Public Health Nutrition: 14(11), 2055–2064 doi:10.1017/S1368980011000395

Antioxidant intake from diet and supplements and elevated serum C-reactive protein and plasma homocysteine concentrations in US adults: a cross-sectional study

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Submitted 27 July 2010: Accepted 23 January 2011: First published online 18 March 2011

Abstract

Objective: To investigate the association of antioxidant intakes from diet and supplements with elevated blood C-reactive protein (CRP) and homocysteine (Hcy) concentrations.

Design: A cross-sectional study. The main exposures were vitamins C and E, carotene, flavonoid and Se intakes from diet and supplements. Elevated blood CRP and Hcy concentrations were the outcome measures.

Setting: The US population and its subgroups.

Subjects: We included 8335 US adults aged ≥19 years from the National Health and Nutrition Examination Survey 1999–2002.

Results: In this US population, the mean serum CRP concentration was 4·14 (95 % CI 3·91, 4·37) mg/l. Intakes of vitamins C and E and carotene were inversely associated with the probability of having serum CRP concentrations >3 mg/l in multivariate logistic regression models. Flavonoid and Se intakes were not associated with the odds of elevated serum CRP concentrations. The mean plasma Hcy concentration was 8·61 (95 % CI 8·48, 8·74) μmol/l. Intakes of vitamins C, E, carotenes and Se were inversely associated with the odds of plasma Hcy concentrations >13 μmol/l after adjusting for covariates. Flavonoid intake was not associated with the chance of elevated plasma Hcy concentrations.

Conclusions: These results suggest that high antioxidant intake is associated with lower blood concentrations of CRP and Hcy. These inverse associations may be among the potential mechanisms for the beneficial effect of antioxidant intake on CVD risk mediators in observational studies.

Keywords
Antioxidants
Vitamin C
Vitamin E
Flavonoids
Carotenes
Selenium
C-reactive protein
Homocysteine
Diet
Supplements

CVD is the leading cause of death in the USA and worldwide[1]. Each day nearly 2300 Americans die of CVD, which equals an average of one death every 38 s[1]. Elevated serum C-reactive protein (CRP) and plasma homocysteine (Hcy) concentrations have been identified as contributing risk factors to CVD[2–5]. CRP is produced by hepatocytes as part of the acute-phase response and represents a sensitive, non-specific marker of inflammation[6]. A recent meta-analysis of twenty-four cohort studies identified CRP as an independent risk factor for CHD after adjusting for the classical Framingham risk variables[4]. Serum CRP concentrations of >3 mg/l were associated with a 58 % increased risk of CHD compared with CRP concentrations <1 mg/l. Hcy is a sulfur-containing intermediate of methionine metabolism and has been associated with an increased risk of CVD and all-cause mortality[3]. A meta-analysis of prospective cohort and retrospective or nested case–control studies confirmed a positive association of Hcy concentrations with cerebrovascular disease and CHD[5].

Dietary intakes of antioxidant-rich foods such as fruit and vegetables, tea and cocoa were associated with decreased CRP concentrations in several population-based studies[7–11]. A cross-sectional study in Japan found that a healthy dietary pattern containing fruits, vegetables and soya products was inversely associated with serum CRP concentrations[12]. Antioxidants present in foods purportedly have anti-inflammatory properties[13,14], inhibit lipid peroxidation in vessel walls[15] and stop pro-atherogenic and pro-thrombotic[16] processes that may be

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relevant for atherosclerosis and CVD. However, results from interventional trials with selected antioxidant-rich foods or antioxidant supplementation remain inconclusive[15,17–22]. Observational studies investigating the associations of antioxidant intake with CVD markers such as CRP on the nutrient level are rare and limited to single antioxidants. These studies favour beneficial effects, but the findings remain inconsistent[23–26]. Most of these previous studies considered plasma antioxidant concentrations instead of the amount of antioxidant intake. Previously, a German study found that high vitamin E intake was associated with reduced CRP concentrations[23]. That study was limited to antioxidant intakes from supplements only. However, in order to investigate the beneficial health effects of antioxidants on CVD risk factors, antioxidant intakes from both diet and supplements need to be considered. As we reported previously, diet as well as supplements are major contributors to total antioxidant intake in the USA[27]. The association of antioxidant intake with plasma Hcy concentrations in a population-based sample has not been reported in the literature.

Therefore, the purpose of the present study was to investigate the association of antioxidant intakes from diet and diet plus supplements with the possibility of elevated serum CRP and plasma Hcy concentrations in a cross-sectional design.

Subjects and methods

Study population

The National Center for Health Statistics (NCHS) conducts the National Health and Nutrition Examination Survey (NHANES) on a regular basis to obtain nationally representative information on the health and nutritional status of the US population, including an over-sampling of people aged ≥60 years, African Americans and Hispanics in order to collect a larger number of certain subgroups of particular public health interest. The NHANES 1999–2002 data sets included a total number of 21 004 participants. We included US adults aged ≥19 years only (n 10 853). Out of this subsample, pregnant and lactating women were excluded (n 666). Inclusion was also limited to individuals who completed a 24 h dietary recall (DR) and an interview on dietary supplement use (n 8809). Finally, individuals with missing data on serum CRP and plasma Hcy concentrations were excluded (n 474). Thus, the final sample included 8335 participants.

The US adult population was grouped by socio-demographic and lifestyle variables: age (19–30, 31–50, 51–70 and >70 years), gender, ethnicity (white, black, Hispanic and others), BMI (< 18·5, 18·5–24·9, 25·0–29·9 and ≥30 kg/m^2), current smoking (yes or no to 'current smoking' and 'smoked cigarettes, cigars or pipes and/or used chewing tobacco or snuff at least once during the past 30 d') and exercise levels (expressed on the metabolic equivalent score calculated by combining the intensity level of leisure-time activities reported and average duration and frequency). Medical condition was assessed by examination and questionnaire.

Food consumption data

Dietary intakes of vitamins C and E, carotenes, Se and flavonoids were estimated on the basis of one 24 h DR (midnight to midnight) of the NHANES 1999–2002[28,29]. DR data contained all foods and beverages consumed by the respondents, except for plain drinking water. Individuals with unreliable or incomplete DR records were excluded from the present study as recommended by the NCHS[30].

The US Department of Agriculture flavonoid databases

Details of the data sets used in the present study were reported in our previous study[31]. Briefly, we created one flavonoid database from two different data sets released in recent years: the US Department of Agriculture (USDA) database for the flavonoid content of selected foods (2007 update)[32] and the USDA–Iowa State University database on the isoflavone content of foods (2008 update)[33]. The combined flavonoid database consisted of twenty-four flavonoid compounds: flavonols (quercetin, kaempferol, myricetin, isorhamnetin), flavones (luteolin, apigenin), flavanones (eriodictyol, hesperetin, naringenin), flavan-3-ols (catechins, epicatechins, theaflavins, thearubigins), anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin) and isoflavones (daidzein, genistein, glycitein, biochanin A, formononetin). In order to improve the coverage of the estimated flavonoid intake, we expanded the flavonoid database according to the pre-established protocol that has been described extensively in a separate publication[31].

Estimation of dietary antioxidant intake

The calculation of dietary antioxidant intake has been described in detail in our preliminary study[27]. In summary, we matched the NHANES food consumption data with the USDA flavonoid database following the same procedure: (i) conversion of food items in NHANES DR to USDA Standard Reference codes using food recipe books and food description data files for NHANES food codes; (ii) weight adjustment using moisture content; (iii) code modification using the USDA food unit conversion search program; and (iv) linking food intake data with the flavonoid database. Daily individual flavonoid intake from selected foods was determined by multiplying the content of the individual flavonoids (mg aglycone equivalent/100 g food) by the daily consumption (g/d) of the selected food item. Estimated total intake of individual flavonoids was the sum of individual flavonoid intakes from all food sources reported in the 24 h DR. Total flavonoid intake was determined by the summation

of total intake of individual flavonoids. Data on individual participant's daily dietary intakes of antioxidant vitamins and Se were available in the NHANES 1999–2002[28,29].

Estimation of antioxidant intake from dietary supplement use

Participants were questioned about their supplement use in an interview in NHANES 1999–2002. Five different dietary supplement data files on supplement counts, supplement records, supplement information, ingredient information and blend information were used to calculate antioxidant intakes from supplements[28,29]. In order to calculate the intakes of antioxidant nutrients from supplements, vitamins C and E, carotenes and Se were selected from the ingredient information file. Even though NHANES dietary supplement data provide comprehensive information on the nutrient intake status of the US population from various dietary supplements, limited information is available on flavonoid composition in those products. Furthermore, flavonoid intake from supplements was reported to be <2 % in US adults[27]. Therefore, flavonoid intake from supplements was not included in the present study.

The antioxidant composition of supplements was obtained using the supplement information file. If the reported serving size units did not match with the labelled serving size units, they were converted according to the labelled serving size units. Ingredient units in the supplements were converted to the Dietary Reference Intake units. Nutrient compounds in the supplements were converted to elemental nutrients[34]. Different nutrient units in the two NHANES data sets were converted and adjusted as described previously[27].

Serum C-reactive protein and plasma homocysteine concentrations

Fasting blood samples were collected from all participants and prepared and analysed by the University of Washington Medical Center according to the NHANES Laboratory/Medical Technologists Procedures Manual[35]. Serum CRP was measured using a Behring nephelometer (Dade Behring Diagnostics Inc., Somerville, NJ, USA). The limit of detection of the assay was 0·2 mg/l; the inter-assay CV ranged from 4 % to 9 %. Total Hcy was measured in plasma using the Abbott Hcy assay, an automated fluorescence polarization immunoassay (Abbott Diagnostics, Abbott Park, IL, USA). The linear range for this method was an Hcy concentration of 2–50 μmol/l and the inter-assay CV was 3–6 %[35].

Statistical analysis

All statistical analyses were performed using the SAS statistical software package version 8·1 (SAS Institute Inc., Cary, NC, USA) and the Survey Data Analysis for multistage sample designs professional software package (SUDAAN, release 8·0·2, 2003; Research Triangle Institute, Research Triangle Park, NC, USA). SUDAAN was used to compute variance estimates and test statistics for a stratified, multistage probability survey design. Sample weights were applied to all analyses to account for the unequal probability of selection, non-coverage and non-response bias resulting from over-sampling of low-income persons, adolescents, the elderly, African Americans and Mexican Americans.

Arithmetic means of dietary, total and individual intakes of antioxidants by subpopulations grouped according to sociodemographic and lifestyle variables were calculated. The SE was calculated by the linearization (Taylor series) variance estimation method for population parameters by SUDAAN. Means for interval scale variables were compared using the *t* test (accounting for the population variance) and the ANOVA technique. The *t* test and ANOVA were used to test for overall differences in antioxidant intake by sociodemographic and lifestyle variables such as age, gender and smoking. Serum CRP and plasma Hcy concentrations were reported as geometric means after logarithmic transformation was performed to normalize the right-skewed data distribution. Because there is no standard definition of a high total Hcy concentration, to determine associations between antioxidant intake and hyperhomocysteinaemia, Hcy values were dichotomized either above or below 13·0 μmol/l. This value has been used in a number of studies as a recommended cut-off point for moderate hyperhomocystenaemia[36–38]. We also used currently recommended cut-off points (>3·0 mg/l) for increased CRP level[39,40]. Since there was no interaction with gender, results were combined for both men and women. Logistic regression analysis was applied to calculate the odds of elevated serum CRP and plasma Hcy concentrations with antioxidant intake as the independent variable. Two statistical models were calculated to adjust for possible confounders: model 1 (for CRP) adjusted for age, gender, ethnicity, total energy intake and BMI; model 2 (for Hcy) adjusted for age, gender, ethnicity, total energy and folate intakes. Continuous variables were age, total energy intake, BMI, exercise level, folate intake, plasma Hcy and serum CRP concentrations; categorical variables were gender, ethnicity (white, black, Hispanic and others), current smoking (yes/no), use of non-steroidal anti-inflammatory drugs (NSAID; yes/no) and taking dietary supplements (yes/no). The level of statistical significance was set at $P < 0{\cdot}05$ for two-tailed tests.

Results

On the basis of the selection criteria a total of 8335 US adults (49·4 % men) were included in the present study. Table 1 shows the baseline characteristics of the participants. The mean age of the participants was 46·1 years, mean BMI was 27·9 kg/m^2 and mean total energy intake

Table 1 Baseline characteristics and supplement use of 8335 US adults aged ≥19 years in NHANES 1999–2002

	Mean or *n*	SE or %
Age (years)*	46·1	0·4
Gender (male)	8335	49·4
Ethnicity	8335	–
White	–	72·6
Black	–	10·1
Hispanic	–	6·9
Others	–	10·5
BMI (kg/m^2; *n* 8115)*	27·9	0·1
Energy intake (kcal/d; *n* 8335)*†	2208·3	14·1
Supplement use‡	8335	52·1
Vitamin C	8335	38·1
Vitamin E	8335	37·6
Carotenes	8335	24·2
Selenium	8335	27·9
Current smokers§	7884	49·8
TC:HDL-C ratio >5	8272	28·3
Arthritis	7891	22·2
CHD	7849	3·6
Diabetes	8213	6·6
Chronic bronchitis	7881	6·6
Taking NSAID‖	8335	8·7

NHANES, National Health and Nutrition Examination Survey; TC, total cholesterol; HDL-C, HDL cholesterol; NSAID, non-steroidal anti-inflammatory drugs.
*Data are presented as mean and SE.
†1 kcal = 4·184 kJ.
‡Supplement use implies taking any dietary supplement including vitamins, minerals or other dietary supplements at the time of interview.
§Current smoking means to have smoked cigarettes, cigars, pipes or to have used chewing tobacco or snuff at least once during the past 30 d.
‖Taking any prescribed NSAID during the past month.

was 9238 kJ (2208 kcal)/d. Of the participants, 52·1 % reported taking dietary supplements.

Serum C-reactive protein concentrations

The mean CRP concentrations in fasting serum samples were 3·38 (95 % CI 3·13, 3·62) mg/l in men and 4·88 (95 % CI 4·54, 5·22) mg/l in women (Table 2). Serum CRP increased with age and BMI, elevated ratio of total cholesterol to HDL cholesterol, current smoking status, any adverse medical condition such as chronic bronchitis, arthritis, CHD and diabetes, and among those taking NSAID. A higher level of exercise was associated with a reduction in serum CRP concentrations.

As shown in Table 3, intake of vitamin C was inversely associated with the odds of serum CRP > 3 mg/l, comparing the highest quintile with the lowest quintile of intake as a reference and after multivariate adjustment for age, gender, ethnicity, total energy intake and BMI (model 1; diet only: OR = 0·74, 95 % CI 0·62, 0·88; diet plus supplement: OR = 0·69, 95 % CI 0·56, 0·86). High vitamin E intake from diet only and also from diet plus supplement use was related to a similar reduction in the odds of elevated serum CRP concentrations (diet only: OR = 0·71, 95 % CI 0·57, 0·89; diet plus supplement: OR = 0·70, 95 % CI 0·54, 0·90). High dietary and total carotene intakes were associated with a reduction in the chance of increased serum CRP concentrations (dietary intake: 0·70, 95 % CI 0·56, 0·87; diet plus supplement: 0·71, 95 % CI 0·58, 0·88). Neither flavonoid nor Se intake showed a statistically significant association with the odds of elevated serum CRP concentrations.

Plasma homocysteine concentrations

The mean Hcy concentrations in fasting plasma samples were higher in men (9·21 μmol/l; 95 % CI 9·04, 9·37 μmol/l) than in women (8·03 μmol/l; 95 % CI 7·86, 8·21 μmol/l; Table 2). Plasma Hcy concentrations increased with age and BMI. Current smoking, lower exercise levels and a diagnosis of arthritis, CHD or diabetes were positively associated with elevated plasma Hcy concentrations. Dietary supplement users showed lower plasma Hcy concentrations than did non-users.

Vitamin C intake was inversely related to the odds of elevated plasma Hcy concentrations after multivariate adjustment for age, gender, ethnicity, total energy and folate intakes according to model 2 and comparing the highest with the lowest quintile (diet only: OR = 0·59, 95 % CI 0·36, 0·97; diet plus supplement: OR = 0·35, 95 % CI 0·24, 0·51; Table 4). High vitamin E intake was associated with a lower chance of elevated plasma Hcy concentrations (diet only: OR = 0·55, 95 % CI 0·36, 0·84; diet plus supplement: OR = 0·34, 95 % CI 0·25, 0·46). High Se intakes from diet and diet plus supplement use were also associated with a reduced chance of elevated plasma Hcy concentrations (dietary intake: OR = 0·42, 95 % CI 0·25, 0·71; diet plus supplement: OR = 0·29, 95 % CI 0·18, 0·49). For carotenes, only intake from diet and supplement use and not intake from diet alone was associated with reduced odds of elevated plasma Hcy concentrations (dietary intake: OR = 0·92, 95 % CI 0·63, 1·35, $P = 0·177$; diet plus supplement: OR = 0·72, 95 % CI 0·48, 1·07; $P < 0·05$). Flavonoid intake showed no statistically significant association with the chance of elevated plasma Hcy concentrations after multivariate adjustment.

Discussion

In the present cross-sectional study, an inverse association of vitamins C, E and carotene intakes with the chance of elevated serum CRP concentrations was observed. Furthermore, people with high intakes of vitamins C and E, Se and carotenes were less likely to have elevated plasma Hcy concentrations.

In addition to our study, an Italian study found an inverse association of dietary total antioxidant capacity with plasma concentrations of CRP[41]. A German study of the MONICA/KORA Augsburg cohort[23] reported an inverse association of vitamin E intake from supplements with plasma CRP concentrations but did not find an association with Se intake. However, the same study was not in accordance with our findings on vitamin C and carotene intakes. In agreement with our study, investigators

Table 2 Geometric means of serum CRP and plasma tHcy concentrations by sociodemographic and lifestyle factors and medical condition of 8335 US adults aged ≥19 years in NHANES 1999–2002

	Serum CRP (mg/l)			Plasma tHcy (μmol/l)		
	Mean	95 % CI	*P* value*	Mean	95 % CI	*P* value*
Total (*n* 8335)	4·14	3·91, 4·37		8·61	8·48, 8·74	
Gender			<0·001			<0·001
Male	3·38	3·13, 3·62		9·21	9·04, 9·37	
Female	4·88	4·54, 5·22		8·03	7·86, 8·21	
Age (years)			<0·001			<0·001
19–30	3·01	2·56, 3·47		7·49	7·32, 7·65	
31–50	3·82	3·51, 4·12		8·05	7·91, 8·20	
51–70	4·97	4·52, 5·43		9·14	8·95, 9·34	
>70	5·53	4·80, 6·26		11·65	11·04, 12·26	
Ethnicity (%)			0·078			<0·001
White	3·96	3·71, 4·21		8·71	8·56, 8·86	
Black	5·48	4·79, 6·18		9·00	8·66, 9·33	
Hispanic	4·33	3·61, 5·06		7·59	7·33, 7·85	
Others	3·94	3·23, 4·65		8·21	7·84, 8·58	
BMI (kg/m^2)			<0·001			<0·001
<18·5	2·02	1·12, 2·91		8·07	7·63, 8·51	
18·5–24·9	2·57	2·33, 2·82		8·37	8·20, 8·54	
25·0–29·9	3·80	3·49, 4·11		8·74	8·58, 8·91	
≥30·0	6·29	5·79, 6·78		8·60	8·35, 8·85	
Dietary supplements			0·649			<0·001
Yes	4·07	3·80, 4·35		8·37	8·17, 8·57	
No	4·21	3·84, 4·58		8·87	8·72, 9·02	
Current smoking†			<0·001			<0·001
Yes	4·47	4·17, 4·77		9·02	8·84, 9·20	
No	3·90	3·61, 4·18		8·26	8·13, 8·39	
Exercise level‡			<0·001			<0·001
1	5·02	4·59, 5·45		8·94	8·72, 9·15	
2	4·12	3·71, 4·54		8·38	8·19, 8·57	
3	3·37	3·08, 3·66		8·33	8·07, 8·60	
4	3·02	2·57, 3·46		8·11	7·89, 8·34	
TC:HDL-C ratio			<0·001			<0·001
≤5	3·97	3·72, 4·23		8·50	8·35, 8·65	
>5	4·56	4·22, 4·90		8·89	8·68, 9·11	
Arthritis			<0·001			<0·001
Yes	5·88	5·37, 6·38		9·68	9·34, 10·01	
No	3·70	3·41, 3·99		8·34	8·23, 8·44	
CHD			<0·001			<0·001
Yes	5·79	4·90, 6·67		11·16	10·53, 11·78	
No	4·11	3·86, 4·36		8·54	8·41, 8·66	
Diabetes			<0·001			<0·001
Yes	6·49	5·56, 7·43		9·71	9·18, 10·24	
No	3·96	3·72, 4·19		8·52	8·39, 8·65	
Chronic bronchitis			<0·001			0·903
Yes	6·37	5·11, 7·64		8·67	8·18, 9·15	
No	4·03	3·77, 4·29		8·63	8·48, 8·78	
Taking NSAID§			<0·001			<0·001
Yes	6·05	5·13, 6·96		9·18	8·68, 9·68	
No	3·96	3·76, 4·16		8·56	8·44, 8·67	

CRP, C-reactive protein; tHcy, total homocysteine; NHANES, National Health and Nutrition Examination Survey; TC, total cholesterol; HDL-C, HDL cholesterol; NSAID, non-steroidal anti-inflammatory drugs.
**P* value for mean difference.
†Current smoking means to have smoked cigarettes, cigars, pipes or used chewing tobacco or snuff at least once during the past 30 d.
‡Exercise levels were calculated into the metabolic equivalent score by intensity level of the leisure-time activities reported, as well as mean duration and frequency.
§Taking any prescribed NSAID during the past month.

from the prospective EPIC–Norfolk cohort reported in a cross-sectional analysis that plasma concentrations of vitamin C were associated with decreased plasma concentrations of CRP[24]. Ford *et al.*[25] found a significantly inverse association of plasma vitamin C, carotenes and Se with blood CRP concentrations on analysing the NHANES 1988–1994 data. The two aforementioned studies used a different approach by choosing blood concentrations of antioxidants instead of antioxidant intake and are therefore not directly comparable to our study. Comparing blood concentrations of antioxidants with blood CRP concentrations offers the advantage of considering bioavailability in human metabolism; however, it is also a limited approach as Ford *et al.*[25] report themselves. Thus, inflammation may depress antioxidant concentrations in blood and thereby mask the potential

Table 3 OR and 95 % CI of serum CRP > 3 mg/l according to quintiles of antioxidant intakes from diet and total antioxidant intakes including supplement use of 8335 US adults aged ≥19 years in NHANES 1999–2002

	Quintile of dietary antioxidant intake*					
Median antioxidant intake	Q1	Q2	Q3	Q4	Q5	*P* value†
Flavonoids‡						
Diet only (mg/d)	2·8	16·8	40·4	92·0	504·8	
n	1670	1663	1668	1667	1667	
Multivariate model 1§						0·212
OR	1·00‖	0·86	0·84	0·81	0·89	
95 % CI	–	0·70, 1·05	0·70, 1·01	0·66, 0·99	0·70, 1·13	
Vitamin C						
Diet only (mg/d)	11·7	34·1	66·4	115·5	228·1	
n	1669	1665	1668	1667	1666	
Multivariate model 1§						<0·001
OR	1·00‖	0·80	0·75	0·70	0·74	
95 % CI	–	0·65, 0·99	0·59, 0·96	0·59, 0·84	0·62, 0·88	
Diet plus supplement (mg/d)	15·5	49·0	98·8	177·4	521·1	
n	1668	1666	1667	1667	1667	
Multivariate model 1§						<0·01
OR	1·00‖	0·78	0·90	0·77	0·69	
95 % CI	–	0·64, 0·95	0·70, 1·16	0·64, 0·93	0·56, 0·86	
Vitamin E						
Diet only (mg ATE/d)	2·2	3·8	5·3	7·5	12·2	
n	1672	1658	1674	1666	1665	
Multivariate model 1§						<0·01
OR	1·00‖	1·00	1·05	0·91	0·71	
95 % CI	–	0·81, 1·25	0·85, 1·29	0·76, 1·10	0·57, 0·89	
Diet plus supplement (mg ATE/d)	2·5	4·6	7·3	11·6	68·5	
n	1669	1668	1663	1669	1666	
Multivariate model 1§						<0·05
OR	1·00‖	0·86	0·87	0·91	0·70	
95 % CI	–	0·67, 1·09	0·72, 1·05	0·76, 1·10	0·54, 0·90	
Carotenes						
Diet only (μg RE/d)	29·6	123·0	274·7	538·5	1263·7	
n	1667	1667	1667	1667	1667	
Multivariate model 1§						<0·05
OR	1·00‖	0·81	0·81	0·90	0·70	
95 % CI	–	0·66, 0·99	0·65, 1·02	0·71, 1·13	0·56, 0·87	
Diet plus supplement (μg RE/d)	37·0	141·5	298·5	573·7	1304·2	
n	1667	1667	1667	1667	1667	
Multivariate model 1§						<0·05
OR	1·00‖	0·87	0·86	0·90	0·71	
95 % CI	–	0·72, 1·04	0·70, 1·05	0·73, 1·12	0·58, 0·88	
Selenium						
Diet only (μg/d)	43·2	69·5	91·4	119·7	177·4	
n	1669	1665	1668	1666	1667	
Multivariate model 1§						0·522
OR	1·00‖	0·95	0·99	1·04	0·86	
95 % CI	–	0·78, 1·14	0·83, 1·19	0·81, 1·33	0·67, 1·12	
Diet plus supplement (μg/d)	45·9	74·6	99·4	132·0	202·6	
n	1667	1668	1666	1668	1666	
Multivariate model 1§						0·262
OR	1·00‖	0·88	0·93	0·99	0·80	
95 % CI	–	0·74,1·03	0·78, 1·10	0·80, 1·22	0·62, 1·03	

CRP, C-reactive protein; NHANES, National Health and Nutrition Examination Survey; ATE, α-tocopherol equivalents; RE, retinol equivalents.
*All participants who did not consume the specific antioxidant nutrient in one 24 h dietary recall were proposed as group 'non-consumers' and included in Q1; all consumers were divided into quintiles by the amount of consumption. Q1, Q2, Q3, Q4 and Q5 stand for the first, second, third, fourth and fifth quintiles, respectively.
†*P* value for linear trend.
‡Flavonoid intake from supplement use was <2 % in US adults. Therefore, only dietary intake was considered for analysis.
§Model 1: adjusted for age, gender, ethnicity, total energy intake and BMI
‖Reference category.

beneficial effect of antioxidants on CRP concentrations. Nevertheless, these findings are consistent with our results, except for Se. Se acts through a different mechanism. In contrast to the other antioxidants, it does not directly scavenge free radicals or interrupt peroxidation chain reactions[13,14]; instead, it is a cofactor of peroxidases and detoxifies lipid peroxides[42]. Therefore, Se intake and blood concentrations may differently impact blood CRP concentrations. Estimation of Se intake from nutritional databases also involves a limitation. Se content of foods may differ depending on geographical region, season of cultivation and also food processing[43]. In the USA, for

Table 4 OR and 95 % CI of plasma tHcy > 13 μmol/l according to quintiles of antioxidant intakes from diet and total antioxidant intakes including supplement use of 8335 US adults aged ≥19 years in NHANES 1999–2002

	Quintile of dietary antioxidant intake*					
Median antioxidant intake	Q1	Q2	Q3	Q4	Q5	*P*-value†
Flavonoids‡						
Diet only (mg/d)	2·8	16·8	40·4	92·0	504·8	
n	1670	1663	1668	1667	1667	
Multivariate model 2§						0·972
OR	1·00‖	0·66	0·73	1·02	0·80	
95 % CI	–	0·45, 0·95	0·50, 1·06	0·68, 1·53	0·56, 1·14	
Vitamin C						
Diet only (mg/d)	11·7	34·1	66·4	115·5	228·1	
n	1669	1665	1668	1667	1666	
Multivariate model 2§						<0·05
OR	1·00	0·84	0·76	0·56	0·59	
95 % CI	–	0·62, 1·13	0·53, 1·10	0·39, 0·80	0·36, 0·97	
Diet plus supplement (mg/d)	15·5	49·0	98·8	177·4	521·1	
n	1668	1666	1667	1667	1667	
Multivariate model 2§						<0·001
OR	1·00‖	0·82	0·61	0·44	0·35	
95 % CI	–	0·61, 1·09	0·42, 0·89	0·30, 0·65	0·24, 0·51	
Vitamin E						
Diet only (mg ATE/d)	2·2	3·8	5·3	7·5	12·2	
n	1672	1658	1674	1666	1665	
Multivariate model 2§						<0·05
OR	1·00	0·78	0·65	0·72	0·55	
95 % CI	–	0·58, 1·04	0·43, 0·98	0·50, 1·04	0·36, 0·84	
Diet plus supplement (mg ATE/d)	2·5	4·6	7·3	11·6	68·5	
n	1669	1668	1663	1669	1666	
Multivariate model 2§						<0·001
OR	1·00‖	0·75	0·65	0·47	0·34	
95 % CI	–	0·53, 1·07	0·47, 0·89	0·32, 0·69	0·25, 0·46	
Carotenes						
Diet only (μg RE/d)	29·6	123·0	274·7	538·5	1263·7	
n	1667	1667	1667	1667	1667	
Multivariate model 2§						0·177
OR	1·00‖	0·91	0·67	0·57	0·92	
95 % CI	–	0·64, 1·29	0·47, 0·96	0·37, 0·87	0·63, 1·35	
Diet plus supplement (μg RE/d)	37·0	141·5	298·5	573·7	1304·2	
n	1667	1667	1667	1667	1667	
Multivariate model 2§						<0·05
OR	1·00	0·72	0·63	0·49	0·72	
95 % CI	–	0·51, 1·02	0·45, 0·88	0·34, 0·72	0·48, 1·07	
Se						
Diet only (μg/d)	43·2	69·5	91·4	119·7	177·4	
n	1669	1665	1668	1666	1667	
Multivariate model 2§						<0·01
OR	1·00‖	0·59	0·61	0·54	0·42	
95 % CI	–	0·44, 0·79	0·46, 0·80	0·38, 0·77	0·25, 0·71	
Diet plus supplement (μg/d)	45·9	74·6	99·4	132·0	202·6	
n	1667	1668	1666	1668	1666	
Multivariate model 2§						<0·001
OR	1·00‖	0·65	0·52	0·49	0·29	
95 % CI	–	0·49, 0·85	0·40, 0·66	0·37, 0·64	0·18, 0·49	

tHcy, total homocysteine; NHANES, National Health and Nutrition Examination Survey; ATE, α-tocopherol equivalents; RE, retinol equivalents.
*All participants who did not consume the specific antioxidant nutrient in one 24 h dietary recall were proposed as group 'non-consumers' and included in Q1; all consumers were divided into quintiles by the amount of consumption. Q1, Q2, Q3, Q4 and Q5 stand for the first, second, third, fourth and fifth quintiles, respectively.
†*P* value for linear trend.
‡Flavonoid intake from supplement use was <2 % in US adults. Therefore, only dietary intake was considered for analysis.
§Model 2: adjusted for age, gender, ethnicity, total energy intake and folate intake.
‖Reference category.

example, the Se concentrations in the same crop could be up to 200 times different depending on the Se concentration in the soil where the crops were cultivated.

Our results on flavonoid intake and serum CRP concentrations are in contrast to our previous findings, in which we reported an inverse association of flavonoid intake and serum CRP[26]. This is most likely explained by the fact that, in the previous study, we used non-consumers as the reference group, whereas in the present study we included non-consumers into the first quintile of intake. This observation may suggest that non-consumers of flavonoids are at higher risk for elevated CRP; however,

a low amount of consumption may be sufficient to reduce CRP concentrations. Nevertheless, our results for flavonoid intake should be interpreted critically. This conceptual limitation, however, is irrelevant for the other antioxidants, because none of the participants were classified as non-consumers.

To our knowledge, no study has ever investigated the relationship between antioxidant intake and plasma Hcy concentrations in a multivariate approach. Previous studies focused on folate and vitamin B intakes only[44,45]. One study that included patients with macular degeneration identified high intakes of dietary antioxidants as one factor related to decreased blood Hcy concentrations using univariate statistics[46]. In our study, on the basis of a sample from the US population, we found an inverse association of vitamins C and E, total carotene and Se intakes with the odds of elevated plasma Hcy concentrations independent of multiple covariates. There was no association for either flavonoid intake or carotene intake from diet.

A biologically plausible mechanism that links antioxidant intake and blood Hcy concentration exists, as Hcy promotes oxidant injury to the vascular endothelium[47]. Antioxidants interfere with oxidative processes at the vessel wall and inhibit pro-atherogenic processes[13,14]. Using a rat model, Joseph *et al.*[48] showed that an antioxidant supplementation with vitamins C and E attenuated hyperhomocysteinaemia-induced myocardial oxidative stress and fibrosis. They concluded that elevated Hcy concentrations act via oxidative stress to directly promote myocardial dysfunction and further suggest that antioxidants could be a preventive and therapeutic treatment for heart failure. In addition, Atamer *et al.*[49] suggest that an oxidant–antioxidant imbalance is closely related to elevated Hcy concentrations in patients with chronic kidney disease. A Tunisian study that included patients with CHD found that high concentrations of Hcy were connected to a low enzyme activity of glutathione peroxidase (GPx)[50]. GPx has the major physiological role to protect tissues from oxidative stress-mediated lipid peroxidation. Interestingly, it is a Se-containing enzyme and GPx activity depends essentially on the presence of Se. Lack of the cofactor Se leads to a loss of function of the enzyme and causes severe oxidative damage[51]. This fact might be one plausible link for the strong inverse association of dietary and total Se intakes with the odds of elevated plasma Hcy concentrations that we observed.

Another interesting observation of our study was that for the odds of elevated Hcy concentration: total carotene intake from diet plus supplement use showed a significant inverse association, whereas intake from diet only did not (Table 4). Considering the other antioxidants, including vitamins C and E and Se, the inverse association was stronger (lower OR) when antioxidant intake was estimated from diet plus supplement use instead of from diet only. This fact suggests that there is some additional benefit from antioxidant supplementation on plasma Hcy concentrations. For CRP concentrations, however, this was not the case, as both antioxidant intake estimations based on diet only and diet plus supplement use resulted in similar OR. It seems as though antioxidant intake from supplement use may have additional beneficial effects on plasma Hcy but not on serum CRP concentrations in the US population. This might not be comparable to the results from other countries. As we reported previously, Americans obtain most of their antioxidants from supplements and not from diet[27]. Nevertheless, the differential effect of antioxidant intake from supplement use on Hcy and CRP requires further investigation.

The strength of our study was that we used a population-based approach and included a large sample size in the analysis by combining the NHANES 1999–2000 and 2001–2002 data sets. We also considered several antioxidants, such as antioxidant vitamins, carotenes, flavonoids and Se, for intake estimations and quantified dietary intake and total intake from diet and supplement use.

Our study has several limitations. First, the implementation of CRP as a biomarker for CVD is being discussed controversially in the literature[52–56]. It is suggested to be a well-proven clinical marker of increased CVD risk that shows good reliability[52]. It has further been suggested as a potential target for CVD diagnosis and prevention[53,54]. However, the major limitation of CRP implementation is its unspecific character as a marker of inflammation[6]. Second, we did not account for the intakes of synthetic antioxidants such as statins, butylated hydroxyanisole and butylated hydroxytoluene. Third, dietary antioxidant intake estimations were based on one 24 h DR that is known to have high within-person variability. Therefore, participants may have been misclassified into lower or higher antioxidant intake categories, which may have produced false positives and negatives and biased the results. However, despite within-person variability, a 24 h DR can produce adequate estimates of mean intake of a group that can be useful for contrasting the dietary status of the group with different levels of risk factors for certain diseases[57]. Furthermore, the cross-sectional design of the present study only allows showing statistical associations and does not prove causality. Large-scale prospective cohort studies and interventional trials are needed to further test a causal relationship between high antioxidant intake and reduction of blood CRP and Hcy concentrations.

In conclusion, we found an inverse association of vitamins C and E and carotene intakes with the chance of elevated serum CRP; further, vitamins C and E, carotene and Se intakes from diet and supplement use were inversely related to the odds of elevated plasma Hcy concentrations. These results support the hypothesis that CRP and Hcy may be mediators of the observed beneficial effects of certain antioxidants on CVD risk. Future studies are warranted to examine whether CRP and Hcy could be considered as mediators.

Acknowledgements

The present study was supported by Beginning Grant-in-Aid no. 0865092E from the American Heart Association. None of the authors reported a conflict of interest. O.K.C., W.O.S. and S.I.K. designed the research; A.F. and O.K.C. conducted the research; S.-J.C., C.E.C. and A.F. performed the statistical analysis; O.K.C., T.P., W.O.S., A.v.R. and M.Y. were responsible for the interpretation of data, as well as for critical review and advice and consultation throughout; A.F. wrote the paper; A.F. and O.K.C. had primary responsibility for the paper. All authors read and approved the final manuscript. The authors thank Dr Seung Ho Kang, Associate Professor of the Department of Applied Statistics from Yonsei University, for his statistical advice throughout the project.

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