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Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects

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Inflammation, a risk factor for cardiovascular disease, is associated with low plasma levels of antioxidant vitamins. In addition to vitamins, other antioxidants modulate the synthesis of inflammatory markers *in vitro* and contribute to the total antioxidant capacity (TAC) of a diet. However, the relationship between dietary TAC and markers of inflammation has never been evaluated *in vivo*. We investigated the relationship between dietary TAC and markers of systemic (high-sensitivity C-reactive protein (hs-CRP), leucocytes) and vascular (soluble intercellular cell adhesion molecule-1) inflammation in 243 non-diabetic subjects. General Linear Model (GLM) analysis showed a significant ($P=0{\cdot}005$) inverse relationship between hs-CRP and quartiles of energy-adjusted dietary TAC, even when recognized modulating factors of inflammation, namely alcohol, fibre, vitamin C, α-tocopherol, β-carotene, BMI, waist circumference, HDL-cholesterol, hypertension, insulin sensitivity and plasma β-carotene, were included in the model as covariates ($P=0{\cdot}004$). The relationship was stronger for subjects with hypertension ($P=0{\cdot}013$ *v.* $P=0{\cdot}109$ for normotensive individuals). Among dietary factors, TAC was significantly higher (5·3 (SD 3·0) *v.* 4·9 (SD 2·7) mmol Trolox/d; $P=0{\cdot}026$) in subjects with low plasma hs-CRP (range: 0·0–4·1 mg/l) than in subjects with high plasma hs-CRP (range: 4·2–27·8 mg/l). We conclude that dietary TAC is inversely and independently correlated with plasma concentrations of hs-CRP and this could be one of the mechanisms explaining the protective effects against CVD of antioxidant-rich foods such as fruits, whole cereals and red wine. This could be of particular significance for subjects with high blood pressure.

Inflammation: Antioxidants: Hypertension: High-sensitivity C-reactive protein

Inflammation and oxidative stress are involved in the pathogenesis of cardiovascular disease (Wattanapitayakul & Bauer, 2001; Lind, 2003). Oxidative damage of the arterial wall by free radicals and the direct stimulation of endothelial cells by the acute-phase C-reactive protein (CRP) promote the expression of cellular adhesion molecules (CAM), which facilitate the adhesion of monocytes and T cells to the arterial wall in the first steps of the atherogenic process (Parhami *et al.* 1993). Oxidative stress appears also responsible for the oxidation of low-density lipoproteins incorporated to the plaque (Parhami *et al.* 1993).

These *in vitro* observations are confirmed by clinical data. In addition to the already established risk factors, the total leukocyte count is an independent predictor of coronary heart disease and myocardial infarction (Danesh *et al.* 1998), the intercellular adhesion molecule-1 (ICAM-1) is consistently elevated in individuals at high risk for atherosclerosis (Demerath *et al.* 2001) and, though to an extent still under debate (Tall, 2004), high plasma concentrations of CRP significantly increase the risk of cardiovascular events (myocardial infarction, stroke, sudden cardiac death and peripheral vascular disease) even among apparently healthy adults (Willerson & Ridker, 2004). Whether these factors of increased risk for CVD are directly modifiable through the diet is an intense area of research. An inverse relationship between plasma levels of certain vitamins (namely vitamin C, carotenoids and α-tocopherol) and markers of inflammation in healthy adults and in patients with myocardial infarction or stroke has been recently observed (Kritchevsky *et al.* 2000; Ford *et al.* 2003; Sanchez-Moreno *et al.* 2004). However, despite the multiple mechanisms by which these vitamins act as anti-inflammatory agents *in vitro* (Calfee-Mason *et al.* 2002; Carcamo *et al.* 2002), supplementation studies show inconsistent results regarding their ability to reduce systemic and vascular inflammation *in vivo*, especially when dietary rather than pharmacological amounts are used (Sanchez-Moreno *et al.* 2003; Van Dam

Abbreviations: CAM, cellular adhesion molecules; CRP, C-reactive protein; GLM, General Linear Model; hs-CRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule-1; OGTT, oral glucose tolerance test; ISI, insulin sensitivity index; PA, physical activity; sICAM-1, soluble intercellular adhesion molecule-1; TAC, total antioxidant capacity.

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et al. 2003; Graziano *et al.* 1995; Goudev *et al.* 2000; Bruunsgaard *et al.* 2003). Concerning dietary studies, epidemiological data link low levels of CRP to high fruit, vegetable, fibre and to moderate alcohol consumption (Albert *et al.* 2003; King *et al.* 2003; Ajani *et al.* 2004; Gao *et al.* 2004). It is known that fruits and vegetables are sources of the antioxidant vitamins cited above, but also contain a great variety of bioactive compounds with antioxidant properties, e.g. polyphenols, able to act at different levels of the inflammatory cascade that leads to plaque formation (Middleton *et al.* 2000). In addition, high concentrations of plant polyphenols are also observed in other foods and beverages, such as red wine, coffee, tea or chocolate, which significantly contribute to the antioxidant capacity of a diet without contributing significantly to vitamin intake (Pellegrini *et al.* 2003*b*). Thus, we hypothesize that the total antioxidant capacity (TAC) of the diet, which summarizes the capacity of the different food antioxidants in scavenging preformed free radicals, rather than single antioxidant molecules, exerts a substantial and independent effect on inflammation. We have tested this hypothesis by exploring the relationship between dietary intake of TAC, circulating antioxidants and markers of inflammation with known pro-atherogenic properties in a non-diabetic, old-adult population, adjusting by other recognized dietary and non-dietary risk factors recently identified in the literature.

Methods

Subjects

A total of 422 workers and ex-workers of the food company Barilla, first recruited in 1981 for a survey on diabetes and CVD and recalled for follow-up in 1993, were invited to join this study. Three hundred and twenty-five responded to the call and 299 underwent the screening visit. Exclusion criteria included type II diabetes, recent cardiovascular events (<6 months), cancer, organ failure and current treatment with statins. All subjects gave their written informed consent at enrolment. The protocol was approved by the Ethics Committee for Human Research of the University of Parma.

Design

Participation in the study included a complete medical history to gather information about health status, current medications including supplements of vitamins and minerals, alcohol drinking, smoking, physical activity (PA) and family history for chronic diseases; a physical examination including height, weight and waist circumference; blood pressure measurements on two different occasions; a blood draw after 12 h fasting for biochemical analyses and an oral glucose tolerance test (OGTT) providing glucose 7 (75g), with blood draws at 0, 60 and 120 min for determination of glucose, insulin and derivate indices of insulin sensitivity. All volunteers were asked to complete a 3 d food record to obtain accurate information on short-term food intake.

Data collection

Data on physical activity was collected using a questionnaire that grades the level of PA into five categories (sedentary, light, moderate, heavy and agonistic) based on the time spent on activities of daily living (TV watching, walking, house cleaning, gardening, driving, etc.), on programmed physical exercise and on the intensity of such activities. Height was measured with a wall-mounted Holtain stadiometer to the nearest 0·1 cm. Weight was measured without shoes and in light clothes to the nearest 0·01 kg. BMI was calculated as weight divided by height squared (kg/m^2). Waist circumference was measured to the nearest 0·1 cm at the narrowest part of the torso between the lowest ribs and the iliac crests while the subject was standing, after a moderate expiration. On two different days, blood pressure was measured twice using a mercury cuff sphygmomanometer. The second blood pressure measurement was obtained after the patient had been quietly seated for 10 min, and this value was used as the experimental variable. Hypertension was defined as active treatment with blood pressure-lowering medications or systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg on the two occasions in which blood pressure was measured. All subjects were trained by a certified dietitian to fill in a 3 d food record which included all foods, beverages and supplements consumed during two non-consecutive working days plus a weekend day the week following the screening visit. The record was checked for completeness and portion sizes by the dietitian in the presence of the patient within 48 h from collection using a book of photographs and standard household measures. Nutrient intake was assessed by entering foods into a customized computer program linked to a database depicting the macro- and micronutrient content of more than 700 Italian foods (Salvini, 1997). Data on TAC were included in the databank on a number of foods directly analysed in our laboratory and representative of the average Northern Italian diet. Such data have been published for 104 items among fruits, vegetables, oils and beverages (Pellegrini *et al.* 2003*b*) and were integrated with fifty further values for cereals and cereal products, pulses, nuts and processed foods, to cover >95 % of total antioxidant-containing foods consumed in the Parma region (N. Pellegrini, M. Serafini, S. Salvatore *et al.*, un published results). The dietary TAC values used in this study have been obtained applying the Trolox Equivalents Antioxidant Capacity assay and are expressed as TAC in mmol Trolox per kg fresh food. This assay is based on the quenching of the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic) acid radical cation ($ABTS^{\bullet+}$) and thanks to a recent improvement in its chemical basis (Pellegrini *et al.* 2003*a*), allows the evaluation of both water-soluble and lipid-soluble antioxidants. In the present study, dietary TAC values are reported as mmol Trolox per day.

Biochemical analyses

hs-CRP was measured using an ELISA kit (ICN Pharmaceuticals, New York, USA), with a minimum detectable concentration of 0·004 mg/l. Intra- and inter-assay CVs were 2·3 % and 2·5 %, respectively. Human soluble intercellular adhesion molecule-1 (sICAM-1) was measured with a specific ELISA kit (Bender MedSystems GmbH, Vienna, Austria) with no cross-reactivity for members of the immunoglobulin superfamily. The minimum detectable concentration was 3·3 ng/ml and intra- and inter-assay CVs were 9·5 % and 12·9 %, respectively. Serum insulin levels were measured by microparticle enzyme immunoassay (IMX; Abbott Laboratories, Abbott Park, IL, USA), with intra- and inter-assay CVs of 3·0 % and 5·3 %, respectively. The insulin sensitivity index (ISI) was calculated as $10{,}000/\sqrt{}$(fasting plasma

glucose (mg/dl) × fasting plasma insulin (μU/ml)) × (mean OGTT glucose concentration (mg/dl) × mean OGTT insulin concentration (μU/ml)) Matsuda & DeFronzo; 1999). Serum total cholesterol and HDL cholesterol were measured enzymatically. Plasma lutein, zeaxanthine, β-criptoxanthine, α-carotene, β-carotene and lycopene were measured by HPLC as described by Riso & Porrini (1997).

Statistical analysis

All continuous variables were checked for normality using the Kolmogorov–Smirnoff test. Data are reported as means and standard deviations for normally distributed variables (unless otherwise noted) except when the distribution was strongly skewed; in that case median and inter-quartile range (IQR) are given. Smoking was coded as current/former or never. Current smoker was defined as regular user of tobacco products in the last 6 months. Hypertension was coded as yes/no. Given the strongly skewed distribution of hs-CRP plasma values, we further created a nominal variable to group subjects as having low hs-CRP (first three quartiles of hs-CRP values) or high hs-CRP (last quartile). Differences between these two groups were tested using unpaired *t* tests for variables with a normal distribution and homogeneity of variances, the U of Mann–Whitney for variables non-normally distributed and the χ^2 statistics for categorical variables.

Dietary TAC was adjusted for energy intake as described by Willet & Stampfer (1986). The association between single continuous variables was explored by Pearson or Spearman correlations as appropriate. Adjusted means of inflammatory markers by quartiles of energy-adjusted dietary TAC were calculated using the General Linear Model (GLM) procedure in SPSS version 12·0. All models were adjusted for covariates using known risk factors of CVD that have been independently related to plasma levels of inflammatory markers in previous publications (Visser *et al.* 1999; Han *et al.* 2002; Saito *et al.* 2003) as well as in our sample: BMI, waist circumference, hypertension, HDL-cholesterol, the ISI as surrogate of insulin sensitivity and plasma concentrations of β-carotene. GLM models we also adjusted for total energy intake, intake of alcohol and fibre, and intake of single antioxidants (vitamin C, α-tocopherol and β-carotene) in order to remove the effects of nutrients known to be highly correlated with TAC intake as well as with CRP concentrations (Goudev *et al.* 2000; Imhof *et al.* 2001; Sanchez-Moreno *et al.* 2003; Van Dam *et al.* 2003; Ajani *et al.* 2004). Polynomial regression techniques were used to assess non-linearity between inflammatory markers and quartiles of energy-adjusted TAC intake and to calculate linear *P* per trend. The contribution of single food groups to total dietary TAC was assessed by stepwise multiple regression analysis. Significance was set at $P<0{\cdot}05$.

Results

Out of the 299 subjects recruited for the study, twenty-two were excluded at screening (two were diabetic, four had an OGTT compatible with diabetes, one had prostate cancer, fifteen were using statins) and thirty-four were withdrawn for missing data in any of the variables considered. Thus, 243 subjects were finally eligible for data analysis.

Table 1. Demographic, clinical and dietary characteristics of the volunteers at admission

	All (*n* 243)		Men (*n* 138)		Women (*n* 105)	
Characteristic	Mean or No. or median	SD or % or IQR	Mean or No. or median	SD or % or IQR	Mean or No. or median	SD or % or IQR
Clinical risk factors						
Age (years)*	60·1	7·0	61·7	7·7	58·0	5·4
BMI (kg/m^2)*	27·1	3·5	27·1	3·1	27·1	4·1
Waist girth (cm)*	95·2	10·4	97·5	9·4	92·6	10·4
Hypertension†	106	44	75	54	31	30
Smoking†	45	19	21	15	24	29
Laboratory variables						
hs-CRP (mg/l)‡	2·2	3·3	2·6	3·4	1·9	2·8
WBC (× 10^9/l)*	5·8	1·4	5·9	1·4	5·7	1·4
sICAM-1 (μg/l)‡	281·7	127·9	286·1	128·1	281·7	119·0
ISI‡	4·43	3·98	4·00	3·44	5·02	3·89
Total cholesterol (mmol/l)*	5·84	1·07	5·69	1·07	6·03	1·06
HDL-cholesterol (mmol/l)*	1·58	0·38	1·47	0·37	1·73	0·36
Plasma β-carotene (mg/l)‡	0·11	0·11	0·09	0·08	0·13	0·11
Dietary variables						
Energy (kcal/d)*	2243	528	2412	535	2021	430
Alcohol (g/d)‡	16·9	22·4	26·8	24·9	9·3	14·7
Fibre (g/d)‡	17·6	6·3	18·7	7·1	16·4	5·9
TAC (mmol Trolox/d)‡	5·2	2·8	6·3	3·2	4·6	1·9
β-Carotene (μg/d)‡	2331	2154	2575	2553	2216	1883
Vitamin C (mg/d)‡	104·2	61·2	103·9	68·6	106·1	54·1
α-Tocopherol (mg/d)‡	10·0	5·0	10·5	5·2	9·1	4·6

hs-CRP, high-sensitivity C-reactive protein; ISI, insulin sensitivity index; sICAM-1, soluble intercellular adhesion molecule-1; IQR, inter-quartile range; TAC, dietary total antioxidant capacity; WBC, white blood cells.

*Mean with standard deviation

†number of subjects having the characteristic with %

‡median with IQR

Table 2. Demographic, clinical and dietary characteristics of subjects by plasma levels of high sensitivity C-reactive protein

	Low hs-CRP (1st to 3rd quartiles) (*n* 179)		High hs-CRP (4th quartile) (*n* 64)		
Characteristic	Mean, No., range or median	SD or % or IQR	Mean, No., range or median	SD or % or IQR	Significance (*P*)
Clinical risk factors					
Age (years)*	60·1	8·0	60·1	6·7	0·988
Sex, males†	99	55	39	61	0·435
BMI (kg/m^2)*	26·7	3·3	28·4	3·9	0·000
Waist girth (cm)*	93·8	10·4	98·8	9·5	0·001
Hypertension†	68	38	38	59	0·003
Smoking†	32	18	13	20	0·667
Laboratory variables					
hs-CRP (mg/l)‡	0–4·1		4·2–27·8		
WBC ($\times 10^9$/l)*	5·5	1·3	6·6	1·5	0·000
sICAM-1 (μg/l)§	276·6	125·6	323·9	141·8	0·016
ISI§	4·7	3·7	3·4	3·1	0·002
Total cholesterol (mmol/l)*	5·84	1·08	5·84	1·07	0·999
HDL-cholesterol (mmol/l)*	1·61	0·40	1·50	0·31	0·024
Plasma β-carotene (mg/l)§	0·11	0·11	0·09	0·07	0·048
Dietary variables					
Energy (kcal/d)*	2258	538	2203	500	0·473
Alcohol (g/d)§	16·5	23·0	19·0	18·0	0·709
Fibre (g/d)§	17·8	6·3	17·4	6·4	0·509
TAC (mmol Trolox/d)§	5·3	3·0	4·9	2·7	0·026
β-Carotene (μg/d)§	2362	2060	2192	2368	0·164
Vitamin C (mg/d)§	107·5	70·5	101·6	61·0	0·101
α-Tocopherol (mg/d)§	9·9	5·1	10·4	5·0	0·889

hs-CRP, high-sensitivity C-reactive protein; ISI, insulin sensitivity index; sICAM-1, soluble intercellular adhesion molecule-1; IQR, inter-quartile range; TAC, dietary total antioxidant capacity; WBC, white blood cells.
*Mean with standard deviation
†number of subjects having the characteristic with %
‡range §median with IQR

Demographic, clinical and dietary data of volunteers by gender (age range 35–88 years) are shown in Table 1. Out of the 105 women eligible for data analysis, eleven were pre-menopausal and fifteen were on hormone replacement therapy. Regarding physical activity, thirteen subjects were classified as sedentary, twenty-nine had a light level of PA, PA was moderate for 169 and thirty-two were engaged in heavy physical exercise on a regular basis. Table 2 characterizes the study population by plasma levels of hs-CRP. Individuals falling into the higher quartile of plasma hs-CRP were heavier, had a higher waist circumference, were less insulin-sensitive, presented lower plasma concentrations of HDL-cholesterol and were more likely to have hypertension, all these parameters being closely related to the metabolic syndrome. They also presented higher levels of all other markers of inflammation and lower plasma levels of β-carotene compared to subjects in the lower quartiles of hs-CRP, whereas PA and plasma levels of lutein, zeaxanthine, β-criptoxanthine, α-carotene and lycopene were not different between groups (data not shown). The only nutritional variable significantly different between the two groups was TAC intake.

We investigated the relationship between dietary TAC intake and inflammation markers adjusting for relevant covariates. Adjusted mean values of hs-CRP, white blood cells (WBC) and sICAM-1 by quartiles of dietary TAC are presented in Table 3. There was a significant inverse dose–response relationship between hs-CRP and quartiles of energy-adjusted dietary TAC. WBC showed a similar association with dietary TAC, whereas no relationship was found for sICAM-1.

Since hypertension appeared as the only predictor of CRP concentrations among all covariates considered in addition to quartiles of TAC, we further explored the interaction among dietary TAC, hypertension and hs-CRP. As shown in Fig. 1, hs-CRP values

Table 3. Adjusted* mean values for markers of inflammation by quartiles of total antioxidant capacity (TAC) daily intake

	Quartiles of energy-adjusted TAC daily intake								
	I		II		III		IV		
Marker	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	*P* for trend
Hs-CRP (mg/l)	5·0	3·8, 6·2	3·8	2·7, 4·9	3·5	2·4, 4·6	2·2	1·0, 3·3	0·004
WBC ($\times 10^9$/l)	6·2	5·8, 6·6	5·9	5·6, 6·3	5·6	5·3, 6·0	5·5	5·2, 5·9	0·035
sICAM-1 (μg/l)	336·2	295·5, 376·9	297·4	260·6, 334·2	323·7	290·2, 363·3	307·2	267·9, 346·6	0·574

Hs-CPR, high-sensitivity C-reactive protein; sICAM-1, soluble intercellular adhesion molecule-1; WBC, white blood cells.
* Adjusted for BMI, waist circumference, HDL - cholesterol hypertension, insulin sensitivity index, plasma β-carotene, total energy intake, and intake of alcohol, fibre, vitamin C, α-tocopherol and β-carotene.

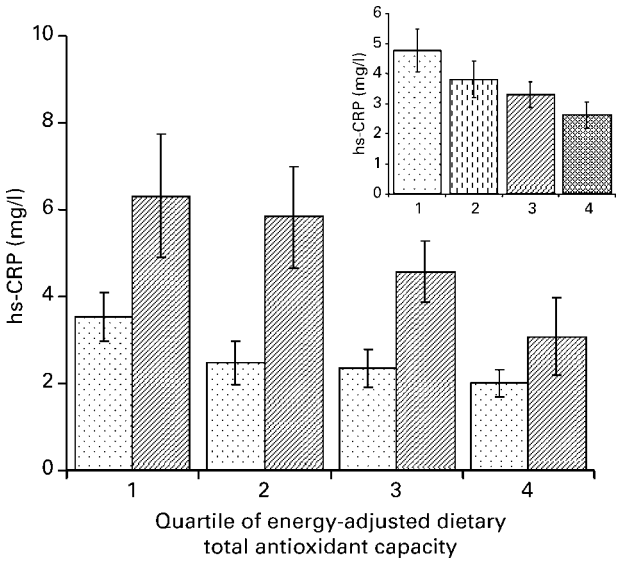

Fig. 1. Mean values (with standard errors of the mean depicted by vertical bars) of high-sensitivity C-reactive protein (hs-CRP) for subjects taking (▨, *n* 106) or not taking (▩, *n* 137) antihypertensive medications and for all subjects (upper frame, *n* 243), stratified according to quartiles of dietary total antioxidant capacity (TAC). Dietary TAC was adjusted for energy intake and expressed as energy-adjusted dietary TAC (mmol Trolox/kg diet) before calculating quartile distribution. *P* values per trend were $P=0{\cdot}013$, $P=0{\cdot}109$ and $P=0{\cdot}005$, respectively, for subjects with hypertension, for normotensive subjects and for all subjects.

were significantly higher across quartiles of energy-adjusted dietary TAC intake in subjects with high blood pressure than in individuals with no diagnosis of hypertension. There was also a strong inverse dose–response relationship between plasma concentrations of hs-CRP and quartiles of energy-adjusted TAC intake in subjects with hypertension. It is noteworthy that hs-CRP values of subjects with high blood pressure falling in the fourth quartile of TAC intake were comparable to those of subjects without hypertension falling in the first quartile of dietary TAC.

In order to explore the relative contribution of single food groups to total dietary TAC, we performed stepwise multiple regression analysis with TAC intake as dependent variable and intake of (1) fruits and fruit juices; (2) vegetables, pulses, oils and nuts; (3) cereals and cereal products; (4) coffee,tea and chocolate; and (5) alcoholic beverages in grams per day as independent variables. As shown in Fig. 2, dietary intake of all mentioned food groups explained as much as 58·5 % of the total dietary TAC, alcoholic beverages, fruits and fruit juices, and coffee (consumption of tea and chocolate was negligible) being the main sources of antioxidants in our population.

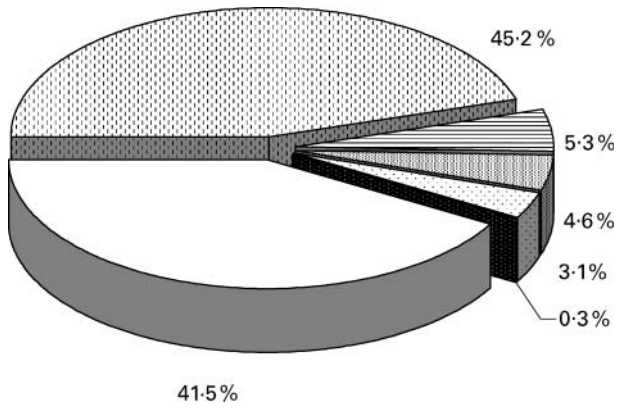

Fig. 2. Proportion of variation (R^2) in dietary total antioxidant capacity explained by the mean intake of contributor food groups, as assessed by multiple regression analysis. (▨) Alcoholic beverages (45·2 %); (▤) fruit and juices (5·3 %); (▥) coffee, tea and chocolate (4·6 %); (▭) cereals (3·1 %); (▩) vegetables, pulses, oils and nuts (0·3 %); (□) unexplained by food quantity (41·5 %).

Discussion

We found a strong inverse association between total antioxidant intake expressed as energy-adjusted dietary TAC and plasma levels of hs-CRP. The association was still significant for WBC but absent for sICAM-1, probably because this is a less-sensitive marker of inflammation.

Gao *et al.* (2004) have recently described decreasing plasma concentrations of CRP and homocysteine across quartiles of fruit and vegetable consumption in US elders with high prevalence of hypertension (70 %), obesity, diabetes and CRP values >6 mg/l (limit of detection of the assay) and, as shown in our study, presumably more sensitive to the anti-inflammatory effects of dietary TAC. However, if the anti-inflammatory properties of fruits and vegetables are to be attributed to their antioxidant components, as authors suggest, these foods are important, but not the only contributors to the TAC of a diet. In our population of White, non-diabetic, adult-elderly subjects, the inverse association between dietary TAC and hs-CRP concentrations was observed for the whole range of hs-CRP values (high sensitivity method). Considering that no other dietary variable but dietary TAC showed this independent relationship with CRP and that consumption of fibre, alcohol, β-carotene, vitamin C and α-tocopherol was comparable between subjects with high and low hs-CRP values, we hypothesize that CRP metabolism may be modulated primarily by the TAC of the diet rather than by single antioxidant compounds. In fact, the anti-inflammatory properties of certain vitamins and other bioactive compounds with antioxidant capacity have been attributed to their ability of modulating the NF-κB DNA-binding activity. NF-κB activation is primarily promoted by oxidative stress and leads to the cytokine-induced expression of CAM molecules in the vascular endothelium and to the TNF-α and IL-6-induced production of CRP by the liver. Since the antioxidant vitamins α-tocopherol and vitamin C, β-carotene (Carcamo *et al.* 2002; Calfee-Mason *et al.* 2002; Palozza *et al.* 2003) and the potent flavonoids quercetin and apigenin (Gerritsein *et al.* 1995; Choi *et al.* 2004) are all able to inhibit NF-κB DNA-binding activity *in vitro*, it is plausible to think that the anti-inflammatory effects of single antioxidants would depend on their redox potential rather than on their particular molecular structure, as described for β-carotene (Palozza *et al.* 2003). This could explain why CRP levels correlated with the TAC of the diet in our study and not with a single class of antioxidants. An alternative explanation is that other nutrients notably contributing to TAC intake and not considered here as single antioxidants (primarily polyphenols) could be responsible for the association.

Regarding the sources of dietary antioxidants, alcoholic beverages (approximately 75 % as red wine) were, by far, the principal factor explaining the variability in TAC intake, followed by fruits and fruit juices, coffee, cereals and vegetables, whereas use of supplements containing antioxidant vitamins was negligible. This observation suggests that the inverse relationship between alcohol

consumption and inflammation reported in previous studies (Imhof *et al.* 2001; Albert *et al.* 2003) could be mediated by the strong antioxidant capacity of certain alcoholic beverages (primarily red wine). It is also remarkable that the intake of all foods and beverages sorted by food group accounted only for 58·5 % of the inter-individual variability in dietary TAC, which means that the remaining 41·5 % must be explained by differences in the antioxidant content among single food items within any given food group. In other words, TAC intake appears to be determined by the type as much as by the amount of alcoholic beverages, fruits, cereals and vegetables consumed with the diet. Practically speaking, the highest quartile of TAC intake (> 6·7 mmol Trolox equivalents/d) could be achieved with relatively low amounts of antioxidant-rich food items. For example, one orange (200 g) or 200 g spinach provide approximately 1·7 mmol Trolox equivalents, whereas 200 g cucumber provides only 0·09 mmol. Similarly, a glass of red wine or a cup of espresso coffee or a cup of green tea contributes as much as 1·5 mmol Trolox equivalents, which is twice the contribution of a glass of common mixed fruit juices (about 0·7 mmol Trolox equivalents; Pellegrini *et al.* 2003*b*).

Finally, it is noteworthy that intake of dietary TAC was found to be significantly related to inflammation despite the short-term dietary assessment (3 d dietary record) used to calculate dietary parameters. This could mean that our population, which mainly included elderly people, has a dietary pattern relatively stable over time or that the anti-inflammatory effect of dietary TAC has a relatively short time of induction, suggesting a true pharmacological effect. The cross-sectional nature of this study precludes a satisfactory answer to this and other questions, such as the cause–effect relationship between TAC intake and CRP values or the possibility that other unmeasured demographic or lifestyle-related factors could mediate the association. Since the study population was quite homogeneous regarding physical activity, socioeconomic extraction and level of education (most were factory workers and ex-workers devoted to manual, non-qualified work), the latter possibility appears unlikely, but this, in turn, limits the generalizability of the results to other, more heterogeneous populations.

In conclusion, intake of foods and beverages rich in antioxidants are associated with low levels of leucocytes and CRP. The increase of TAC brought about by such diets could be one of the mechanisms that mediate the protective effects of fruits, vegetables and red wine against chronic disease (primarily CVD), and this could be of particular significance in subjects with hypertension. However, intervention studies are needed to clarify the nature and extent of the association between dietary TAC, CRP and other markers of inflammation.

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