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

Furio Brightenti1, Silvia Valtueña2*, Nicoletta Pellegrini1, Diego Ardigò2, Daniele Del Rio1, Sara Salvatore1, PierMarco Piazzì3, Mauro Serafini4 and Ivana Zavaroni2

1Department of Public Health, University of Parma, Parma, Italy
2Department of Internal Medicine and Biomedical Sciences, University of Parma, Via Gramsci 14, 43100 Parma, Italy
3Cardiovascular and Metabolic Rehabilitation Unit, IRCCS H. San Raffaele, Milan, Italy
4Antioxidant Research Laboratory, National Institute for Food and Nutrition Research, Rome, Italy

(Received 24 August 2004 – Revised 10 December 2004 – Accepted 20 December 2004)

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 \( P=0.005 \) inverse relationship between hs-CRP and quartiles of energy-adjusted dietary TAC, even when recognized modulating factors of inflammation, namely alcohol, fibre, vitamin C, \( \beta \)-carotene, BMI, waist circumference, HDL-cholesterol, hypertension, insulin sensitivity and plasma \( \beta \)-carotene, were included in the model as covariates \( (P=0.004) \). The relationship was stronger for subjects with hypertension \( (P=0.013 \text{ vs } P=0.109 \text{ for normotensive individuals}) \). Among dietary factors, TAC was significantly higher \( (5.3 \text{ (SD 3.0)} \text{ mmol Trolox/d}) \) in subjects with low plasma hs-CRP \( (\text{range: } 0.0–4.1 \text{ mg/l}) \) than in subjects with high plasma hs-CRP \( (\text{range: } 4.2–27.8 \text{ mg/l}) \). 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 \( \alpha \)-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.

*Corresponding author: Dr Silvia Valtueña, fax +39 0521 903271, email valtuena@libero.it
et al. 2003; Graziano et al. 1995; Goudev et al. 2000; Bruuns-
gaard 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 differ-
ent levels of the inflammatory cascade that leads to plaque for-
mation (Middleton et al. 2000). In addition, high concentrations
of plant polyphenols are also observed in other foods and bev-
erages, such as red wine, coffee, tea or chocolate, which signifi-
cantly contribute to the antioxidant capacity of a diet without
contributing significantly to vitamin intake (Pellegrini et al.
2003b). 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 inde-
pendent effect on inflammation. We have tested this hypothesis
by exploring the relationship between dietary intake of TAC, cir-
culating 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 sub-
jects gave their written informed consent at enrolment. The proto-
col 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 differ-
ent occasions; a blood draw after 12 h fasting for biochemical
analyses and an oral glucose tolerance test (OGTT) providing glu-
cose 7 (75g), with blood draws at 0, 60 and 120 min for determi-
nation 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/m²). 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 stand-
ing, after a moderate expiration. On two different days, blood
pressure was measured twice using a mercury cuff sphygmoman-
ometer. 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 medi-
cations 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 cer-
tified diettian 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 follow-
ing 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. 2003b) 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., unpublished 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-sulpho-
ic) acid radical cation (ABTS⁺⁺) and thanks to a recent
improvement in its chemical basis (Pellegrini et al. 2003a),
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/√(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-cryptoxanthine, 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 tests for variables non-normally distributed and the χ² 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. 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<0.05.

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n 243)</th>
<th>Mean or No.</th>
<th>sd or % or IQR</th>
<th>Mean or No.</th>
<th>sd or % or IQR</th>
<th>Mean or No.</th>
<th>sd or % or IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60-1</td>
<td>7-0</td>
<td></td>
<td>61-7</td>
<td>7-7</td>
<td>58-0</td>
<td>5-4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27-1</td>
<td>3-5</td>
<td></td>
<td>27-1</td>
<td>3-1</td>
<td>27-1</td>
<td>4-1</td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td>95·2</td>
<td>10·4</td>
<td></td>
<td>97·5</td>
<td>9-4</td>
<td>92·6</td>
<td>10-4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>106</td>
<td>44</td>
<td></td>
<td>75</td>
<td>54</td>
<td>31</td>
<td>30</td>
</tr>
<tr>
<td>Smoking†</td>
<td>45</td>
<td>19</td>
<td></td>
<td>21</td>
<td>15</td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td>Laboratory variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP (mg/l)‡</td>
<td>2·2</td>
<td>3-3</td>
<td></td>
<td>2·6</td>
<td>3-4</td>
<td>1·9</td>
<td>2-8</td>
</tr>
<tr>
<td>WBC (x 10³/l)*</td>
<td>5·8</td>
<td>1-4</td>
<td></td>
<td>5·9</td>
<td>1-4</td>
<td>5·7</td>
<td>1-4</td>
</tr>
<tr>
<td>sICAM-1 (µg/l)¶</td>
<td>281·7</td>
<td>127·9</td>
<td></td>
<td>286·1</td>
<td>128·1</td>
<td>281·7</td>
<td>119·0</td>
</tr>
<tr>
<td>ISI‡</td>
<td>4·43</td>
<td>3-98</td>
<td></td>
<td>4·00</td>
<td>3-44</td>
<td>5·02</td>
<td>3-89</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)*</td>
<td>5·84</td>
<td>1·07</td>
<td></td>
<td>5·69</td>
<td>1·07</td>
<td>6·03</td>
<td>1-06</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)†</td>
<td>1·58</td>
<td>0·38</td>
<td></td>
<td>1·47</td>
<td>0·37</td>
<td>1·73</td>
<td>0·36</td>
</tr>
<tr>
<td>Plasma β-carotene (mg/l)¶</td>
<td>0·11</td>
<td>0·11</td>
<td></td>
<td>0·09</td>
<td>0·08</td>
<td>0·13</td>
<td>0·11</td>
</tr>
<tr>
<td>Dietary variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/d)*</td>
<td>2243</td>
<td>528</td>
<td></td>
<td>2412</td>
<td>535</td>
<td>2021</td>
<td>430</td>
</tr>
<tr>
<td>Alcohol (g/d)‡</td>
<td>16·9</td>
<td>22-4</td>
<td></td>
<td>26·8</td>
<td>24-9</td>
<td>9·3</td>
<td>14-7</td>
</tr>
<tr>
<td>Fibre (g/d)†</td>
<td>17·6</td>
<td>6-3</td>
<td></td>
<td>18·7</td>
<td>7-1</td>
<td>16·4</td>
<td>5-9</td>
</tr>
<tr>
<td>TAC (mmol Trolox/d)‡</td>
<td>5·2</td>
<td>2-8</td>
<td></td>
<td>6-3</td>
<td>3-2</td>
<td>4·6</td>
<td>1-9</td>
</tr>
<tr>
<td>β-Carotene (µg/d)¶</td>
<td>2331</td>
<td>2154</td>
<td></td>
<td>2575</td>
<td>2553</td>
<td>2216</td>
<td>1883</td>
</tr>
<tr>
<td>Vitamin C (mg/d)‡</td>
<td>104·2</td>
<td>61·2</td>
<td></td>
<td>103·9</td>
<td>68·6</td>
<td>106·1</td>
<td>54-1</td>
</tr>
<tr>
<td>α-Tocopherol (mg/d)‡</td>
<td>10·0</td>
<td>5-0</td>
<td></td>
<td>10·5</td>
<td>5-2</td>
<td>9·1</td>
<td>4-6</td>
</tr>
</tbody>
</table>

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
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 \( \beta \)-carotene compared to subjects in the lower quartiles of hs-CRP, whereas PA and plasma levels of lutein, zeaxanthine, \( \beta \)-cryptoxanthine, \( \alpha \)-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>
<thead>
<tr>
<th>Characteristics</th>
<th>Low hs-CRP (1st to 3rd quartiles) ( (n = 179) )</th>
<th>High hs-CRP (4th quartile) ( (n = 64) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)*</td>
<td>60.1 (8.0)</td>
<td>60.1 (6.7)</td>
</tr>
<tr>
<td>Sex, males†</td>
<td>99 (55)</td>
<td>39 (61)</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>26.7 (3.3)</td>
<td>28.4 (3.9)</td>
</tr>
<tr>
<td>Waist girth (cm)*</td>
<td>93.8 (10.4)</td>
<td>98.8 (9.5)</td>
</tr>
<tr>
<td>Hypertension†</td>
<td>68 (38)</td>
<td>38 (59)</td>
</tr>
<tr>
<td>Smoking†</td>
<td>32 (18)</td>
<td>13 (20)</td>
</tr>
<tr>
<td>Laboratory variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP (mg/l)‡</td>
<td>0–4.1</td>
<td>4.2–27.8</td>
</tr>
<tr>
<td>WBC (&lt;10⁹/l)*</td>
<td>5.5 (1.3)</td>
<td>6.6 (1.5)</td>
</tr>
<tr>
<td>sICAM-1 (µg/l)§</td>
<td>276–6 (125–6)</td>
<td>323–9 (141–8)</td>
</tr>
<tr>
<td>ISI§</td>
<td>4.7 (3.7)</td>
<td>3.4 (3.1)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)*</td>
<td>5.8–4 (1.0–8)</td>
<td>5.8–4 (1.0–7)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)*</td>
<td>1.6–1 (0.4–0)</td>
<td>1.5 (0.3–1)</td>
</tr>
<tr>
<td>Plasma ( \beta )-carotene (mg/l)§</td>
<td>0.1–1 (0.1–1)</td>
<td>0.0–9 (0.0–7)</td>
</tr>
<tr>
<td>Dietary variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/d)*</td>
<td>2258 (538)</td>
<td>2203 (500)</td>
</tr>
<tr>
<td>Alcohol (g/d)§</td>
<td>16.5 (23.0)</td>
<td>19.0 (18.0)</td>
</tr>
<tr>
<td>Fibre (g/d)§</td>
<td>17.8 (6.3)</td>
<td>17.4 (6.4)</td>
</tr>
<tr>
<td>TAC (mmol Trolox/d)§</td>
<td>5–3 (3–0)</td>
<td>4.9 (2.7)</td>
</tr>
<tr>
<td>( \beta )-Carotene (µg/d)§</td>
<td>2362 (2060)</td>
<td>2192 (2368)</td>
</tr>
<tr>
<td>Vitamin C (mg/d)§</td>
<td>107–5 (70–5)</td>
<td>101–6 (61–0)</td>
</tr>
<tr>
<td>( \alpha )-Tocopherol (mg/d)§</td>
<td>9–9 (5–1)</td>
<td>10–4 (5–0)</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Marker</th>
<th>Quartiles of energy-adjusted TAC daily intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Mean</td>
<td>Mean 95% CI</td>
</tr>
<tr>
<td>Hs-CRP (mg/l)</td>
<td>5.0</td>
</tr>
<tr>
<td>WBC (&lt;10⁹/l)</td>
<td>6.2</td>
</tr>
<tr>
<td>sICAM-1 (µg/l)</td>
<td>336.2</td>
</tr>
</tbody>
</table>

Hs-CRP, high-sensitivity C-reactive protein; sICAM-1, soluble intercellular adhesion molecule-1; WBC, white blood cells.

*Adjusted for BMI, waist circumference, HDL-cholesterol, hypertension, ISI, plasma \( \beta \)-carotene, total energy intake, and intake of alcohol, fibre, vitamin C, \( \alpha \)-tocopherol and \( \beta \)-carotene.
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.

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 (Gerritsen 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. 2003b).

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.

Acknowledgements

We wish to thank Prof. Marisa Porrini and Dr Patrizia Riso, from the Department of Food Sciences, University of Milan, for the analyses of plasma carotenoids and Mrs Nadia Anelli from the Department of Public Health, University of Parma, for her skilful collection of dietary data. This research was supported by the European Community IST-2001-33 204 ‘Healthy Market’, the Italian Ministry of University and Research COFIN 2001 and the National Research Council CU01.00923.2 CT26 research projects.

References


