 1986) and serum Se by electrothermal atomic absorption spectrometry with the Zeeman effect (Arnaud *et al.* 1993). Cut-off limits for serum concentrations of the different antioxidant vitamins and minerals were as follows: 0.3  $\mu$ mol/l for  $\beta$ -carotene; 11.4  $\mu$ mol/l for vitamin C; 15  $\mu$ mol/l for  $\alpha$ -tocopherol (vitamin E); 0.75  $\mu$ mol/l for Se; 10.7  $\mu$ mol/l for Zn (Pilch & Senti, 1985; van Dael & Deelstra, 1993; Hercberg *et al.* 1994).

We had previously reported that randomisation yielded intervention and placebo groups balanced at baseline for factors related to nutritional status and/or cancer risk. For the groups, the mean age was 46.6 (SD 6.6) years for females and 51.3 (SD 4.6) years for males. Smoking habits, alcohol consumption, occupation, capsule compliance, BMI, cholesterol, blood levels of  $\beta$ -carotene, vitamin C, vitamin E, Zn and Se were identically distributed across the groups (Hercberg *et al.* 2004). Seventy-four percent of the participants reported at the end of follow-up having taken at least two-thirds of the capsules. There were no differences in capsule consumption between the groups (mean percentage of capsules taken was 79% in each).

### Cancer ascertainment and review

Participants were asked to complete a monthly questionnaire, summarising treatment compliance and health events, via Minitel (a phone-based French terminal), the Internet or mail. If there was no contact with the participant for a long period, or if the participant failed to appear at the yearly visit, an investigation was launched to determine the reasons. If necessary, an inquiry was made among neighbours and/or the participant's physician. Whatever the source of information, once a cancer was suspected, all relevant records, including results of diagnostic tests and procedures, were collected from the physicians and hospitals involved or directly from participants.

All data were reviewed by an expert committee, whose members were blinded for supplementation assignment. Cancers (ICD10 codes C00–C97 and D00–D09; World Health Organization, 1993) were validated by pathology reports.

Causes of death were confirmed by information from relatives or physicians. At the end of the follow-up, vital status of all subjects and causes of death were checked at the national death registry.

### Statistical analysis

All analyses were stratified by sex. Follow-up duration for each subject was calculated from the date of randomisation until the date of cancer diagnosis or the date of death on 1 September 2002. All cases of cancer were retained for analysis, except for basal cell carcinoma of the skin.

Differences in serum concentrations of antioxidants between sexes were evaluated with the *t* test, and the relationship between antioxidant status and its determinants (age, smoking, alcohol consumption and BMI) was evaluated using the Fisher test.

Because of skewed distribution, serum  $\beta$ -carotene and vitamin C were log-transformed for analysis to obtain a normal distribution.

To evaluate the relationship between baseline concentrations of antioxidant vitamins and minerals and risk of cancer occurrence, Cox proportional hazard models were used. The same method was applied to assess the effect of antioxidant supplementation on cancer incidence, and these analyses were stratified according to baseline antioxidant status. To evaluate a possible interaction between the antioxidant supplementation group and antioxidant status at baseline, an interaction term (supplementation  $\times$  serum concentration) was included in the model (without stratification).

Statistical analyses were performed using SAS software version 8.2 (SAS Institute, Inc., Cary, NC, USA).

## Results

### Baseline status of antioxidants in the placebo group and their determinants

At baseline, women had significantly higher baseline serum  $\beta$ -carotene and vitamin C concentrations ( $P < 0.0001$ ) and lower concentration of serum vitamin E, Zn and Se ( $P < 0.0001$ ) than men (Table 1); 36.8% of men and 15.0% of women in the placebo group had serum  $\beta$ -carotene concentrations  $< 0.30 \mu\text{mol/l}$ ; respectively 1.9 and 0.5% had serum vitamin C levels  $< 11.4 \mu\text{mol/l}$ ; 0.6% of both sexes exhibited serum  $\alpha$ -tocopherol  $< 15 \mu\text{mol/l}$ ; 4.5 and 9.5% had serum Zn  $< 10.7 \mu\text{mol/l}$  and 1.3 and 1.6% had serum Se  $< 0.75 \mu\text{mol/l}$ .

**Table 1.** Baseline levels and percentage of values under cut-off points in men and women of the placebo group (Mean values and standard deviations)

	Men		Women		P
	Mean	SD	Mean	SD	
$\beta$ -Carotene					
n	2128		3287		
Serum concentration ( $\mu\text{mol/l}$ )	0.46	0.36	0.67	0.47	0.0001
Percentage $< 0.30 \mu\text{mol/l}$	36.8		15.0		
Vitamin C					
n	1836		2847		
Serum concentration ( $\mu\text{mol/l}$ )	49.8	23.8	59.4	28.6	0.0001
Percentage $< 11.4 \mu\text{mol/l}$	1.9		0.5		
Vitamin E					
n	2128		3288		
Serum concentration ( $\mu\text{mol/l}$ )	32.2	8.2	30.9	7.5	0.0001
Percentage $< 15.0 \mu\text{mol/l}$	0.6		0.6		
Zn					
n	2404		3664		
Serum concentration ( $\mu\text{mol/l}$ )	13.5	1.9	12.9	1.8	0.0001
Percentage $< 10.7 \mu\text{mol/l}$	4.5		9.5		
Se					
n	2395		3643		
Serum concentration ( $\mu\text{mol/l}$ )	1.13	0.20	1.08	0.19	0.0001
Percentage $< 0.75 \mu\text{mol/l}$	1.3		1.6		

A higher percentage of women with concentration of serum  $\beta$ -carotene under the cut-off limit ( $P < 0.03$ ) was observed at a younger age (Table 2). The prevalence of subjects with antioxidant concentrations under the cut-off limit was higher in current smokers than in non-smokers for  $\beta$ -carotene ( $P < 0.0001$ ), vitamin C ( $P < 0.0001$  in men;  $P < 0.03$  in women) and Se ( $P < 0.001$  in men;  $P < 0.007$  in women). In alcohol consumers, low values were more frequently observed for serum  $\beta$ -carotene ( $P < 0.0001$  in men;  $P < 0.03$  in women), whereas for vitamin E, the prevalence of low values was lower ( $P < 0.0004$ ) among men consumers. The percentage of subjects with serum  $\beta$ -carotene under cut-off limits was much higher in obese than in overweight or normal-to-low-weight subjects ( $P < 0.0001$  with a significant linear relationship in both sexes).

### Risk of cancer according to baseline status of antioxidants in the placebo group

In the placebo group, mean baseline serum  $\beta$ -carotene and vitamin C concentrations were lower in men who developed a cancer than in those who did not (Table 3). No significant difference was found for serum vitamin E, Zn or Se. Baseline status of the five antioxidant markers was not related to cancer in women. The percentages of men with serum concentrations under the cut-off limits were higher in those who developed a cancer when compared with those who did not for vitamin C, vitamin E and  $\beta$ -carotene (Table 3).

Relative risks for cancer according to baseline serum antioxidants concentrations below or above cut-off limits in the placebo group are presented in Table 4.

In men, a low serum  $\beta$ -carotene, serum vitamin C or vitamin E concentration was positively associated with the risk of cancer. Adjustment for age, smoking, alcohol consumption and BMI did not modify the relationship for vitamin E; however, the relationship with  $\beta$ -carotene and vitamin C disappeared after adjustment. No association was observed in women.

### Effect of supplementation on risk of cancer according to baseline cut-off

In men, we found a significant interaction between antioxidant supplementation and baseline serum vitamin C concentration (significant for interaction term in the model;  $P = 0.04$ ; Table 5). No such interaction was observed for  $\beta$ -carotene ( $P = 0.20$ ), vitamin E ( $P = 0.55$ ), Zn ( $P = 0.90$ ) or Se ( $P = 0.97$ ), nor were there any interactions in women.

In men, the effect of supplementation was stronger in those with low serum vitamin C concentrations than in those with higher concentration; relative risk for cancer in the supplemented group of men with serum levels of vitamin C  $< 11.4 \mu\text{mol/l}$  was 0.10 (95% CI 0.01, 0.77;  $P < 0.03$ ) and 0.83 (95% CI 0.60, 1.16;  $P = 0.28$ ) in those with serum levels of vitamin C  $\geq 11.4 \mu\text{mol/l}$  when compared with the placebo.

The relative risk of developing a cancer in the supplemented group of men with serum concentrations of  $\beta$ -carotene  $< 0.3 \mu\text{mol/l}$  was 0.59 (95% CI 0.37, 0.95;  $P < 0.03$ ) v. 0.88 (95% CI 0.61, 1.28;  $P = 0.50$ ) in the group with initial concentrations of serum  $\beta$ -carotene  $> 0.3 \mu\text{mol/l}$ . For vitamin E, the relative risks in men with serum levels below or above the cut-off limits of  $15 \mu\text{mol/l}$  were respectively 0.39 (95% CI 0.04, 3.83;  $P = 0.42$ ) and 0.76 (95% CI 0.57, 1.02;  $P = 0.07$ ).

**Table 2.** Percentage of subjects with baseline serum vitamin and mineral concentrations below cut-off limits in men and women according to age, tobacco and alcohol consumption and BMI

	Serum $\beta$ -carotene < 0.30 $\mu\text{mol/l}$		Serum vitamin C < 11.4 $\mu\text{mol/l}$		Serum vitamin E < 15 $\mu\text{mol/l}$		Serum Zn < 10.7 $\mu\text{mol/l}$		Serum Se < 0.75 $\mu\text{mol/l}$	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
<b>Age</b>										
< 45 years	–	16.7	–	0.5	–	0.8	–	9.0	–	2.2
45–50 years	34.1	15.9	1.2	0.4	0.9	0.7	3.9	8.8	0.8	1.5
50–55 years	39.7	12.4	2.5	0.8	0.3	0.5	4.8	10.3	1.8	0.9
> 55 years	37.2	12.5	2.0	0.6	0.6	0.4	5.1	10.9	1.6	1.3
<i>P</i>	0.09	0.03	0.25	0.76	0.42	0.76	0.45	0.42	0.14	0.17
<b>Tobacco</b>										
Non-smokers	26.9	12.7	1.1	0.3	0.4	0.5	3.6	9.7	0.7	1.2
Former smokers	38.8	13.7	1.4	0.5	0.5	1.0	4.8	9.8	1.1	1.7
Current smokers	51.4	24.5	5.5	1.4	1.2	0.6	5.9	7.9	3.4	3.2
<i>P</i>	0.0001	0.0001	0.0001	0.03	0.25	0.30	0.20	0.37	0.001	0.007
<b>Alcohol consumption</b>										
Non-drinkers	26.9	12.5	1.2	0.8	1.3	0.8	3.7	9.0	1.2	1.8
Drinkers	39.1	16.4	2.0	0.3	0.4	0.7	4.6	9.5	1.4	1.6
<i>P</i>	0.0001	0.003	0.36	0.12	0.04	0.75	0.44	0.53	0.74	0.56
<b>BMI (kg/m<sup>2</sup>)</b>										
< 25	25.1	11.1	1.5	0.4	0.7	0.7	3.9	10.1	1.6	1.6
25–30	39.3	15.7	0.9	0.5	0.4	0.2	5.6	8.7	1.3	2.0
> 30	63.0	31.5	3.2	0.7	0.7	0.6	3.1	6.6	0.6	1.7
<i>P</i>	0.0001	0.0001	0.14	0.87	0.81	0.50	0.16	0.22	0.61	0.81

For Zn, the relative risk of developing cancer in the supplemented group of men with serum levels  $\geq 10.7 \mu\text{mol/l}$  was 0.74 (95 % CI 0.55, 0.98;  $P=0.034$ ) v. 0.81 for those <  $10.7 \mu\text{mol/l}$  (95 % CI 0.24, 2.80;  $P=0.74$ ). For Se, the relative risk of developing a cancer in the supplemented group of men with serum levels  $\geq 0.75 \mu\text{mol/l}$  was 0.73 (95 % CI 0.55, 0.97;  $P=0.027$ ) v. 0.84 (95 % CI 0.05, 13.45;  $P=0.90$ ),

No difference in the effect of supplementation according to baseline levels was shown in women.

### Discussion

The present study revealed lower concentrations of  $\beta$ -carotene and vitamin C at baseline in men than in women, which is in line with the hypothesis that the difference in the effect of antioxidant supplementation that we observed between men and women is dependent on baseline antioxidant status. This observation is consistent with the results of numerous studies performed in different countries with subjects in the same age range (Herberg *et al.*

**Table 3.** Baseline vitamin and mineral status in men and women, and subjects who developed or did not develop a cancer in the placebo group

(Mean values and standard deviations)

	Men					Women				
	Cancer		No cancer		<i>P</i>	Cancer		No cancer		<i>P</i>
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
<b><math>\beta</math>-Carotene</b>										
<i>n</i>	104		2024			134		3153		
Serum concentration ( $\mu\text{mol/l}$ )	0.42	0.34	0.47	0.36	0.03	0.67	0.47	0.64	0.45	0.87
Percentage < 0.30 $\mu\text{mol/l}$	45.2		36.3		0.07	11.2		15.2		0.20
<b>Vitamin C</b>										
<i>n</i>	86		1750			124		2723		
Serum concentration ( $\mu\text{mol/l}$ )	45.2	21.7	50.0	23.9	0.003	55.9	19.5	59.5	28.9	0.16
Percentage < 11.4 $\mu\text{mol/l}$	9.3		1.5		0.0001	1.6		0.5		0.09
<b>Vitamin E</b>										
<i>n</i>	104		2024			134		3154		
Serum concentration ( $\mu\text{mol/l}$ )	31.5	7.8	32.2	8.2	0.39	30.8	6.5	30.9	7.6	0.88
Percentage < 15.0 $\mu\text{mol/l}$	2.9		0.5		0.002	0.7		0.6		0.87
<b>Zn</b>										
<i>n</i>	116		2288			162		3502		
Serum concentration ( $\mu\text{mol/l}$ )	13.6	1.9	13.5	1.8	0.57	12.8	2.1	12.9	1.0	0.57
Percentage < 10.7 $\mu\text{mol/l}$	4.3		4.5		0.91	12.5		9.3		0.20
<b>Se</b>										
<i>n</i>	117		2278			160		3483		
Serum concentration ( $\mu\text{mol/l}$ )	1.14	0.21	1.13	0.19	0.62	1.08	0.16	1.08	0.20	0.68
Percentage < 0.75 $\mu\text{mol/l}$	0.2		1.2		0.64	0.9		1.4		0.30

**Table 4.** Relative risk (RR) of cancer according to baseline serum antioxidant concentrations below or above cut-off limits in the placebo group (RR and 95% CI)

	Men					Women					
	Crude RR	95% CI	P	Adjusted* RR	95% CI	P	Crude RR	95% CI	Adjusted* RR	95% CI	P
Serum $\beta$ -carotene	1			1			1		1		
$\geq 0.30 \mu\text{mol/l}$	1.48	0.996, 2.18	0.047	1.43	0.86, 2.36	0.17	0.73	0.43, 1.26	1.02	0.52, 1.99	0.96
$< 0.30 \mu\text{mol/l}$											
Serum vitamin C	1			1			1		1		
$\geq 11.4 \mu\text{mol/l}$	6.31	3.05, 13.07	0.0001	2.47	0.58, 39.1	0.22	3.26	0.81, 13.20	3.61	0.49, 26.51	0.21
$< 11.4 \mu\text{mol/l}$											
Serum vitamin E	1			1			1		1		
$\geq 15.0 \mu\text{mol/l}$	5.86	1.78, 19.30	0.004	8.21	1.72, 10.60	0.008	1.32	0.18, 9.50	2.28	0.31, 16.62	0.41
$< 15.0 \mu\text{mol/l}$											
Serum Se	1			1			1		1		
$\geq 0.75 \mu\text{mol/l}$	0.74	0.10, 5.33	0.77	†	†	-	0.38	0.05, 2.72	0.57	0.08, 4.13	0.57
$< 0.75 \mu\text{mol/l}$											
Serum Zn	1			1			1		1		
$\geq 10.7 \mu\text{mol/l}$	0.96	0.39, 2.36	0.93	0.84	0.27, 2.68	0.77	1.36	0.85, 2.18	0.71	0.38, 1.30	0.26
$< 10.7 \mu\text{mol/l}$											

\* Adjusted for age, BMI, alcohol consumption and smoking.

† Too few cases to perform adjustment.

1994; Olmedilla *et al.* 1994, 2002; Curran-Celentano *et al.* 2001; De Waart *et al.* 2001; Wallström *et al.* 2001). Differences in nutritional intake (especially fruit and vegetable consumption) may explain the difference in status of these vitamins and carotenoids. It is possible that hormonal factors or lifestyle factors (alcohol consumption, smoking habits, etc.) also contribute to this difference.

The percentage of subjects with high risk of biological deficiency is generally low, except for  $\beta$ -carotene. These percentages, as well as the serum concentrations, are in agreement with previous studies conducted in France and other European countries (Millet *et al.* 1989; Hercberg *et al.* 1994; Carbajal *et al.* 1996; Benton *et al.* 1997).

In the placebo group, baseline serum concentrations of vitamin C, vitamin E and  $\beta$ -carotene were significantly lower in men who developed cancer than in the control group, but not in women. Furthermore, we observed a trend toward higher cancer incidence among men with low baseline serum concentration of vitamin C, vitamin E and  $\beta$ -carotene, but this relationship was confounded for  $\beta$ -carotene and vitamin C by other risk factors, and did not remain after adjustment. We observed a similar tendency in women for vitamins C and E, but no statistically significant relationship was found. It should be noted that the number of women with serum concentrations under the cut-off limits was very small.

Prospective analysis in the placebo group suggested a protective effect against cancer for men with baseline serum concentrations of vitamin C,  $\beta$ -carotene and vitamin E above the cut-off. Numerous prospective studies have been published showing an inverse association between blood concentrations of  $\beta$ -carotene (Nomura *et al.* 1985; Wald *et al.* 1988; Connett *et al.* 1989; Comstock *et al.* 1991; Orentreich *et al.* 1991; Stähelin *et al.* 1991; van Poppel & Goldbohm, 1995), vitamin C (Hsing *et al.* 1990; Eischholzer *et al.* 1996) and vitamin E (Knekt, 1988; Knekt *et al.* 1988a,b; Hsing *et al.* 1990; Comstock *et al.* 1991; Stähelin *et al.* 1991) and the incidence of cancer or cancer mortality. Such an association appears most consistent for lung and stomach cancers, whereas the association seems inconsistent for breast and prostate cancer. The lack of relationship with breast cancer may explain why we did not find any relationship in women. Indeed, in the present study, breast cancer represented the main cancer site in females; 195 out of 350 total cancers after 7.5 years of follow-up (Hercberg *et al.* 2004). Results of studies relating blood concentrations of vitamin E are less consistent. Most prospective studies on blood markers of antioxidants and cancers involved only male populations. In few prospective studies performed on both sexes, plasma ascorbic acid was inversely related to cancer mortality in men, but not in women (Enstrom *et al.* 1992; Loria *et al.* 2000; Khaw *et al.* 2001).

Several strengths of the present study should be mentioned. First, we had the opportunity to prospectively assess the association with baseline antioxidant levels and cancer incidence in a large sample of subjects. However, one concern is that our subjects were enrolled in a nutritional intervention study. Although a previous report on baseline characteristics of participants in the SU.VI.MAX study showed that the study sample was close to the national population with regard to geographic density and socio-economic status, these subjects may have a healthier lifestyle (Hercberg *et al.* 1998b). Second, in the present study we used cut-off limits rather than percentiles to assess the risk of developing cancer. These cut-off limits do not correspond to major deficiency (with clinical signs), but are usually considered to define a risk of moderate deficiency with impaired biological functions. In our analysis these cut-off limits seem



**Table 5.** Relative risk (RR) of cancer according to baseline serum antioxidant concentrations (below or over cut-off limits) in supplemented group compared with placebo, and interactions between sex and serum baseline level (RR and 95 % CI)

	Men			Women		
	RR	95 % CI	<i>P</i>	RR	95 % CI	<i>P</i>
Serum $\beta$ -carotene						
< 0.30 $\mu\text{mol/l}$	0.59	0.37, 0.95	0.0285	1.15	0.58, 2.28	0.6941
$\geq$ 0.30 $\mu\text{mol/l}$	0.88	0.61, 1.28	0.4965	1.18	0.92, 1.51	0.1918
Interaction			0.20			0.95
Serum vitamin C						
< 11.4 $\mu\text{mol/l}$	0.10	0.01, 0.77	0.0271	0.55	0.05, 6.09	0.6274
$\geq$ 11.4 $\mu\text{mol/l}$	0.83	0.60, 1.16	0.2776	1.09	0.86, 1.40	0.4778
Interaction			0.04			0.58
Serum vitamin E						
< 15.03 $\mu\text{mol/l}$	0.39	0.04, 3.83	0.4219	0.38	0.01, 15.29	0.6058
$\geq$ 15.03 $\mu\text{mol/l}$	0.76	0.57, 1.02	0.0706	1.17	0.93, 1.47	0.1863
Interaction			0.55			0.97
Serum Se						
< 0.75 $\mu\text{mol/l}$	0.84	0.05, 13.45	0.8987	3.09	0.32, 29.67	0.3291
$\geq$ 0.75 $\mu\text{mol/l}$	0.73	0.55, 0.97	0.0270	1.06	0.86, 1.32	0.5738
Interaction			0.97			0.35
Serum Zn						
< 10.7 $\mu\text{mol/l}$	0.81	0.24, 2.80	0.7416	0.97	0.53, 1.77	0.9142
$\geq$ 10.7 $\mu\text{mol/l}$	0.74	0.55, 0.98	0.0349	1.08	0.86, 1.36	0.5195
Interaction			0.90			0.74

appropriate for categorising subjects with higher risk of cancers, suggesting a threshold effect.

These cut-off limits were also good predictors of the effects of antioxidant supplementation. The reduction in the risk of cancer in the supplemented group of men with serum levels of vitamin C, E or  $\beta$ -carotene below cut-off limits was much higher than the risk reduction observed in the supplemented group when the population was studied independently of the initial baseline status or in subjects with concentration above cut-off limits (Herberg *et al.* 2004).

In women, a tendency toward reduction in risk in subjects with serum vitamin levels below cut-off limits for vitamin C and E was found, but the number of subjects with cancer in this group was too low for statistical power. The more favourable status in women compared with men for vitamin C and  $\beta$ -carotene at inclusion, and the too-small number of cancers, mainly breast cancers, may explain why, unlike men, we were unable to find a specific relationship between these biomarkers and the risk of cancers in the placebo group, nor a specific effect of supplementation according to the initial level of the antioxidant.

For Zn and Se, a decrease in the risk of cancer in the supplemented group was seen only in men with serum values above the cut-off limit concentrations. This supplementation does not seem to affect men with low values, and no relationship was observed in women.

The baseline level of antioxidants may be an important factor for explaining discrepancies between intervention trials on this topic, which involved different populations. The only study which found protective effects of antioxidant supplementation on cancer (the Linxian trial), included a population with a very low baseline micronutrient status, due to poor living conditions (Blot *et al.* 1993). As baseline mean levels of antioxidants suggest, it is likely that many subjects presented very low levels, which was not the case in the other studies not demonstrating a protective effect (Anonymous, 1994; Hennekens *et al.* 1996; Omenn *et al.* 1996; Lee *et al.* 1999).

These observations agree with the present results as we found no beneficial effects in females who had higher concentrations of serum antioxidants at baseline than males; in addition, in males the beneficial effect of supplementation was more pronounced in vitamin- and  $\beta$ -carotene-sub-deficient subjects. The differences in cancer risk according to supplementation and basal antioxidant status in men may be related to the fact that we corrected biological deficiencies important enough to increase the risk of cancer. In subjects with adequate status (above the cut-off limits), supplementation was not useful. However, we do not have a clear explanation as to why baseline antioxidant status is related to the risk of cancer in men but not in women. It could be related to the type of cancer observed in women (mainly breast cancers), the higher serum values of vitamin C and E in women with too-small a number of women with biochemical evidence of deficiency. The difference in baseline antioxidant status does not totally explain the differences observed in the effect of antioxidant supplementation on cancer risk between sexes in the SU.VI.MAX study. Therefore, further investigation should be undertaken in the future.

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S. H. was the principal investigator. S. H., P. G., P. P., A. F. and S. B. designed the study. H. F. and J. A. guaranteed the quality of analyses. P. G., P. P., S. H. and S. B. collected the data. S. B., P. P., P. G., S. H., S. B., L. M. and N. A. carried out data analysis. P. G., S. H., L. M. and S. B. prepared the manuscript, which was reviewed by all authors. P. G. is the guarantor. There are no competing interests.

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