

## Vitamin E supplementation is associated with lower levels of C-reactive protein only in higher dosages and combined with other antioxidants: The Cooperative Health Research in the Region of Augsburg (KORA) F4 study

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### Abstract

The aim of the present study was to examine the association between intake of five common antioxidative nutrients from supplements and medications (vitamin E, vitamin C, carotenoids, Se, and Zn) and levels of high-sensitivity C-reactive protein (hs-CRP) in the general population. For this purpose, a total of 2924 participants of the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study (2006–8) were investigated cross-sectionally. Intake of dietary supplements and medication during the last 7 d was recorded in a personal interview, when participants were asked to show product packages of ingested preparations. Linear regression models were calculated; first, the exposure to regular nutrient intake was treated with a binary response (yes/no); then regularly ingested amounts were divided into quartiles to examine dose–response relationships. Effect of single *v.* combined supplementation of antioxidants was assessed through the inclusion of interaction terms into the models. Regular intake of any of the five investigated antioxidants *per se* was not associated with hs-CRP levels. However, dose–response analyses revealed that participants who regularly ingested more than 78 mg vitamin E/d, which corresponds to the upper quartile, had 22% lower hs-CRP levels (95% CI 0.63, 0.97) compared to those of persons who were not exposed to any vitamin E supplementation. Stratified analyses showed that this association was found only in persons who took vitamin E in combination with other antioxidants. The combined supplementation of vitamin E with other antioxidants could thus be a promising strategy for the prevention of inflammation-related diseases in the general population, if further studies could confirm that the proposed association is causal.

**Key words:** Vitamin E supplementation: Antioxidative supplements: High-sensitivity C-reactive protein: Population-based studies

Subclinical inflammation processes are closely related to the development of common chronic diseases. Healthy subjects with higher concentrations of the inflammatory marker C-reactive protein (CRP) have a higher risk of developing CVD and type 2 diabetes within the next years<sup>(1,2)</sup>. Thus, dietary supplements with antioxidative or immune modulating properties might be an effective strategy for the prevention of inflammation-related diseases at the population level.

However, results from epidemiological studies on antioxidant intake and CRP levels present conflicting scenarios<sup>(3,4)</sup>. Low intake of vitamin C and carotenoids was associated with elevated CRP levels in one study<sup>(4)</sup>, whereas their association was found to be non-significant in another study<sup>(3)</sup>. Furthermore, another study found a significant inverse association of vitamin E and CRP only in women<sup>(3)</sup>, while yet another study reported a such significant association in both men

**Abbreviations:** CRP, C-reactive protein; hs-CRP, high-sensitivity C-reactive protein; KORA, Cooperative Health Research in the Region of Augsburg.

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and women<sup>(4)</sup>. Therefore, it is not clear if there are sex specific effects. As intervention studies are usually conducted on diseased or high-risk samples<sup>(5–9)</sup>, conclusions cannot be drawn from them for the general healthy population. Moreover, it is possible that effects of intakes of single nutrients differ from those of combined intakes, because nutrients might interact with each other. Besides, there might be dose–effect relationships, which need close investigation<sup>(3)</sup>. Intervention studies which are most reliable to detect effects of nutrient supplementation should begin with sufficient evidence from observational studies on specific target populations, most effective nutrient combinations, and possible dose–response effects. The aim of the present study was, therefore, to investigate the association between intakes of common antioxidative vitamins and minerals from supplements and medications (vitamin E, vitamin C, carotenoids, Se, and Zn)<sup>(10)</sup> with levels of high-sensitivity C-reactive protein (hs-CRP) in the general population. In this respect, dose–response relationships, effects of single *v.* multiple use of antioxidants, and potential sex-specific effects were examined.

## Methods

Data are based on the German Cooperative Health Research in the Region of Augsburg (KORA) F4 study (2006–8), a follow-up examination of the population-based KORA S4 survey (1999–2001)<sup>(11)</sup>. Exclusion criteria for the present investigation were hs-CRP levels  $>10\text{ mg/l}^{(1)}$ , pregnancy and missing values in any exposure, outcome or confounder ( $n\ 155$ ). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee of the Bavarian Chamber of Physicians (Munich, Germany). Written informed consent was obtained from all participants.

For assessment of the outcome hs-CRP, fasting blood samples were collected in the morning, kept at  $4^{\circ}\text{C}$  until centrifugation and stored at  $-80^{\circ}\text{C}$  until laboratory measurements. Hs-CRP was measured of EDTA plasma samples with a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Siemens); intra-assay and inter-assay CV were  $<5\%$  and  $<10\%$ , respectively.

For exposure assessment, intake of dietary supplements during the last 7 d was recorded together with medication used through computer-based software<sup>(12)</sup> in a personal interview, when participants were asked to show product packages of ingested preparations<sup>(13)</sup>. Vitamins and minerals not only from supplements, but also from medications were taken into consideration for the investigation of the etiological association between nutrients and hs-CRP levels, as the same ingredients (e.g.  $\alpha$ -tocopherol) can be marketed either as supplements or as medications in Germany. Relative serum  $\alpha$ -tocopherol levels were measured in a subgroup of 1685 subjects (CV: 19.3%), as part of a non-targeted metabolomics experiment using GC coupled with MS (GC–MS). Serum samples that were collected after overnight fasting during the KORA F4 examinations were analysed at Metabolon,

Inc., a commercial supplier of metabolomics measurements<sup>(14)</sup>. The measurement followed established protocols as described earlier<sup>(15,16)</sup>. Raw quantification values (ion counts) were normalized to the median of the samples measured at the same run day to account for inter-day variation of the instrument. Normalized values were log-transformed and values above or below four standard deviations from the mean were excluded from further analysis.

Confounders were selected on the basis of practices followed in literature. Fully adjusted models included the following covariables: age (continuous), sex, education (low/high), BMI (continuous), BMI<sup>2</sup> (continuous), physical activity (active/inactive), smoking (current/former/never), alcohol consumption (none/moderate/high), cholesterol:HDL ratio (continuous), actual hypertension (yes/no), CVD (yes/no), diabetes (yes/no), non-steroidal anti-inflammatory drug use (yes/no), and statin use (yes/no). In sensitivity analyses, regression models were additionally adjusted for the use of other dietary supplements, and for the use of hormone therapy and oral contraceptives in women.

## Data collection and description of covariables

Covariables were collected through a personal interview or measured during an examination at the study centre. For age, the actual age at examination was recorded. Information about years of education was collected in the baseline S4 study (1999–2001)<sup>(11)</sup> and summarized as years at school plus professional education. The variable was dichotomized into low (8–10 years) and high (11–17 years) education. For calculating the BMI, participants' weight and height were measured. Categories as per the WHO standard were applied to the classification in Table 1<sup>(17)</sup>. Physical activity included frequency and duration of activity in summer and in winter and was divided into active and inactive<sup>(18)</sup>. Smoking was defined as smoking cigarettes and categorized as current (further subdivided into 'regularly' and 'irregularly'), former and never. Irregular smoking was defined as usually smoking less than 1 cigarette/d. Alcohol intake was assessed as daily average intake based on the consumption of last weekend and the last weekday before the examination, following the validated recall method of Döring *et al.*<sup>(19)</sup>. Moderate consumption was defined as more than zero and less than 40 g alcohol/d in men, and more than zero and less than 20 g/d in women; and high consumption was defined as 40 g/d or more in men, and 20 g/d or more in women<sup>(20)</sup>. For serum cholesterol measurement, blood was drawn from sedentary participants in the morning after an overnight fast of at least 8 h. Total cholesterol was measured using the Boehringer CHOD-PAP method (inter-assay CV:  $\leq 2.1\%$ ; Roche Diagnostics). HDL-cholesterol was measured using the phosphotungstic acid method (inter-assay CV:  $\leq 3.5\%$ ; Boehringer Mannheim). Cut-off points for total:HDL-cholesterol ratio used for description in Table 1 were defined according to the American Heart Association<sup>(21)</sup>. A participant was classified as hypertensive if the mean of the second and the third blood pressure measurements met the criteria of the WHO for hypertension, i.e. a systolic pressure

**Table 1.** C-reactive protein (CRP) levels by categories of covariables in KORA F4 (Cooperative Health Research in the Region of Augsburg) (Geometric mean values and antilog standard deviations, *n* 2924)

Characteristic	<i>n</i>	hs-CRP (mg/l)				<i>P</i> for mean difference‡
		Unadjusted		Adjusted†		
		Geometric mean	Antilog SD	Geometric mean	Antilog SD	
Age (years)						< 0.0001
31–43	659	0.84	2.89	0.84	2.67	
> 43–52	569	0.93	2.75	0.92	2.68	
> 52–60	557	1.19	2.63	1.18***	2.67	
> 60–70	636	1.43	2.56	1.43***	2.67	
> 70–82	503	1.46	2.50	1.46***	2.67	
Sex						0.0082
Female	1511	1.18	2.82	1.18	2.67	
Male	1413	1.08	2.66	1.08	2.67	
Education (years)						< 0.0001
8–10	1299	1.37	2.62	1.28	2.72	
11–17	1625	0.97	2.77	1.02	2.70	
BMI (kg/m <sup>2</sup> )§						< 0.0001
< 25	937	0.69	2.69	0.71	2.57	
25–< 30	1230	1.16	2.49	1.16***	2.51	
≥ 30	757	1.99	2.39	1.91***	2.52	
Physical activity						< 0.0001
Active	1604	0.99	2.72	1.00	2.65	
Inactive	1320	1.33	2.71	1.31	2.65	
Smoking						< 0.0001
Current	528	1.17	2.79	1.33	2.72	
Former	1091	1.15	2.74	1.13**	2.68	
Never	1305	1.09	2.72	1.05***	2.71	
Alcohol consumption¶						< 0.0001
None	882	1.31	2.77	1.28	2.71	
Moderate	1532	1.06	2.69	1.08***	2.67	
High	510	1.05	2.79	1.05***	2.66	
Cholesterol:HDL ratio						< 0.0001
< 3.5	1012	0.86	2.76	0.85	2.69	
≥ 3.5– < 5.0	1330	1.22	2.67	1.20***	2.61	
≥ 5.0	582	1.55	2.55	1.60***	2.64	
Hypertension††						< 0.0001
No	1826	0.95	2.74	1.00	2.79	
Yes	1098	1.50	2.57	1.37	2.86	
CVD‡‡						0.0003
No	2628	1.09	2.73	1.10	2.67	
Yes	296	1.60	2.66	1.38	2.73	
Diabetes						0.0053
No	2725	1.10	2.75	1.11	2.67	
Yes	199	1.64	2.53	1.37	2.73	
NSAID§§						0.0108
No	2834	1.12	2.75	1.12	2.67	
Yes	90	1.61	2.52	1.46	2.67	
Statins						0.7008
No	2575	1.10	2.77	1.12	2.69	
Yes	349	1.38	2.48	1.15	2.79	
Hormone therapy						0.7552
No	1384	1.17	2.84	1.18	2.74	
Yes	127	1.24	2.60	1.15	2.75	
Use of oral contraceptives						< 0.0001
No	1415	1.15	2.80	1.12	2.71	
Yes	96	1.65	3.03	2.35	2.83	

hs-CRP, high sensitivity C-reactive protein; NSAID, non-steroidal anti-inflammatory drugs.

 Mean value was significantly different compared with the first category: \*\* *P* < 0.01, \*\*\* *P* < 0.001.

† Adjusted for age (continuous) and sex; age was only adjusted for sex and sex was only adjusted for age.

 ‡ *P* for age- and sex-adjusted mean difference (ANCOVA).

 § BMI < 18.5 kg/m<sup>2</sup> (*n* 10) and 18.5 ≤ BMI < 25 (*n* 927) were combined.

 || Smoking regularly (*n* 450) and irregularly (*n* 78) were combined.

¶ Moderate: men &gt; 0 to &lt; 40 g/d; women &gt; 0 to &lt; 20 g/d; high: men ≥ 40 g/d; women ≥ 20 g/d.

†† Actual hypertension (≥ 140/90 mmHg) or intake of antihypertensive medication and awareness of being hypertensive.

‡‡ History of myocardial infarction, stroke or angina pectoris.

§§ Without acetylsalicylic acid in dosages of 100 or 300 mg.

||| In women only.

$\geq 140$  mmHg and/or diastolic  $\geq 90$  mmHg<sup>(22)</sup>, or if the participant was taking antihypertensive medication and was aware of being hypertensive. CVD was defined as a participant's having a history of myocardial infarction or stroke (treated as an inpatient, self-reported), or a history of angina pectoris (self-reported). Diabetes mellitus was defined according to self-report or current use of antidiabetic drugs. Medication intake during the last 7 d was collected from product packages showed by participants, and recorded through a database supported computer software<sup>(12)</sup>. For the definition of non-steroidal anti-inflammatory drugs, regular use was considered, and acetylsalicylic acid use in dosages of 100–300 mg was not included. Hormone therapy was defined as use of systemic estrogens with or without gestagen intake. The adjustment variable dietary supplement intake, that was used for sensitivity analysis, was built including supplementation of any of the following nutrients which have been found to be associated with CRP levels: vitamin A, D, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, folic acid, pantothenic acid, niacin, biotin, Mg, Fe, DHA, EPA,  $\alpha$ -linolenic acid, glucosamine and chondroitin<sup>(3,23–34)</sup>.

### Statistical analysis

Age- and sex-adjusted mean levels of hs-CRP by categories of covariables and exposure variables were compared using ANCOVA. If the *P*-value for the group was  $< 0.05$ , *post hoc* analyses were performed with the first category as reference group, to clarify where the differences are. For statistical analyses, hs-CRP was log-transformed to approximate a normal distribution, and back-transformed for presentation of results. These are displayed as ratio (95% CI) of geometric mean hs-CRP levels among nutrient users *v.* non-users. The association of vitamin E, vitamin C, carotenoids, Se and Zn intake with hs-CRP was investigated cross-sectionally using linear regression models; initially total regular nutrient intake was treated binary (yes/no), then regularly ingested amounts were divided into quartiles to investigate dose–response effects. The reference group comprised persons with a zero intake of the nutrients of interest from supplements or medications. Assumptions of linear regression models were checked and fulfilled. Effect modification was tested for sex, and intake of at least one of the other four antioxidants, respectively. Through the latter we examined the effect of single *v.* combined supplementation of antioxidants, *i.e.*, if the association of each nutrient with hs-CRP depends on the intake of at least one of the other four antioxidants, or if supplementation of each nutrient in isolation (without other antioxidants) is associated with hs-CRP. An independent effect of each antioxidant was assessed via adjustment for use of at least one of the other four antioxidants, respectively. We used a spline, *i.e.* a piecewise polynomial function, to examine a non-linear relationship between regularly ingested vitamin E amounts and hs-CRP levels. The Spearman correlation coefficient was calculated to assess the correlation between average daily intake amounts of vitamin E from supplements, and serum levels of  $\alpha$ -tocopherol, in regular vitamin E supplement users. Linear regression analyses were conducted for regular vitamin E supplement users dividing

the exposure serum  $\alpha$ -tocopherol levels into quartiles with the first quartile as reference group; and effect modification through intake of at least one of the other four antioxidants (vitamin C, carotenoids, Se, and Zn) was investigated by inclusion of the respective interaction terms in the models. The quadratic term of BMI (BMI<sup>2</sup>) was used in the analyses in addition to BMI, because of a non-linear relationship of BMI with hs-CRP. Statistical analyses were performed using Statistical Analysis Systems version 9.3 (SAS Institute, Inc.).

### Results

The final study population comprised 2924 participants (1413 men, 1511 women) with a mean age of 56 years, ranging from 31 to 82 years. Frequency of regular antioxidant intake was 9.1% for vitamin E, 9.2% for vitamin C, 4.0% for carotenoids, 5.0% for Se, and 6.9% for Zn. Frequencies of sex-specific intakes and intakes as needed are presented in Table 2. The geometric mean hs-CRP concentration in the study population was 1.13 (antilog *SD* 2.74) mg/l. Unadjusted and adjusted geometric means by categories of covariables are provided in Table 1, and by categories of exposure variables in Table 3.

Regular intake of any of the five investigated antioxidative nutrients *per se* was not associated with hs-CRP (Table 4). However, dose–effect analyses revealed that regular intake of more than 78 mg vitamin E, which corresponds to the top quartile, was significantly associated with lower hs-CRP levels. In fully adjusted analyses hs-CRP levels of persons who ingested more than 78 mg vitamin E/d were 22% lower compared to those of participants who did not ingest any vitamin E from supplements or medications (Table 4). This association persisted after additional adjustment for the use of at least one of the other four antioxidants (Table 4) and

**Table 2.** Use of antioxidative supplements in KORA F4 (Cooperative Health Research in the Region of Augsburg)

(Number of subjects and percentages, *n* 2924)

Nutrient	Men		Women		Total	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
<b>Vitamin E</b>						
No intake	1281	90.7	1325	87.7	2606	89.1
Regular intake	109	7.7	156	10.3	265	9.1
Intake as needed	23	1.6	30	2.0	53	1.8
<b>Vitamin C</b>						
No intake	1241	87.8	1285	85.0	2526	86.4
Regular intake	114	8.1	156	10.3	270	9.2
Intake as needed	58	4.1	70	4.6	128	4.4
<b>Carotenoids</b>						
No intake	1360	96.3	1433	94.8	2793	95.5
Regular intake	48	3.4	68	4.5	116	4.0
Intake as needed	5	0.4	10	0.7	15	0.5
<b>Se</b>						
No intake	1346	95.3	1417	93.8	2763	94.5
Regular intake	58	4.1	88	5.8	146	5.0
Intake as needed	9	0.6	6	0.4	15	0.5
<b>Zn</b>						
No intake	1321	93.5	1363	90.2	2684	91.8
Regular intake	76	5.4	127	8.4	203	6.9
Intake as needed	16	1.1	21	1.4	37	1.3

**Table 3.** C-reactive protein (CRP) levels by categories of exposure variables in KORA F4 (Cooperative Health Research in the Region of Augsburg) (Geometric mean values and antilog standard deviations, *n* 2924)

Antioxidative nutrient	Intake	Quartile boundaries	<i>n</i>	hs-CRP (mg/l)				<i>P</i> for mean difference†
				Unadjusted		Adjusted*		
				Geometric mean	Antilog SD	Geometric mean	Antilog SD	
Vitamin E		mg/d					0.0646	
	No intake	–	2606	1.13	2.75	1.14	2.67	
	Quartile 1	0– ≤ 10	81	1.10	2.70	1.01	2.67	
	Quartile 2	10– ≤ 20	55	1.16	2.65	1.07	2.67	
	Quartile 3	20– ≤ 78	63	1.40	2.56	1.20	2.68	
	Quartile 4	> 78	66	0.98	2.59	0.81	2.69	
Vitamin C		mg/d					0.1215	
	No intake	–	2526	1.15	2.74	1.15	2.67	
	Quartile 1	0– ≤ 60	84	1.08	2.76	0.98	2.67	
	Quartile 2	60– ≤ 120	62	1.40	2.46	1.26	2.67	
	Quartile 3	120– ≤ 275	57	1.01	2.54	0.93	2.67	
	Quartile 4	> 275	67	0.99	2.57	0.99	2.67	
Carotenoids		mg/d					0.7030	
	No intake	–	2793	1.13	2.75	1.13	2.67	
	Quartile 1	0– ≤ 1	29	1.29	2.41	1.20	2.67	
	Quartile 2	1– ≤ 3	29	1.15	2.68	1.05	2.67	
	Quartile 3	3– ≤ 8	31	1.08	2.59	0.95	2.67	
	Quartile 4	> 8	27	1.41	2.41	1.16	2.68	
Se		µg/d					0.1665	
	No intake	–	2763	1.13	2.75	1.14	2.67	
	Quartile 1	0– ≤ 25	69	1.25	2.53	1.12	2.67	
	Quartile 2	25– ≤ 30	17	1.13	2.89	1.07	2.67	
	Quartile 3	30– ≤ 60	27	0.85	2.32	0.73	2.67	
	Quartile 4	> 60	33	0.96	2.84	0.89	2.67	
Zn‡							0.2652	
	No intake	–	2684	1.14	2.74	1.14	2.67	
	Regular intake	–	203	1.09	2.69	1.02	2.68	
	Intake as needed	–	37	0.96	3.11	1.06	2.67	

hs-CRP, high sensitivity C-reactive protein.

\* Adjusted for age (continuous) and sex.

† *P* for age- and sex-adjusted mean difference (ANCOVA).

‡ Division into quartiles was not possible due to distribution, because 40% of persons with regular intakes ingested 5 mg Zn/d.

other nutrient supplements (ratio 0.79 (95% CI 0.63, 0.99)). A spline ( $P=0.25$ ,  $\chi^2$  test) did not improve significantly the description of the association between regularly ingested vitamin E amounts and hs-CRP compared to the linear model.

The interaction term of regular vitamin E use in the top quartile and use of any of the other four antioxidants was significant with a *P*-value of 0.028. Stratification according to single vitamin E supplementation (without the use of any of the other four antioxidants) and supplementation of vitamin E in combination with at least one of the other antioxidants showed that associations were confined to those, who ingested vitamin E in combination with at least one other antioxidant (ratio 0.66 (95% CI 0.48, 0.90)), but the same could not be seen in those with supplementation of vitamin E alone (ratio 1.09 (95% CI 0.77, 1.56)) (see Fig. 1).

Participants in the highest vitamin E quartile (*n* 66) were 54.6% female and ingested mean vitamin E amounts of 256 (SD 141) mg/d. In persons who ingested vitamin E in combination with other antioxidants (*n* 42), the most frequent combination was use of all five antioxidants (*n* 14), followed by vitamin E with vitamin C (*n* 7). Only five participants of the forty-two did not supplement vitamin E together with vitamin C.

Effect modification by sex using quartiles of vitamin E as exposure was not seen ( $P=0.80$ ). However, stratified analyses

revealed a stronger association in women, whereas the association was weaker and non-significant in men (ratios for highest vitamin E quartiles: 0.72 (95% CI 0.52, 0.98) and 0.88 (95% CI 0.63, 1.23), respectively). Further adjustment for hormone therapy and use of oral contraceptives in women did not alter results substantially (data not shown).

As a sensitivity analysis, the relationship between serum levels of  $\alpha$ -tocopherol and hs-CRP concentrations in persons who regularly ingested vitamin E supplements was investigated (*n* 165) (see online Supplementary Table S1). The correlation coefficient between average intake amounts of vitamin E and serum levels of  $\alpha$ -tocopherol was 0.53 ( $P<0.001$ ). Ratios for the association of serum  $\alpha$ -tocopherol concentrations with hs-CRP from linear regression analyses decreased non-significantly for  $\alpha$ -tocopherol quartile numbers two, three and four *v.* one as follows respectively: 1.03 (95% CI 0.70, 1.52),  $P=0.88$ ; 0.83 (95% CI 0.56, 1.23),  $P=0.36$ ; 0.79 (95% CI 0.53, 1.17),  $P=0.23$  (see online Supplementary Table S1). The *P*-value for the interaction with intake of at least one of the other four antioxidants was 0.0260. In persons who ingested other antioxidants as well, higher  $\alpha$ -tocopherol levels compared to lower values showed a tendency to be associated with lower hs-CRP levels (ratios for quartiles three and four *v.* one, respectively: 0.85 (95% CI 0.56, 1.29),

**Table 4.** Dose–response relationship between regular nutrient intake with high sensitivity C-reactive protein (hs-CRP) levels (linear regression)  
(Ratios and 95 % confidence intervals of geometric means)

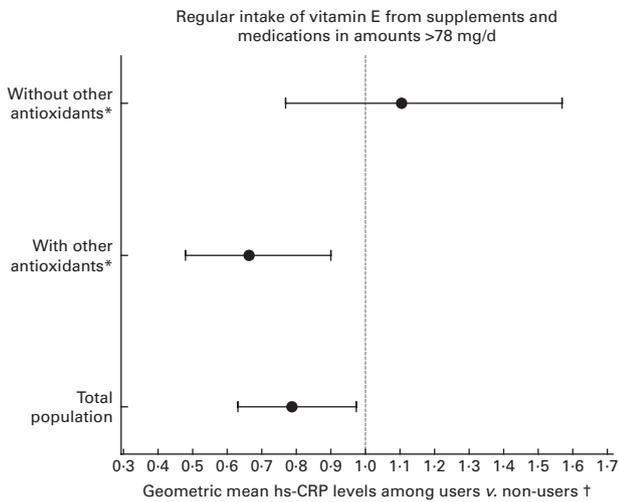
Antioxidant nutrient	Model	hs-CRP levels*									
		Regular intake†		Quartile 1		Quartile 2		Quartile 3		Quartile 4	
		Ratio	95 % CI	Ratio	95 % CI	Ratio	95 % CI	Ratio	95 % CI	Ratio	95 % CI
Vitamin E				0 < – ≤ 10 mg/d		10 < – ≤ 20 mg/d		20 < – ≤ 78 mg/d		> 78 mg/d	
	<i>n</i>	265		81		55		63		66	
	Unadjusted	1.01	0.89, 1.15	0.97	0.77, 1.21	1.02	0.78, 1.34	1.23	0.96, 1.59	0.87	0.68, 1.11
	Age- and sex-adjusted	0.88	0.78, 1.00	0.88	0.71, 1.10	0.93	0.72, 1.21	1.05	0.82, 1.34	0.71	0.56, 0.90
	Fully adjusted‡	0.96	0.86, 1.07	0.94	0.77, 1.14	0.99	0.79, 1.26	1.19	0.95, 1.48	0.78	0.63, 0.97
	Fully adjusted‡ + other antioxidants	0.96	0.83, 1.10	0.95	0.77, 1.17	1.00	0.78, 1.29	1.20	0.95, 1.53	0.79	0.63, 0.99
Vitamin C				0 < – ≤ 60 mg/d		60 < – ≤ 120 mg/d		120 < – ≤ 275 mg/d		> 275 mg/d	
	<i>n</i>	270		84		62		57		67	
	Unadjusted	0.96	0.85, 1.09	0.94	0.75, 1.17	1.22	0.95, 1.58	0.88	0.68, 1.15	0.86	0.68, 1.10
	Age- and sex-adjusted	0.90	0.79, 1.02	0.86	0.69, 1.06	1.10	0.86, 1.41	0.81	0.63, 1.05	0.87	0.68, 1.10
	Fully adjusted‡	1.02	0.91, 1.14	0.98	0.81, 1.18	1.17	0.94, 1.46	0.96	0.76, 1.21	1.00	0.81, 1.23
	Fully adjusted‡ + other antioxidants	1.08	0.93, 1.25	1.03	0.83, 1.28	1.24	0.97, 1.59	1.01	0.79, 1.31	1.05	0.83, 1.33
Carotenoids				0 < – ≤ 1 mg/d		1 < – ≤ 3 mg/d		3 < – ≤ 8 mg/d		> 8 mg/d	
	<i>n</i>	116		29		29		31		27	
	Unadjusted	1.08	0.90, 1.30	1.14	0.79, 1.65	1.01	0.70, 1.47	0.96	0.67, 1.37	1.25	0.85, 1.83
	Age- and sex-adjusted	0.95	0.79, 1.15	1.06	0.74, 1.52	0.93	0.65, 1.33	0.84	0.59, 1.18	1.03	0.71, 1.49
	Fully adjusted‡	1.09	0.93, 1.29	1.21	0.88, 1.67	1.11	0.80, 1.53	1.04	0.76, 1.42	1.02	0.73, 1.42
	Fully adjusted‡ + other antioxidants	1.13	0.95, 1.36	1.26	0.90, 1.76	1.15	0.83, 1.61	1.08	0.78, 1.48	1.05	0.75, 1.48
Se				0 < – ≤ 25 µg/d		25 < – ≤ 30 µg/d		30 < – ≤ 60 µg/d		> 60 µg/d	
	<i>n</i>	146		69		17		27		33	
	Unadjusted	0.96	0.81, 1.13	1.10	0.86, 1.40	0.99	0.61, 1.61	0.75	0.51, 1.11	0.85	0.60, 1.20
	Age- and sex-adjusted	0.86	0.73, 1.01	0.99	0.78, 1.25	0.94	0.59, 1.50	0.64	0.44, 0.93	0.78	0.56, 1.10
	Fully adjusted‡	0.99	0.86, 1.15	1.13	0.92, 1.40	0.95	0.63, 1.45	0.83	0.59, 1.16	0.89	0.66, 1.21
	Fully adjusted‡ + other antioxidants	1.03	0.87, 1.22	1.18	0.94, 1.47	0.99	0.64, 1.51	0.86	0.61, 1.21	0.92	0.67, 1.26
Zn§				–		–		–		–	
	<i>n</i>	203		–		–		–		–	
	Unadjusted	0.96	0.83, 1.11	–	–	–	–	–	–	–	
	Age- and sex-adjusted	0.89	0.78, 1.03	–	–	–	–	–	–	–	
	Fully adjusted‡	1.04	0.91, 1.18	–	–	–	–	–	–	–	
	Fully adjusted‡ + other antioxidants	1.12	0.96, 1.30	–	–	–	–	–	–	–	

\* Reference group: no intake of nutrient of interest from supplements or medications.

† Binary coded as yes/no.

‡ Adjusted for age (continuous), sex, education (low/high), BMI (continuous), BMI<sup>2</sup> (continuous), physical activity (active/inactive), smoking (current/former/never), alcohol consumption (none/moderate/high), cholesterol:HDL ratio (continuous), actual hypertension (yes/no), CVD (yes/no), diabetes (yes/no), non-steroidal anti-inflammatory drug use (yes/no), and statin use (yes/no).

§ Division into quartiles was not possible due to distribution, because 40 % of persons with regular intakes ingested 5 mg Zn/d.



**Fig. 1.** Interaction between regularly ingested vitamin E amounts in the upper quartile and intake of other antioxidants. Values are ratios of geometric mean hs-CRP, with their 95% confidence intervals represented by horizontal bars. \* Intake of at least one of the other investigated antioxidants (vitamin C, carotenoids, selenium, and zinc). † Estimates result from fully adjusted linear regression models (adjusted for age (continuous), sex, education (low/high), BMI (continuous), BMI<sup>2</sup> (continuous), physical activity (active/inactive), smoking (current/former/never), alcohol consumption (none/moderate/high), cholesterol:HDL ratio (continuous), actual hypertension (yes/no), CVD (yes/no), diabetes (yes/no), non-steroidal anti-inflammatory drug use (yes/no), and statin use (yes/no)). hs-CRP, high sensitivity C-reactive protein.

$P=0.45$ ; 0.76 (95% CI 0.49, 1.17),  $P=0.21$ ) (see online Supplementary Table S1). On the contrary, in persons who ingested vitamin E supplements only without any other antioxidants, higher  $\alpha$ -tocopherol levels tended to be associated with higher hs-CRP levels (ratios for quartiles three and four *v.* one, respectively: 1.62 (95% CI 0.33, 8.01),  $P=0.54$ ; 1.47 (95% CI 0.34, 6.39),  $P=0.59$ ) (see online Supplementary Table S1). Overall, associations were not significant possibly due to small numbers especially in the stratified analyses ( $n \leq 36$  in the group of subjects who did ingest other antioxidants besides vitamin E, and  $n \leq 11$  in the group of subjects who did not).

### Discussion

Regular vitamin E intake in amounts above 78 mg/d was significantly associated with lower hs-CRP levels in the present study. Stratified analyses showed this association was found only in persons who used vitamin E in combination with other antioxidants. The interaction term for sex was not significant; however, associations were stronger in women and non-significant in men after stratification.

Vitamin E is a potent antioxidant that can reduce oxidative stress and influence inflammatory processes<sup>(35)</sup>. Direct anti-inflammatory effects are triggered through several mechanisms including down regulation of the proinflammatory transcription factor NF- $\kappa$ B<sup>(36)</sup>, reduction of lipopolysaccharide-stimulated levels of the proinflammatory cytokine TNF- $\alpha$ <sup>(37)</sup>, and decrease of proinflammatory IL-1 $\beta$  by inhibition of 5-lipoxygenase,

an enzyme involved in synthesis of inflammatory prostaglandins<sup>(38)</sup>. It is noteworthy that there is an interaction between inflammation and oxidative stress, implying that one condition triggers the other<sup>(39)</sup>. Oxidative stress is increased through the production of reactive oxygen and nitrogen species by immune cells. And these species do promote inflammation through the activation of NF- $\kappa$ B and the subsequent transcription of proinflammatory cytokines<sup>(39)</sup>. Therefore, an anti-inflammatory effect of vitamin E may be reasonable via both direct anti-inflammatory and also antioxidative pathways.

Supplementation of vitamin E alone might not be as favourable as compared to its combined supplementation with other antioxidants, because antioxidative action of vitamin E leads to the formation of oxidized vitamin E, which has pro-oxidative potential if not regenerated. Vitamin C as a hydrophilic antioxidant is involved in the regeneration of oxidized vitamin E<sup>(40)</sup>. This could explain why vitamin E used particularly in combination with the other antioxidants, especially vitamin C, mostly supplemented in the present study, was most strongly associated with lower hs-CRP levels. Accordingly, Garcia-Bailo *et al*<sup>(39)</sup> suggested that the combined administration of vitamin C and vitamin E might be more effective in reducing oxidative stress and inflammation than utilising either micronutrient on its own. Furthermore, it was proposed that there is a 'vitamin E regeneration system' based on the complex interaction of several antioxidants, which has to be considered when investigating vitamin E effectiveness<sup>(41)</sup>. Results from the present investigation support this hypothesis.

$\alpha$ -Tocopherol is the only form of vitamin E used in supplements<sup>(42)</sup>, and a moderate-to-strong correlation was observed in the present study between average daily intake amounts of vitamin E from supplements and  $\alpha$ -tocopherol levels in serum. Results from linear regression analyses for serum levels of  $\alpha$ -tocopherol as exposure were comparable to those found for intake amounts of vitamin E from supplements, i.e., higher  $\alpha$ -tocopherol levels tended to be associated with lower levels of hs-CRP in regular vitamin E supplement users, especially when they ingested other antioxidants as well. On the contrary, the association tended to be positive if other antioxidants were not ingested. Results regarding serum  $\alpha$ -tocopherol levels were, however, not significant, possibly because the number of subjects in each quartile was low since measurements were available only in a subsample of the study population. In previous studies both positive and negative associations between  $\alpha$ -tocopherol levels and CRP concentrations were found, whereas the concomitant use of (antioxidant) supplements was not considered in those studies<sup>(43,44)</sup>. Results from the present study suggest that the simultaneous use of other antioxidant supplements could be an effect modifier that needs to be taken into account.

In line with the present investigation, supplementation of vitamin E alone was not associated with reduced CRP levels in two intervention studies, where 800 and 400 IU levels of vitamin E/d were used respectively<sup>(45,46)</sup>, with the exception of another trial that used an extremely high amount of 1200 IU vitamin E/d<sup>(47)</sup> (1 IU equating to 0.67 mg *RRR*- $\alpha$ -tocopherol).

However, combined supplementation of 800 IU vitamin E together with twenty-three other ingredients (among them vitamin C,  $\beta$ -carotene, Se, and Zn) reduced CRP levels in a randomized, double-blind, placebo-controlled trial<sup>(48)</sup>.

As in the present analysis, other observational studies examining associations between nutrients and hs-CRP also reported for antioxidants stronger inverse associations among women as compared to those in men<sup>(3)</sup>, and similar associations for glucosamine and chondroitin<sup>(32)</sup>. Furthermore, in two intervention studies, no effect of vitamin E in combination with vitamin C and with vitamin C and  $\beta$ -carotene was observed in men with high cholesterol or with elevated homocysteine levels<sup>(49,50)</sup>. However, Floegel *et al.*<sup>(4)</sup> found in the US National Health and Nutrition Examination Survey no significant interaction on the basis of sex, and reported combined results for an inverse association of antioxidant intake from diet plus supplements with CRP concentrations. Further randomized controlled trials especially in women are warranted to investigate the association between supplementation of antioxidants and CRP levels.

Vitamin E intake amounts of 78 mg/d are well above the German reference values for daily vitamin E intake, which is set at 13 mg for men and 14 mg for women in the age group of 51 to 64 years<sup>(51)</sup>. The actual European Tolerable Upper Intake Level for vitamin E was set at 300 mg/d in 2003<sup>(52)</sup>. During the subsequent years, meta-analyses of randomized trials reported an increased risk of mortality among users of high-dosage vitamin E supplements<sup>(53,54)</sup>. According to a review article published in the year 2005, several large or long-term clinical trials support the safety of vitamins E and C in combination<sup>(55)</sup>. However, the safety of antioxidative supplements is still debated<sup>(56)</sup>, and should further be investigated.

The major limitation of the present analysis is the cross-sectional design; therefore, the observed association might not be causal. Furthermore, because of the inherent nature of observational studies, the said effect could be due to residual confounding, i.e., the subgroup of people with intake of several antioxidants could represent a healthier group particularly interested in health. However, analyses were adjusted for common lifestyle factors and use of other supplements. Moreover, a multitude of further important covariables was available for multivariable adjustment. Intake of dietary supplements over the previous 7 d may not truly reflect usual irregular intake behaviour. However, interview questions elicited details of participants' 'regular intake' and 'intake as needed', respectively, and only 'regular intake' of supplements was considered for analyses. The causal role of CRP levels and CVD progression is controversial<sup>(57)</sup>. Nevertheless, CRP is still widely used as a clinical marker in contemporary studies<sup>(33,34,58,59)</sup>.

The major strength of the present study is the thorough assessment of supplements and medications used with detailed data on the composition of individual preparations, which allowed the conduct of dose–effect analyses. Moreover, serum levels of  $\alpha$ -tocopherol were additionally measured which supported the main findings. Also a hs-CRP assay was used to detect lower CRP levels which have the potential to

predict CVD events<sup>(1)</sup>. Finally, the KORA F4 study represents a population-based survey; therefore, results are applicable to the general Southern German population.

## Conclusion

Regular supplementation of vitamin E was associated with lower hs-CRP levels only in higher amounts and in combination with other antioxidants. Further intervention studies are needed to establish whether this association is causal. Furthermore, the safety of this kind of supplementation should be confirmed. If done so, the combined supplementation of vitamin E with other antioxidants could be a promising strategy for the prevention of inflammation-related diseases in the general population.

## Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0007114515000902>

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