The importance of dietary antioxidants on plasma antioxidant capacity and lipid peroxidation in vivo in middle-aged men

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Abstract

Background: High intake of foods rich in antioxidants is associated with a reduced risk of several diseases, including coronary heart disease and some cancers.

Objective: To study associations between dietary antioxidants and antioxidant-rich food items and antioxidant capacity (AOC), as well as lipid peroxidation in vivo.

Design: A total of 86 men, with a mean age of 60 years, who were part of a larger population-based study in Sweden, participated. Fourteen 24 h recalls were collected by telephone, evenly distributed during 1 year. AOC was measured in plasma using an enhanced chemiluminescence assay and biomarkers of non-enzymic lipid peroxidation were determined by measuring F2-isoprostanes (8-iso-prostaglandin F2α) in plasma.

Results: Higher intakes of ascorbic acid, tocopherols and β-carotene, and of the combined intake of different antioxidant-rich foods, were related to a higher plasma AOC. The levels of F2-isoprostanes were related neither to dietary intake of antioxidants nor to antioxidant-rich foods.

Conclusions: Individuals with a higher intake of several different antioxidant-rich foods and of dietary antioxidants had a higher plasma AOC than those with the lowest intake, supporting the importance of a balanced diet rich in various antioxidants. Moreover, the results indicate that non-enzymic lipid peroxidation in vivo, measured as F2-isoprostanes in plasma, is not easily affected by the diet.

Keywords: antioxidant status; diet; food habits; isoprostanes; oxidative stress

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Introduction

A high intake of foods rich in antioxidants is associated with a reduced risk of several diseases, including coronary heart disease and some cancers (1–3). Since antioxidants are suggested to interact in a complex network, measurement of antioxidant capacity (AOC) in plasma may be a good approach by which to study the integrated effect of a number of antioxidants in humans in vivo. Several methods have been developed to measure the AOC of different foods, as well as in plasma or serum (4–8). Recent studies have reported the AOC of various foods (9, 10) and others have calculated AOC in the diet (11–13), but studies investigating the effect of food habits or long-term food intake on plasma AOC are sparse (14, 15).

Oxidative stress is a condition with increased production of free radicals or reactive oxygen species associated with reduced antioxidant defence, which might lead to different types of oxidative damage in the body and contribute to the development of several chronic diseases (16). Oxidative stress can be assessed by measuring lipid peroxidation in the body. When lipid peroxidation in the body is increased, the antioxidant levels may be either reduced owing to an increased consumption or increased owing to an up-regulation of the endogenous antioxidant production (17, 18). It is of interest to investigate further the impact of dietary factors on the AOC and biomarkers of lipid peroxidation in vivo.

This study investigated the associations between dietary antioxidants (ascorbic acid, tocopherols and β-carotene) and antioxidant-rich foods, and AOC and non-enzymic lipid peroxidation in vivo,
measured as F₂-isoprostanes, in plasma in middle-aged men.

Subjects and methods
A total of 86 apparently healthy men, who took part in a large population-based study in central Sweden, was included in the study. None of the subjects was taking dietary supplements. Nineteen were current smokers. The study was approved by the Ethics Committee of the Faculty of Medicine at Uppsala University.

Dietary assessment
For each participant, fourteen 24 h recalls were collected by telephone during a period of 1 year. Each participant was interviewed approximately once a month, and every day of the week was covered twice in a random order. An average daily intake of foods, food groups and beverages rich in antioxidants – fruits and berries, vegetables, root vegetables, leguminous plants, tea, coffee, red wine, white wine, juices, jam and marmalade – was calculated. The database from the Swedish National Food Administration (PC-Kost 1996; SLV, Uppsala, Sweden) was used for calculation of the nutrients in the 24 h recalls. Intake of ascorbic acid, tocopherols and β-carotene was calculated, as well as the density of these antioxidants per 1000 kcal.

Blood sampling
Blood samples were taken from an antecubital vein after an overnight fast. All plasma or serum samples were kept frozen at −70°C until analysed, or fresh samples were used.

Biochemical analyses
The plasma AOC was measured using an enhanced chemiluminescence assay (5). This technique is based on the measurement of light emission from a chemiluminescent substrate, which is suppressed by radical scavenging antioxidants, and the duration of light suppression is related to the amount of antioxidant present. Light emissions from the chemiluminescent reaction were measured with a luminometer (1251 Luminometer; BioOrbit, Turku, Finland). Antioxidant capacities of samples were quantified by comparing the duration of light suppression to that induced by the tocopherol analogue Trolox (Aldrich Chemie, Steinheim, Germany). The concentration is expressed in μmol Trolox equivalents per litre of plasma. Plasma AOC in the absence of uric acid was determined by adding uricase (Boehringer, Mannheim, Germany) to remove the uric acid content in the plasma sample before measuring the AOC. The coefficient of variation between duplicate plasma samples was 3.2%. AOC without uric acid is partly correlated with the concentration of plasma lipids, probably reflecting the transport of lipid-soluble antioxidants by lipid molecules. Therefore, the AOC was also calculated with and without adjustment for the sum of cholesterol and triglycerides in serum (AOC without uric acid divided with sum of cholesterol and triglyceride concentrations in serum), as tocopherol concentration is often expressed (19). The concentration of uric acid in plasma was measured enzymically (Instrumentation Laboratories, Lexington, MA, USA) in a Monarch centrifugal analyser (Instrumentation Laboratories). The coefficient of variation between duplicate plasma samples was 1.4%.

The plasma levels of 8-iso-prostaglandin F₂α (8-iso-PGF₂α) was analysed using a validated radioimmunoassay developed by Basu (20). The detection limit of the assay was 8 pg ml⁻¹.

The concentrations of cholesterol and triglycerides in serum and in the isolated lipoprotein fractions were determined by enzymic colorimetric methods (Instrumentation Laboratories) in a Monarch centrifugal analyser (Instrumentation Laboratories). Low-density lipoprotein (LDL) cholesterol was calculated according to Friedewald (21).

Statistics
Variables with a skewed distribution (Shapiro Wilk’s W-test <0.05) were log-transformed before statistical analysis. Otherwise, non-parametric tests were used. The Student’s unpaired t-test or Mann–Whitney test was used to analyse differences in AOC between the mean of the three highest quartiles and the lowest one. This calculation is based on results from a previous study, which found that only subjects with the lowest AOC exhibited a significant increase after intake of foods rich in antioxidants (unpublished observations). For F₂-isoprostanes the difference between the lowest and the highest quartile was tested. The correlation coefficients (Pearson’s or Spearman’s as appropriate) were calculated when correlations between variables were tested. p-Values <0.05 were regarded...
as statistically significant. Statistical analyses were performed using JMP, version 3.2 (SAS Institute, Cary, NC, USA).

Results
The clinical characteristics of the study participants are shown in Table 1 and the estimated dietary intake of macronutrients, antioxidants and antioxidant-rich foods is shown in Table 2.

The summarised intake of ascorbic acid, tocopherols and β-carotene was higher in the three upper quartiles of AOC than in the lowest quartile, as shown in Table 3. A higher intake of ascorbic acid was related to higher levels of AOC, whereas the associations for tocopherols and β-carotene were weaker, when considered separately. A significant difference in intake of foods between the lowest and the higher quartiles of AOC was found only when different food groups were combined (Table 4). No relationship was found between the intake of tea and coffee, and AOC. The combined intake of ascorbic acid, tocopherols and β-carotene [mean (SD), mg 1000 kcal⁻¹] related to quartiles of AOC was, in quartile 1: 32 (10); quartile 2: 45 (15); quartile 3: 42 (16); and quartile 4: 42 (16), and the combined intake of fruits, berries, vegetables and root vegetables (g per day) as related to quartiles of AOC was, in quartile 1: 151 (72); quartile 2: 225 (85); quartile 3: 185 (68); and quartile 4: 190 (125). Furthermore, intake of food groups calculated per 1000 kcal per day showed a similar pattern, with a difference between the lowest and the higher quartiles (for fruits, berries, vegetables and root vegetables, \( p < 0.01 \), and for total intake of selected foods excluding tea and coffee, \( p < 0.001 \)).

The relationships between dietary intake and AOC, when uric acid was removed and adjusted for lipid concentration, were similar, but the differences were smaller and there were no significant differences between the higher and lowest quartiles (results not shown).

The relationship between the intake of ascorbic acid and AOC without uric acid was \( r = 0.20, p = 0.065 \), and between the combined intake of ascorbic acid, tocopherols and β-carotene \( r = 0.21, p = 0.056 \), and the intake of β-carotene was significantly correlated with AOC without uric acid (\( r = 0.25, p < 0.05 \)).

AOC without uric acid was not significantly correlated with any of the food groups, but lipid-

| Table 1. Characteristics of study participants |
|-----------------------------------------------|
| Mean (SD)                                      |
| (n = 86)                                       |
| Age (years) 59.8 (8.1)                         |
| BMI (kg m⁻²) 26.5 (2.9)                       |
| Total cholesterol (mmol l⁻¹) 5.92 (1.13)       |
| HDL cholesterol (mmol l⁻¹) 1.27 (0.35)         |
| LDL cholesterol (mmol l⁻¹) 4.02 (0.98)         |
| LDL/HDL 3.35 (1.06)                           |
| Triglycerides (mmol l⁻¹) 1.40 (0.70)           |
| AOC (μmol Trolox equivalents l⁻¹) 487.1 (80.5) |
| AOC without uric acid (μmol Trolox equivalents l⁻¹) 104.5 (23.2) |
| Uric acid (μmol l⁻¹) 276.1 (61.5)              |
| Lipid-adjusted AOC without uric acid (μmol Trolox equivalents l⁻¹) 14.49 (2.75) |
| \( F_2 \)-isoprostanes (pg ml⁻¹) 32.5 (23.1)   |

Data are presented as means (SD).

BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; AOC: antioxidant capacity.

| Table 2. Mean daily dietary intake of selected nutrients and foods based on fourteen 24 h recalls in the 86 men in the study |
|---------------------------------------------------------------------------------------------------------------|
| Mean (SD)                                                                                                      |
| Nutrients                                         | Energy (kcal) 2088 (401) |
| Protein (E %)                                      | 14.9 (2.1) |
| Carbohydrate (E %)                                 | 47.6 (5.4) |
| Fat (E %)                                          | 33.4 (3.8) |
| SFA (E %)                                          | 14.2 (2.1) |
| MUFA (E %)                                         | 12.1 (1.5) |
| PUFA (E %)                                         | 4.9 (0.9) |
| Alcohol (E %)                                      | 4.2 (4.3) |
| Ascorbic acid (mg)                                  | 72.9 (30.3) |
| Tocopherols (mg)                                   | 7.8 (1.6) |
| β-Carotene (mg)                                    | 2.2 (1.6) |
| Food groups                                        |                                        |
| Fruits and berries (g)                             | 123 (78) |
| Vegetables (g)                                     | 54 (32) |
| Root vegetables (g)                                | 11 (14) |
| Leguminous plants (g)                              | 3 (11) |
| Fruits and berries + vegetables + root vegetables (g)          | 188 (92) |
| Black tea (g)                                      | 153 (231) |
| Coffee (g)                                         | 463 (234) |
| Red wine (g)                                       | 25 (39) |
| White wine (g)                                     | 20 (39) |
| Juices (g)                                         | 29 (59) |
| Jam/marmalade (g)                                  | 15 (14) |
| Total intake of selected foods excluding tea and coffee (g)      | 279 (145) |

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

\(^a\)Sum of daily intake of fruit and berries, vegetables, root vegetables, leguminous plants, red wine, white wine, juices, and jam and marmalade.
adjusted AOC without uric acid was correlated with intake of vegetables \( r = 0.23, p < 0.05 \), intake of root vegetables \( r = 0.34, p < 0.01 \), and total intake of selected foods excluding tea and coffee \( r = 0.24, p < 0.05 \). The intake of vegetables was significantly higher in the higher quartiles of lipid-adjusted AOC without uric acid than in the lowest quartile \( p < 0.05 \).

There were no associations between the levels of F2-isoprostanes and intake of ascorbic acid, tocopherols or β-carotene, either when considered separately or when combined. None of the studied food groups was correlated with F2-isoprostanes. However, subjects in the lowest quartile of F2-isoprostanes had a higher intake of white wine compared with subjects in the highest quartile, with a significant difference between the lowest and the highest quartile \( p < 0.05 \).

There was no significant correlation between AOC and F2-isoprostanes or between AOC without uric acid and F2-isoprostanes.

Furthermore, the concentration of uric acid was not correlated with intake of ascorbic acid, tocopherols or β-carotene or with any of the food groups.

Intake of ascorbic acid was correlated with intake of fruits and berries \( r = 0.54, p < 0.0001 \) and vegetables \( r = 0.26, p < 0.05 \), and intake of β-carotene was correlated with intake of root vegetables \( r = 0.77, p < 0.0001 \) and vegetables \( r = 0.31, p < 0.01 \).

**Discussion**

A higher dietary intake of ascorbic acid, tocopherols and β-carotene, when considered together, as well as a higher dietary intake of ascorbic acid, was

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**Table 3. Nutrient density of selected dietary antioxidants by quartiles of plasma antioxidant capacity (AOC) in the 86 men in the study**

|                | Quartile 1 (lowest) of AOC, 327–423 (μmol Trolox equivalents l⁻¹) | Quartiles 2–4 of AOC, 424–675 (μmol Trolox equivalents l⁻¹) | p for difference between lowest and three higher quartiles of AOC | Correlation with AOC |
|----------------|---------------------------------------------------------------|----------------------------------------------------------|---------------------------------------------------------------|---------------------|
|                | \( n = 21 \)                                                   | \( n = 65 \)                                              |                                                               |                     |
| Ascorbic acid  | 27.8 (9.5)                                                    | 38.1 (15.5)                                               | 0.006                                                         | 0.24    0.026       |
| Tocopherols    | 3.7 (0.8)                                                     | 3.8 (0.5)                                                 | 0.198                                                         | 0.19    0.086       |
| β-carotene     | 0.91 (0.61)                                                   | 1.12 (0.77)                                               | 0.135                                                         | 0.12    0.274       |
| Ascorbic acid + tocopherols + β-carotene | 32.3 (9.7) | 43.1 (15.8) | 0.005 | 0.25    0.021 |

Data are presented as means (SD).

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**Table 4. Daily consumption of antioxidant-rich foods by quartiles of plasma antioxidant capacity (AOC) in the 86 men in the study**

|                | Quartile 1 (lowest) of AOC, 327–423 (μmol Trolox equivalents l⁻¹) | Quartiles 2–4 of AOC, 424–675 (μmol Trolox equivalents l⁻¹) | p for difference between lowest and three higher quartiles of AOC | Correlation with AOC |
|----------------|---------------------------------------------------------------|----------------------------------------------------------|---------------------------------------------------------------|---------------------|
|                | \( n = 21 \)                                                   | \( n = 65 \)                                              |                                                               |                     |
| Fruits and berries (g) | 96 (55) | 132 (83) | 0.097 | 0.04    0.740 |
| Vegetables (g) | 46 (27) | 56 (33) | 0.133 | 0.09    0.420 |
| Root vegetables (g) | 9 (12) | 12 (15) | 0.763 | 0.09    0.478 |
| Leguminous plants (g) | 3 (8) | 4 (18) | 0.295 | 0.06    0.678 |
| Fruits and berries + vegetables + root vegetables (g) | 151 (72) | 200 (95) | 0.016 | 0.12    0.278 |
| Black tea (g) | 152 (165) | 153 (250) | 0.637 | –0.13   0.225 |
| Coffee (g) | 507 (223) | 448 (237) | 0.069 | –0.08   0.446 |
| Red wine (g) | 14 (26) | 28 (42) | 0.180 | 0.11    0.335 |
| White wine (g) | 10 (23) | 24 (42) | 0.602 | 0.27    0.134 |
| Juices (g) | 11 (21) | 34 (66) | 0.183 | 0.21    0.055 |
| Jam/marmalade (g) | 20 (19) | 13 (12) | 0.272 | 0.15    0.208 |
| Total intake of selected foods excluding tea and coffee (g) | 210 (87) | 302 (153) | 0.005 | 0.19    0.074 |

Data are presented as means (SD).
associated with a higher plasma AOC. Similarly, a
significant association between antioxidant-rich
foods and AOC was apparent when a number
of antioxidant-rich food groups was considered
together. These findings may support the synergistic
and integrated effects between different types of
antioxidants, and the importance of a variety of
antioxidants from different food sources for the
AOC in plasma.

The AOC in various foods can differ enormously,
by more than a 1000-fold (9). Berries generally have
high antioxidant capacities (9, 10), but the difference
in intake of fruit and berries between the
lowest and the three higher quartiles of AOC did
not differ significantly in the present study. How-
ever, when intakes of vegetables and root vegetables
were considered together with intake of fruits and
berries, there was a significant difference in intake
between the lowest and the higher quartiles of AOC.
Halvorsen and co-workers reported that fruits
and berries in a Norwegian diet contributed most
to the total intake of plant antioxidants, but
beverages were not included in these calculations
(9). Beverages such as tea, wine and coffee also have
high antioxidant capacities, owing to a high content
of polyphenols and other phenolic compounds.
Thus, although a high AOC in beverages such as
tea, coffee and wine is reported (10), as well as
increases in AOC after intake (22–24), this was
not reflected in the AOC measured in plasma in
this study.

AOC in plasma is affected not only by exogenous
antioxidants from the diet, but also by endogenous
antioxidants such as uric acid and bilirubin, as well
as by ongoing oxidation processes in the body. In
most of the AOC assays, uric acid is a major
contributor, with clear correlations between AOC
and uric acid content (25, 26), but the function of
uric acid as an antioxidant is unclear. In a previous
study, therefore, uricase was used to remove uric
acid in the sample (in press). In this study a similar
pattern was found, with an association between
higher intakes of antioxidants and antioxidant-rich
foods and higher levels of AOC, also when uric acid
was removed. AOC without uric acid was also
associated with the intake of β-carotene, as well as
with the intake of vegetables and root vegetables,
which are the most important sources of β-carotene
in Sweden (27).

In the present study the AOC in plasma was
measured. This depends on the intake, as well as on
the bioavailability, of the dietary antioxidants. The
associations between AOC in plasma and dietary
factors were also studied. Others have calculated the
AOC in the diet, which does not account for the
bioavailability of different antioxidants (11–13).
Both food groups and nutrients were investigated,
since the well-known antioxidants including ascor-
ic acid, α-tocopherol and β-carotene are suggested
to reflect only a minor part of the dietary antiox-
didants. However, cereals, nuts and spices, which may
also contribute to the AOC, were not included in
this study.

The plasma concentration of F2-isoprostanes is
considered as a gold-standard indicator of lipid
peroxidation in vivo (28, 29). F2-isoprostanes are
increased in clinical conditions associated with
increased oxidative stress, such as diabetes (30,
31), and in smokers (32, 33). The results of this
study showed that there were no differences in
plasma F2-isoprostane concentrations when related
to quartiles of ascorbic acid, tocopherols or β-
carotene intake, or when they were combined.
Reduced levels of F2-isoprostanes were not seen in
groups reporting a high intake of foods rich
in antioxidants, which is in line with other studies
(34, 35). In contrast, Block and co-workers reported
an inverse correlation between plasma F2-isopros-
tanes and fruit intake (36). Results from interven-
tion studies, where fruits and vegetables were added
to the diet, have reported conflicting findings, with
unaltered (34, 35) as well as decreased (17, 37) levels
of F2-isoprostanes. However, in the present study,
subjects in the lowest quartile of F2-isoprostanes
had a higher intake of white wine, which is
compatible with the high content of polyphenols
in wine possibly decreasing the levels of F2-isopros-
tanes. Although the AOC is higher in red wine than
in white wine (10), reflecting the higher content of
polyphenols in red wine, which might decrease
further the formation of F2-isoprostanes, this was
not reflected in the current study. The relationships
between dietary antioxidants and isoprostanes are
complex (38) and the biological variation between
individuals and day-to-day variation in F2-isopros-
tanes is high (39, 40).

A strength of this study was the use of repeated
24 h recalls, including every day of the week, twice.
A limitation of the study was that blood samples
were collected on only one occasion.

In summary, the results of this study showed
that individuals with a higher intake of various
antioxidant-rich foods, including different antioxidants, had a better plasma AOC. The associations tended to be stronger between plasma AOC and different antioxidant-rich foods, when the foods were combined. An optimal antioxidant status may be achieved by eating a balanced diet rich in a mix of various antioxidant-containing foods. Moreover, the results indicate that lipid peroxidation in vivo is not easily affected by the diet.

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