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Relationship of fruit and vegetable intake to dietary antioxidant capacity and markers of oxidative stress: A sex-related study

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ABSTRACT

Objectives: Oxidative stress contributes to the development of chronic diseases. Fruits and vegetables contain several phytonutrients (carotenoids, polyphenols) that exert antioxidant effects. The aim of this study was to investigate sex differences in fruit and vegetable intake, and the relationship to plasma levels of carotenoids as well as to total antioxidant capacity (pTAC). We studied also sex differences in the relationship between fruit and vegetables intake and plasma levels of lipid hydroperoxides, as well as of oxidized low-density lipoprotein (ox-LDL).
Methods: This study included 83 healthy adults (35 men and 48 women, mean age 40 ± 10 y). Dietary intake of carotenoids and total antioxidant capacity (dTAC) were evaluated on the basis of a 15-d food frequency questionnaire. Plasma levels of β-carotene, lutein, and pTAC were studied. Moreover, levels of plasma lipid hydroperoxides and ox-LDL were evaluated using the ferrous oxidation-xylenol orange 2 (FOX2) assay and a monoclonal antibody–based enzyme-linked immunosorbent assay procedure, respectively.
Results: Dietary habits were sex-related with a higher intake of fruits and vegetables ($P < 0.05$) and β-carotene ($P < 0.001$) in women than in men. Mean values of plasma lutein and β-carotene were higher in women than in men. Mean values of ox-LDL and lipid hydroperoxides were higher in men than in women ($P < 0.05$). Significant negative correlations were established between the individual values of ox-LDL and the levels of lutein versus β-carotene and versus pTAC values in plasma in both groups. Individuals belonging to the tertile with the highest daily intake of fruits and vegetables or the highest daily dTAC showed the lowest levels of plasma ox-LDL. In each category, sex-related differences were observed with men showing higher levels of ox-LDL than women. Moreover, lower levels of plasma β-carotene were observed in men in each tertile of daily intake of fruits and vegetables compared with females.
Conclusions: Based on the data obtained, we confirm that high fruit and vegetable consumption exerts a positive effect on antioxidant defenses and decreases oxidative damage of plasma lipoproteins for both sexes. The results suggest that the protective effect can be found to a higher extent in women than in men. Sex-based differences are apparent in many chronic diseases. Thus, a higher consumption of antioxidant-rich fruits and vegetables should be recommended in efforts to prevent diseases in which sex-related differences in oxidative stress play a considerable role.

Introduction

According to the World Health Organization (WHO) [1], daily intake of adequate amounts of plant foods is inversely associated with the development of chronic diseases, such as cardiovascular diseases [2]. The WHO recommends eating ≥400 g of fruits and vegetables (FV) per day, not counting starchy tubers [3]. It also estimates that in >50% of the countries of the European Region the consumption of FV is <400 g/d, and in 33% of the countries, the average intake is <300 g/d. Sex differences in dietary intakes and eating behaviors have been reported. Literature data suggest that women consume more FV, legumes, and whole foods. Men tend to consume more fat- and protein-rich foods and to drink more wine, beer, spirits, and sweet carbonated drinks [4,5].

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Among molecular mechanisms involved in the protective effects exerted by a high intake of plant foods, it has been proposed that phytochemicals contained in FV play a key role [6,7]. In particular, some phytochemicals behave as antioxidants, modulate gene expression, and prevent oxidative stress, which is involved in the pathogenesis and progression of several chronic diseases, including cardiovascular diseases (CVDs) [7–9], cancer [10], and aging-associated diseases. Among phytochemicals contained in FV, the nutritional roles of carotenoids [11] and polyphenols have been mainly investigated [7]. In addition, FV are an important source of vitamin C, fiber, and magnesium—nutrients that have been negatively associated with oxidative stress [12–14].

Circulating markers of oxidative stress and inflammation are known to play a complex role in the development of age-related chronic diseases [15]. Lipid peroxidation of plasma high-density lipoproteins (HDL) and low-density lipoproteins (LDL) occurs *in vivo*. In particular, the attention given to plasma levels of oxidized low-density lipoproteins (ox-LDL) is supported by their physiopathologic roles [16]. In fact, ox-LDL are related to atherosclerotic events [16] and are considered a marker for CVDs [16,17]. Among antioxidant extracellular molecules, a key role is played by paraoxonase-1 (PON1). The enzyme is associated with HDL and exerts an antioxidant activity by protecting *in vitro* both lipoproteins and biological membranes against lipid peroxidation [18,19], therefore there is growing interest in the dietary factors that modulate PON1 expression and activity [20,21]. Previous studies have shown that a high intake of FV is related to lower levels of oxidative stress markers, such as ox-LDL and F_2-isoprostanes, in adolescents, middle-aged men, and women [12,13,22–25]. Sex-related differences have been less investigated. A growing interest is devoted to sex as an important biological variable in biomedical research [26–29]. Sex differences are present in many chronic diseases. Men tend to suffer from myocardial infarctions earlier than women, and a woman's risk for CVD increases after menopause [27]. The interest in investigating nutritional aspects and sex-related differences in the effect of dietary intake of FV on plasma lipid peroxidation is also supported by recent studies that have demonstrated sex-related differences in the production and metabolic deactivation of reactive oxygen metabolites mainly in animal models [27]. To our knowledge, few studies have described sex differences in response to diet or antioxidant therapy in humans [29].

The aim of the present study was to investigate sex-related relationships between FV intake, total dietary antioxidant capacity (dTAC), and markers of antioxidants or oxidative stress in plasma of 83 healthy adults (35 men and 48 women). Plasma levels of carotenoids (lutein and β-carotene), widely used as biomarkers for FV intake [30], were evaluated. We also studied the effect of FV intake and dietary antioxidants on the activity of the antioxidant enzyme PON1. Plasma levels of ox-LDL and lipid hydroperoxides, widely used to investigate lipid peroxidation of plasma lipids [17], were evaluated as biochemical parameters of oxidative stress.

The relationship between dietary habits and plasma levels of markers of antioxidant or oxidative stress of the participants included also was examined by a multivariate statistical analysis including partial least squares regression (PLS) and principal component analysis (PCA). PLS and PCA are widely used in various disciplines, including medicine and nutritional sciences, especially when a large number of predictors is necessary [31].

Materials and methods

Participants

There were 83 healthy adults enrolled in the study. Volunteers were recruited from the Polytechnic University of Marche (UNIVPM), Italy. There were 35 men and 48 women. The mean age was 40 ± 10 y and the mean body mass index (BMI) was 23.1 ± 2 kg/m^2 (Table 1). Inclusion criteria for participants were as follows:

Table 1
Anthropometric data and plasma lipids

Parameters	Total participants (N = 83)	Men (n = 35)	Women (n = 48)
Age (y)	40 ± 10	41 ± 8	38 ± 11
BMI (kg/m^2)	23.1 ± 2	24.3 ± 1.5	22.2 ± 1.9*
TC (mmol/L)	4.6 ± 0.5	4.8 ± 0.4	4.4 ± 0.5*
HDL-C (mmol/L)	1.5 ± 0.3	1.4 ± 0.2	1.6 ± 0.3*
LDL-C (mmol/L)	3 ± 0.5	3.2 ± 0.4	2.6 ± 0.5*
HDL-C/LDL-C ratio	0.6 ± 0.2	0.4 ± 0.1	0.6 ± 0.2*
Glucose (mmol/L)	4.8 ± 0.4	5.1 ± 0.42	4.6 ± 0.4*

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol
Values expressed as mean ± SD
*$P < 0.05$ vs men

- Not taking vitamins, minerals, or other types of supplements during the previous 2 mo;
- Nonsmoking;
- BMI within the normal range according to the WHO criteria (18.5–25.5 kg/m^2);
- Normal biochemical and hematologic profile (serum cholesterol <6.8 mmol/L, serum triacylglycerols [TGs] <2.8 mmol/L, glucose <6.11 mmol/L).

Exclusion criteria were:

- Diagnosed diseases such as allergies, cancer, diabetes, obesity, hypertension, mental diseases, gastrointestinal or renal diseases, as well as intake of drugs related to these pathologies;
- Alcohol consumption >30 g/d;
- Consumers of vegetarian diet.

None of the women were pregnant or lactating. The study was conducted according to the Declaration of Helsinki and all procedures were approved by the Ethics Committee of the "Azienda Ospedaliero-Universitaria Ospedali Riuniti" Ancona (Italy). All participants gave informed consent.

Dietary assessment

Each participant was asked to complete a 15-d dietary record to evaluate energy and nutrient intake. Dietary data were estimated by the software MètaDieta (version 3.1, Meteda, Ascoli Piceno, Italy), validated by the Italian Association of Dietetic and Clinical Nutrition in 2009. Food composition tables were derived from the European Institute of Oncology (Milan, Italy). FV intake was assessed from data of the quantitative questionnaire, which included evaluation of fresh fruits and fresh or cooked vegetables. Fruit juice was not considered in this study. Serving size information was provided for each food group and participants ticked a box representing how many servings they consumed on an average day. A portion of fruit corresponded to 150 g, 50 g for salad, and 250 g for vegetables [32]. Daily food consumption was estimated as frequency × portion × size for each consumed food item.

The MètaDieta software also contains an antioxidant database (USDA Database for the Oxygen Radical Absorbance Capacity [ORAC] of selected foods, Release 2 [2010]). The method used to evaluate ORAC levels was developed by Prior et al. [33] and measures both hydrophilic (H-ORAC) and lipophilic ORAC (L-ORAC) for water-soluble and fat-soluble antioxidant compounds. The USDA Database contains total ORAC (T-ORAC) values for 326 food items. The values of dTAC are expressed as mmol Trolox equivalent/100 g (mmol TE/100 g) [34].

Blood samples and evaluation of plasma lipids and glycemia

Fasting blood samples (10 mL) were collected from each participant by venipuncture from the antecubital vein: 5 mL was placed in tubes containing heparin and 5 mL was placed in tubes without any anticoagulant and centrifuged at 1500 *g* for 10 min at 4°C for serum separation. Plasma and serum aliquots were prepared and stored at −80°C until analysis.

Serum glucose, TGs, total cholesterol (TC), and HDL cholesterol (HDL-C) were analyzed by commercial kits (Chemadiagnostica, Jesi, Italy). LDL cholesterol (LDL-C) was calculated by the Friedewald formula [35].

Plasma levels of carotenoids

Carotenoids (β-carotene and lutein) were quantified in plasma of participants by high-performance liquid chromatography (HPLC) system using a single dilution step after extraction with propanol (1:5, v/v) and vigorous vortexing of 250 μL of extraction mixture. This mix was centrifuged for 2 min at 20 000 *g* at 4°C. Forty microliters of supernatant were injected into the high-performance liquid chromatography with electrochemical detector by Shiseido (Tokyo, Japan), using a preseparation concentrating column 50 × 2.0 mm ID 5 μm, separation C18 column 150 × 2.0 mm ID 3 μm, and a post-separation–reducing column CQR 20 × 2.0 mm, all from Shiseido. For each carotenoid quantified, two mobile phases were used. Mobile phase 1 for loading and concentrating the sample (50 mM sodium perchlorate in methanol/water 95:5, v/v) was the same for both molecules, whereas mobile phase 2 was 50 mM sodium perchlorate in methanol/isopropanol (80:20, v/v) for lutein and 50 mM sodium perchlorate in methanol/ isopropanol (98:2, v/v) for β-carotene. Moreover, the flow rate was 200 μL/min for phase 1 in both analyses. Flow rates for phase 2 were 300 and 80 μL/min for β-carotene and lutein, respectively. Total chromatographic run times and retention times were 24 min/12.3 min for β-carotene and 21 min/9.8 min for lutein, as previously described [36].

Biomarkers of oxidative stress

Lipid hydroperoxides

The levels of lipid hydroperoxides were determined in plasma samples using ferrous oxidation-xylenol orange 2 (FOX2) assay as previously described [19]. The levels of lipid hydroperoxides were quantified using a stock solution of t-butyl hydroperoxide. The results are shown as μmol of lipid hydroperoxides for liter of plasma.

Ox-LDL

Oxidized-LDL was determined in plasma by a sandwich enzyme-linked immunosorbent assay procedure using the murine monoclonal antibody mAB-4 E6 as the capture antibody and a peroxidase-conjugated antibody against oxidized apolipoprotein B bound to the solid phase (ox-LDL, Mercodia AB, Uppsala, Sweden). Intra- and interassay CV were 2.82% and 7.29%, respectively. Because LDL-C is considered a major determinant of absolute ox-LDL levels, plasma values of ox-LDL (U/L) were adjusted by the plasma levels of LDL-C (mmol/L) by calculating their ratio (units of ox-LDL/mmol of LDL-C), in agreement with Zuliani et al. [37].

Plasma total antioxidant capacity

Plasma total antioxidant capacity (pTAC) was assessed using the ORAC assay adapted for semiautomated measurement on a 96-well microplate reader (Synergy HT; BioTek, Winooski, VT, USA) [38]. The ORAC of plasma samples employs the oxidative loss of the intrinsic fluorescence of fluorescein induced by the free radical initiator 2,2′-azobis(2-amidinopropane)hydrochloride. Fluorescein fluorescence decay shows a lag or retardation in the presence of antioxidants, related to the antioxidant capacity of the sample. Trolox was used as a reference antioxidant for calculating the ORAC values. Results are expressed as mmol TE/L.

PON1 activity

Paraoxonase activity of PON1 was measured in plasma using paraoxon as substrate [19]. The basal assay mixture included 5 mM Tris-HCl, pH 7.4 containing 0.15 M NaCl, 4 mM $MgCl_2$, 2 mM $CaCl_2$ and 1 mmol/L paraoxon. Paraoxon hydrolysis was spectrophotometrically monitored for 8 min (every 15s) at 412 nm. Non-enzymatic hydrolysis of paraoxon was subtracted from the total rate of hydrolysis. One unit of PON1 paraoxonase activity is equivalent to 1 nmol of paraoxon hydrolyzed·min·mL^{-1}.

Statistical analysis

Values of nutrient intake and biochemical markers are shown as mean ± standard deviation (SD). Statistical differences were calculated by using Student's *t* test. $P = 0.05$ was considered statistically significant. Pearson correlation coefficients and *P*-value were used to show correlations and their significance using the software Microcal Origin 5.0 (OriginLab).

To assess the association among FV consumption, dTAC, and biochemical markers, we categorized the participants by tertiles using the Excel program (Microsoft Excel®, version 2013). Data analysis was carried out through PLS and PCA using the software Simca p8.0 (Umetrics, Umea, Sweden).

One of the goals of PCA is to reduce the number of variables to enable visualization of the information in the multivariate data set. In PCA, a linear combination of the original variables is constructed to obtain principal components while preserving the largest possible variation in X. Scatter plots are used to visualize object similarities and clustering tendencies, whereas loading plots reveal the contributions of the original variables. In the present study, the variables used in the PCA were BMI, energy intake, FV intake, dietary β-carotene, dTAC, plasma levels of lipid hydroperoxides, plasma levels of ox-LDL, plasma PON1 activity, pTAC, and plasma levels of lutein and β-carotene. PLS can analyze data with strongly collinear, noisy, and numerous X-variables (X BLOCK), and also simultaneously model several response variables (Y) (Y BLOCK). The objective of PLS regression is to find the relations between two blocks (X and Y). In the present study, X BLOCK contained variables associated with diet of the participants (FV intake, dietary β-carotene, dTAC), whereas Y BLOCK contained variables associated with their levels of antioxidant/oxidant status in plasma (lipid hydroperoxides, pTAC, ox-LDL, lutein and β-carotene). The joint score vectors (t, u) were obtained from a PLS fit using X BLOCK (represented by t) and Y BLOCK (represented by u) and plotted against each other.

Results

Anthropometric and clinical data of participants are summarized in Table 1. Plasma lipids (TC and LDL-C) and glucose levels were lower in women than in men. Higher values of HDL-C and of the ratio of HDL-C to LDL-C were observed in women than in men ($P < 0.05$).

Dietary habits

The assessment of dietary habits demonstrated sex-related differences with lower energy intake and lower protein and lipid intake in women (Table 2). β -carotene intake was in agreement with previous studies in Italian individuals [39]. The study of micronutrient intake showed a higher intake of vitamin C ($P < 0.05$) and β-carotene ($P < 0.001$) and higher dTAC in women than in men, but the difference was not statistically significant (Table 2).

Plasma levels of carotenoids and total antioxidant capacity

The average values of plasma levels of β-carotene and lutein were in agreement with those reported in other studies [40] (Table 3). Higher levels of carotenoids were observed in plasma of women than in men (Table 3). pTAC was higher in women but the difference was not statistically significant.

Biochemical markers of lipid peroxidation and paraoxonase activity

Levels of ox-LDL ranged between 5.1 and 66.0 U/mL with a mean value of 34.4 ± 12.1 U/mL; levels of hydroperoxides ranged

Table 2
Dietary habits

	Men (n = 35)	*Women* (n = 48)
Food		
Fruits and vegetable (portions)	2.4 ± 0.7	3 ± 1.1*
Meat (portions/d)	0.9 ± 0.1	0.7 ± 0.29*
Fish (portions/d)	0.3 ± 0.1	0.3 ± 0.1
Components		
Energy (kcal/d)	1778.3 ± 77.6	1480.5 ± 219.4†
Proteins (g/d)	70 ± 4.2	57. 1 ± 8.3†
Available carbohydrate (g/d)	221 ± 14.9	190.5 ± 18.6
Total fiber (g/d)	20.1 ± 1.36	20.3 ± 2.1
Lipids (g/d)	59.8 ± 1.3	51.2 ± 9*
Total saturated (g/d)	17.2 ± 1.9	13.1 ± 3.5*
Total monounsaturated (g/d)	24.6 ± 0.8	23.3 ± 3.4
Total polyunsaturated (g/d)	6.7 ± 0.7	5.6 ± 0.8
Total ω-3 (g/d)	1.05 ± 0.07	1.02 ± 0.09
Total ω-6 (g/d)	5.3 ± 0.9	4.4 ± 0.7
Vitamin C (mg/d)	65.3 ± 16	76.7 ± 7.3*
Vitamin E (mg/d)	7.9 ± 0.7	8.1 ± 0.7
Thiamine (mg/d)	0.77 ± 0.13	0.63 ± 0.10
Folic acid (mcg/d)	193.5 ± 16.6	174.8 ± 29.8
β-carotene (mg/d)	2.3 ± 1.8	3.2 ± 1.4†
dTAC (mmol TE/d)	5.3 ± 1.6	5.9 ± 1.1

dTAC, dietary total antioxidant capacity
Values expressed as mean ± SD
*$P < 0.05$ vs men.
†$P < 0.001$ vs men.

Table 3
Plasma levels of carotenoids, total antioxidant capacity (pTAC), biochemical markers of lipid peroxidation, and paraoxonase-1 activity

Plasma levels	*Men* (n = 35)	*Women* (n = 48)
β-carotene (μg/mL)	0.36 ± 0.19	0.47 ± 0.22*
Lutein (μg/mL)	0.24 ± 0.07	0.28 ± 0.09†
pTAC (mmol TE/L)	18.10 ± 1.82	18.43 ± 3.74
ox-LDL (U/mL)	37.05 ± 6.16	32.49 ± 11.5†
Lipid hydroperoxides (μmol/L)	1.73 ± 0.24	1.50 ± 0.29†
Paraoxonase activity (U/mL)	82 ± 31	72 ± 35

ox-LDL, oxidized low-density lipoprotein; pTAC, plasma total antioxidant capacity
Values are expressed as mean ± standard deviation
*$P < 0.001$ vs men.
†$P < 0.05$ vs men.

between 0.76 and 3.08 μmol/L with a mean value of 1.57 ± 0.47 μmol/L. The average values of ox-LDL and lipid hydroperoxides were in agreement with values reported by other authors in a healthy population [37]. Both markers of lipid peroxidation (ox-LDL and lipid hydroperoxides) were higher in men than in women (Table 3). The activity of PON1 was not significantly different in the two groups (Table 3).

Correlations between plasma lipids and markers of lipid peroxidation

A positive correlation was established between levels of ox-LDL and lipid hydroperoxide in all 83 participants ($r = 0.42$; $P < 0.005$). A positive correlation was also established between levels of ox-LDL and LDL-C levels ($r = 0.71$; $P < 0.001$) and TC ($r = 0.51$; $P < 0.001$). Conversely, levels of ox-LDL were inversely related to HDL-C levels ($r = -0.41$; $P < 0.05$). No correlation was established between levels of ox-LDL and PON1 activity (data not shown).

Correlations among dietary habits, carotenoid intake, and plasma levels of carotenoids

To investigate whether dietary intake of FV is related to levels of phytochemicals in plasma, we analyzed correlations between dietary carotenoid intake and dTAC versus plasma levels of β-carotene, lutein, and pTAC values. As summarized in Table 4, a significant positive correlation was established between daily intake of FV and daily intake of β-carotene *versus* plasma levels of β-carotene in all participants. Even levels of plasma lutein positively correlated with daily intake of FV in both sexes. The results also showed a positive relationship between daily consumption of FV and dietary intake of β-carotene versus pTAC values. No significant correlation was observed between dTAC and pTAC in all participants and by sex.

Correlations among dietary habits, plasma carotenoids, and markers of lipid peroxidation

As reported in Table 4, a significant negative correlation was observed in both sexes between daily intake of FV and daily intake of β-carotene and dTAC versus plasma levels of markers of lipid peroxidation (ox-LDL and lipid hydropexides).

Moreover, significant correlations were established between individual values of markers of lipid peroxidation (ox-LDL and lipid hydroperoxides) versus plasma levels of lutein versus β-carotene and versus pTAC values of individuals included in the study (Table 5).

To better investigate the effect of dietary habits on markers of oxidative stress in men and women, we reported the levels of plasma ox-LDL by sex-specific tertile of daily FV intake and daily dTAC. As shown in Figures 1 and 2, the men and women belonging to the tertile with the highest daily FV intake or the highest daily dTAC showed lowest levels of plasma ox-LDL. In each category, sex-related differences were observed; men showed higher levels of ox-LDL than women (Figs. 1 and 2). As summarized in Figure 3, lower levels of plasma β-carotene were observed in men in each tertile of FV intake.

The relationship between dietary habits and markers of antioxidant or oxidative stress in plasma was investigated using PLS and PCA.

As summarized in Fig. 4A, the X BLOCK included variables related to dietary habits (FV intake, dietary β-carotene, and dTAC) and Y BLOCK included variables associated to antioxidant or oxidative stress

Table 5
Correlations between plasma antioxidants and markers of lipid peroxidation

Variables	*Plasma antioxidant*		
	β-carotene	Lutein	pTAC
ox-LDL			
Total participants (n = 83)	−0.30*	−0.52†	−0.37†
Men (n = 35)	−0.26	−0.48*	−0.36*
Women (n = 48)	−0.28*	−0.50†	−0.31*
Lipid hydroperoxides			
Total participants (n = 83)	−0.31*	−0.40†	−0.31*
Men (n = 35)	−0.26	−0.37*	−0.29*
Women (n = 48)	−0.29*	−0.35*	−0.28*

ox-LDL, oxidized low-density lipoprotein; pTAC, plasma total antioxidant capacity
*$P < 0.05$.
†$P < 0.001$.

Table 4
Correlations between dietary factors, plasma antioxidants, and markers of lipid peroxidation

Variables	*Plasma levels*				
	β-carotene	Lutein	pTAC	ox-LDL	Lipid hydroperoxides
Fruit and vegetable portions					
Total participants (n = 83)	0.44*	0.40*	0.35*	−0.71*	−0.37†
Men (n = 35)	0.38†	0.42†	0.42†	−0.65*	−0.34†
Women (n = 48)	0.42†	0.34†	0.31†	−0.76*	−0.40†
Dietary β-carotene					
Total participants (n = 83)	0.37*	0.31†	0.34†	−0.41†	−0.37†
Men (n = 35)	0.35†	0.34†	0.39†	−0.43†	−0.33†
Women (n = 48)	0.33†	0.29†	0.32†	−0.39†	−0.39†
dTAC					
Total participants (n = 83)	0.31†	0.32†	0.1	−0.51*	−0.42†
Men (n = 35)	0.29	0.34†	0.24	−.53*	−0.42†
Women (n = 48)	0.30†	0.31†	0.04	−0.47*	−0.35†

dTAC, dietary total antioxidant capacity; ox-LDL, oxidized low-density lipoprotein; pTAC, plasma total antioxidant capacity
*$P < 0.001$.
†$P < 0.05$.

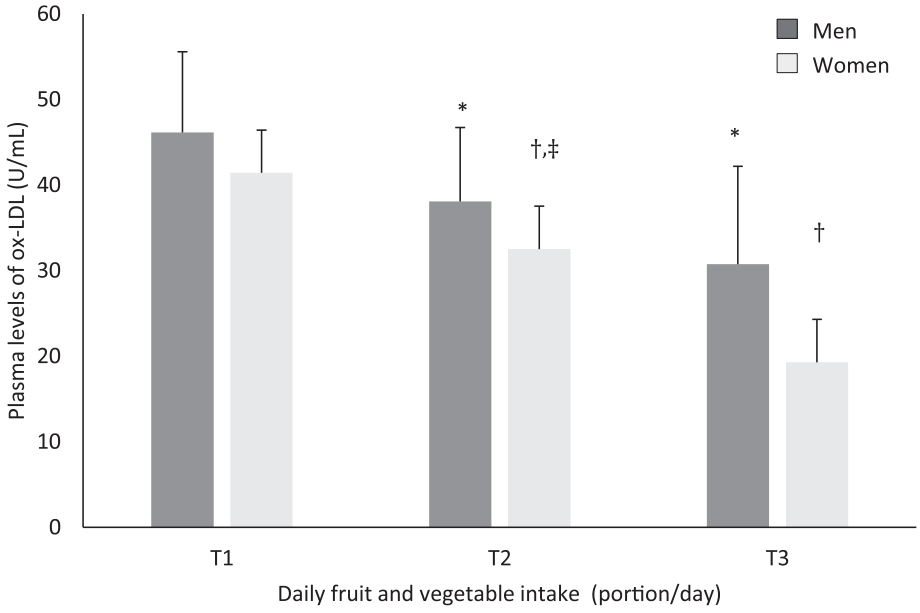

Fig. 1. Plasma levels of ox-LDL in sex-specific tertiles of daily fruit and vegetable intake. Men: T1 (n = 12, 0.46–1.93), T2 (n = 11, 1.9–2.86), T3 (n = 12, 2.86–5.80). Women: T1 (n = 16, 1.10–2.3), T2 (n = 16, 2.30–3.33), T3 (n = 16, 3.33–5.80). ox-LDL, oxidized low-density lipoprotein. *$P < 0.05$ versus men belonging to tertile with lowest daily fruit and vegetable intake (T1). †$P < 0.05$ for the comparison between men and women belonging to the same tertile. ‡$P < 0.05$ versus women belonging to tertile with lowest daily fruit and vegetable intake (T1).

in plasma (lipid hydroperoxides, ox-LDL, pTAC, lutein, and β-carotene). X- and Y-loadings, which are the linear coefficients that link the terms to the x or y scores, respectively, are also reported.

Figure 4B, showing the graph obtained by PLS analysis, confirms the general correlation among dietary factors (FV intake, dietary β-carotene, and dTAC), levels of antioxidants (pTAC, plasma levels of β-carotene, and lutein), and lipid peroxidation markers (plasma levels of ox-LDL and lipid hydroperoxides) in plasma.

Moreover, a PCA was carried out. This method reduces data dimensions because the new low-dimensional variables are used instead of the original high-dimensional ones. Figure 5A and B show the scatter plot of the principal components (PC1 and PC2) after PCA and loading plot, respectively, considering the first two components. PC1 components had the highest data variance, with a variance contribution ratio of 41%, followed by PC2 components with variance contribution rates of 11%. Therefore, the first two

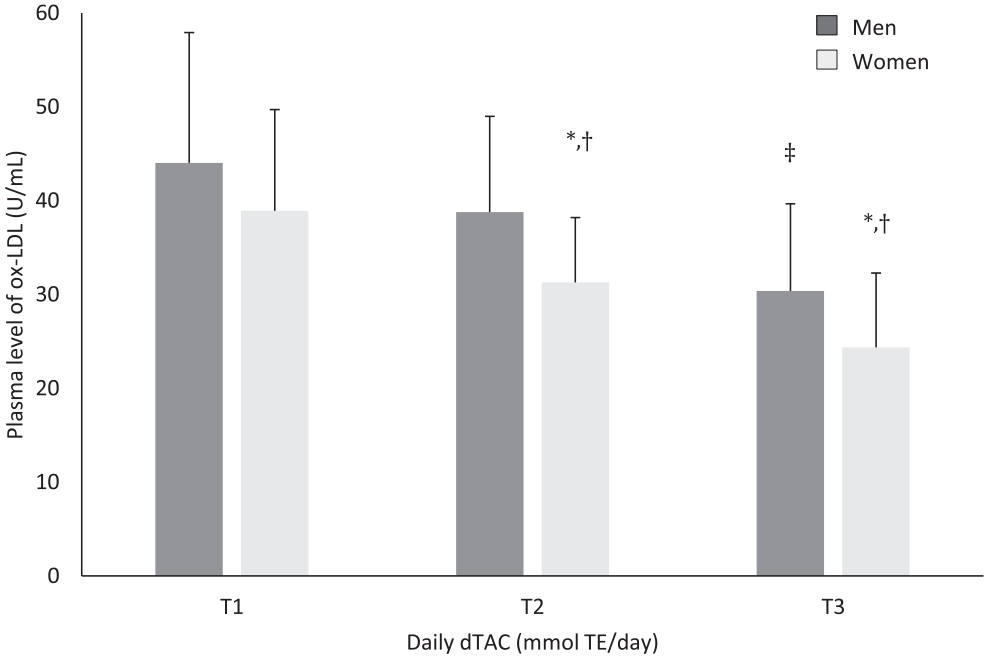

Fig. 2. Plasma levels of ox-LDL in sex-specific tertile of daily dTAC. Men: T1 (n = 12, 2.34–4.63), T2 (n = 11, 4.36–6.29), T3 (n = 12, 6.29–9.60). Women: T1 (n = 16, 3.02–5.18), T2 (n = 16, 5.18–6.61), T3 (n = 16, 6.61–9.65). dTAC, dietary total antioxidant capacity; ox-LDL, oxidized low-density lipoprotein; TE, Trolox equivalent. *$P < 0.05$ versus women belonging to tertile with lowest daily dTAC (T1). †$P < 0.05$ for the comparison between men and women belonging to the same tertile. ‡$P < 0.05$ versus men belonging to tertile with lowest daily dTAC (T1).

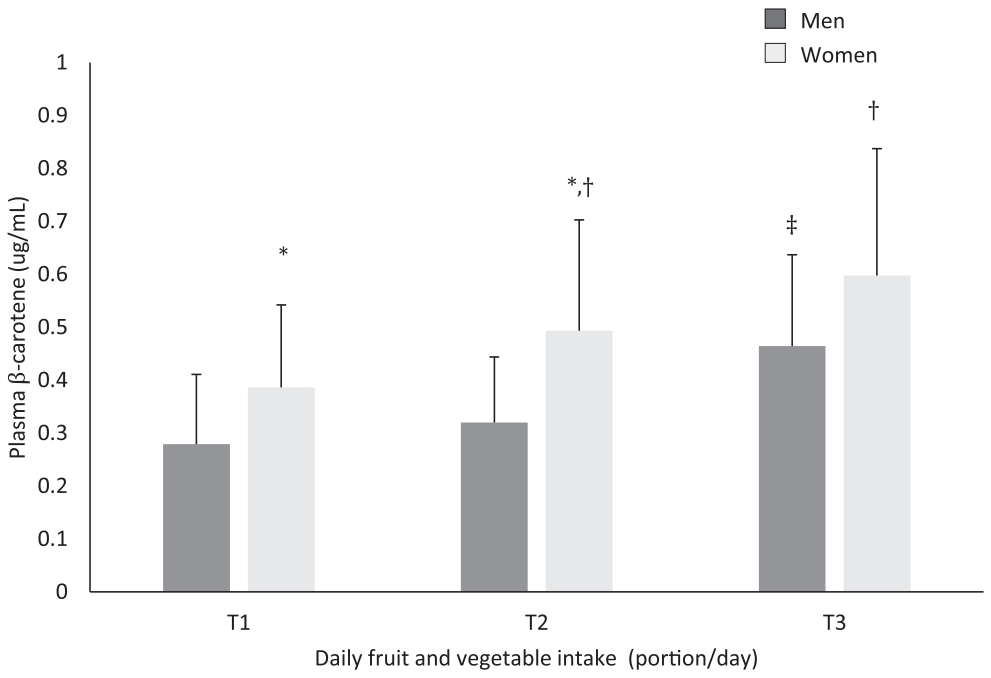

Fig. 3. Plasma levels of β-carotene in sex-specific tertiles of daily fruit and vegetable intake. Men: T1 (n = 12, 0.46–1.93), T2 (n = 11, 1.9–2.86), T3 (n = 12, 2.86–5.80). Women T1 (n = 16, 1.10–2.3), T2 (n = 16, 2.30–3.33), T3 (n = 16, 3.33–5.80). *$P < 0.05$ versus women belonging to tertile with lowest daily fruit and vegetable intake (T1). †$P < 0.05$ for the comparison between men and women belonging to the same tertile. ‡$P < 0.05$ versus men belonging to tertile with lowest daily fruit and vegetable intake (T1).

principal components reflect the majority of the information (which explains 52% of the total variance).

The results revealed that men and women have a different trend, mainly owing to higher FV intake and higher concentrations of plasma antioxidants (β-carotene, lutein, and pTAC) in women and higher plasma levels of markers of lipid peroxidation (plasma lipid hydroperoxides and ox-LDL) in men.

Discussion

In this study, we confirmed sex-related differences in dietary habits [4]. Higher intakes of FV, vitamin C, and β-carotene were observed in women than in men. In the overall group of participants and in subgroups, FV intake was positively associated with levels of carotenoids and TAC in plasma. The present results are in agreement with previous studies, which have demonstrated that FV intake and related nutrients are associated with lower levels of oxidative stress [22–25]. In detail, the negative significant correlations established, both in men and women, between dietary carotenoids intake and dTAC versus plasma levels of markers of lipid peroxidation (ox-LDL and lipid hydroperoxides) of participants included in the present study confirm a protective role of dietary lipophilic antioxidants against lipid peroxidation of LDL. The present results demonstrated that higher levels of plasma carotenoids were associated with lower values of oxidized LDL. The results are in agreement with previous studies that have demonstrated that lutein and β-carotene are molecules able to inhibit lipid peroxidation [40,41]. Previous studies also found that levels of carotenoids in plasma correlate with carotenoid levels associated with LDL. In fact, because of their hydrophobic nature, carotenoids are transported by lipoproteins. Other studies have demonstrated that carotenoid intake is related to lower lipid oxidation and lower oxidative damage to DNA in middle-aged men [41]. A relationship also has been shown [41] between a high FV intake and an increased resistance of plasma lipoproteins to oxidation owing to a higher level of plasma antioxidants, which exert an inhibitory role against lipid peroxidation. Ox-LDL play an atherogenic role and is able to induce a proinflammatory status by activating the nuclear factor-κB, a redox-sensitive and proinflammatory transcriptional factor [42]. The present results confirm that antioxidant-rich foods, expressed by dTAC values, can play a protective role against lipid peroxidation of LDL and might prevent inflammatory pathways triggered by ox-LDL.

The present study also found that the sex-related differences in dietary habits reflect in modifications in markers of lipid peroxidation (ox-LDL and lipid hydroperoxides). Within each category of FV or dietary TAC tertiles, levels of ox-LDL were significantly higher in men than in women. These results are likely related to the lower levels of carotenoids in the plasma of men with respect to women observed in each tertile. These results were confirmed by a multivariate statistical analysis. In fact, PLS regression revealed a relationship between dietary habits and plasma levels of markers of antioxidant or oxidative stress of the participants. PCA confirmed a different trend, mainly owing to higher FV intake and higher concentrations of plasma antioxidants in women and higher plasma levels of markers of lipid peroxidation in men.

Dietary and genetic factors influence digestion and absorption of carotenes. A wide variability in response to ingested β-carotene has been previously reported [43].

The present data contribute to this discussion and suggest sex-related potential effects, which deserve to be better investigated in both sexes.

The correlation coefficient between FV intake and ox-LDL was higher ($r = -0.71$) compared with the coefficients calculated for the relationship between dietary β-carotene or dTAC versus ox-LDL (−0.41 and −0.51, respectively; Table 4). Oxidative damage of LDL occurs *in vivo* and is modulated by exogenous and endogenous factors. Regarding this aspect, the role of dietary factors, as

A

DIETARY FACTORS	**X BLOCK**	
	Variable	**Loading**
	Fruit and vegetable intake	0.652
	β-Carotene	0.580
	dTAC	0.489
PLASMA FACTORS	**Y BLOCK**	
	Variable	**Loading**
	Lipid Hydroperoxides	-0.29
	pTAC	0.21
	ox-LDL	-0.48
	Lutein	0.29
	β-Carotene	0.28

B

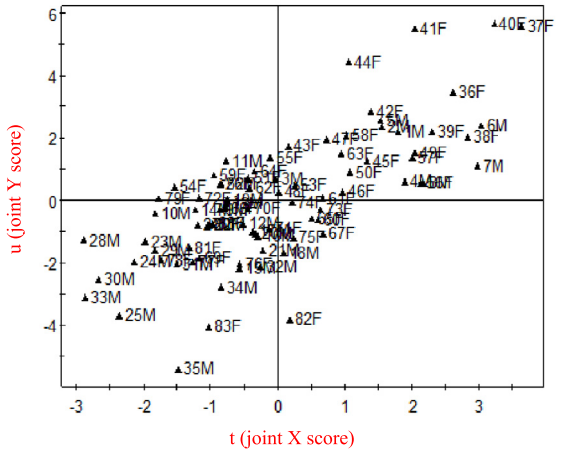

Fig. 4. The data analysis through partial least squares (PLS) regression among dietary factors (X BLOCK) and plasma factors (Y BLOCK). (**A**) Variables included in X BLOCK and Y BLOCK and their X-loadings and Y-loadings. (**B**) Scatterplot joint score vectors. The joint score vectors (t, u) obtained from an PLS fit using X BLOCK (represented by t) and Y BLOCK (represented by u) are plotted against each other.

mentioned previously, is fundamental, with carotenoids being the key factor. However, in addition to carotenoids, other nutrients or bioactive molecules such as fiber, vitamin C, vitamin E, and polyphenols could be involved in the modulatory effect shown by FV consumption. Most polyphenols have low bioavailability and are detected in small amounts both in plasma and tissues [44]. Cooperation among antioxidants with different chemical characteristics may work in an integrated and complementary network and a synergistic effect has been proven *in vitro* [6,7]. No correlation between dTAC and pTAC was observed in the present study. The contribution of dTAC to pTAC is a matter of debate. Several factors such as bioavailability of bioactive molecules and methods to evaluate pTAC could explain the results. In the present experimental conditions, dTAC was evaluated using the ORAC assay, which measures the radical chain-breaking ability of antioxidants by monitoring the inhibition of peroxyl radical–induced oxidation. Peroxyl radicals are the predominant free radicals found in lipid oxidation in foods and biological systems under physiologic conditions. Hence, ORAC values are considered by some to be of biological relevance as a reference for antioxidant effectiveness. Other mechanisms can be involved in the antioxidant effects exerted by dietary bioactive molecules such as chelating effects, modulator of cellular signaling processes, and control of gene expression contributing to oxidative stress [45].

Many dietary antioxidants work in a network, and the total amount of antioxidants derived from the combinations of individual antioxidants is considered a better concept than individual dietary antioxidants. Therefore, databases containing levels of phytochemicals and TAC of several foods have been developed to estimate antioxidant vitamin intake from diet and dietary supplements [46–48] and to assess the beneficial effects of dietary antioxidants. Although the applicability of dTAC data and its *in vivo* physiological relevance is debated, it has been demonstrated that dTAC represents a relevant tool in epidemiologic studies. In fact, it has been demonstrated that an increase of dTAC was associated with a lower risk for ischemic stroke in an Italian cohort [49] and lower inflammatory status [50] in middle-aged people.

Regarding PON1, several studies have demonstrated a protective effect against CVDs and a decrease of its activity has been observed in several pathologic conditions [19,51,52], therefore particular attention has been devoted to dietary factors that may modulate PON1 activity or expression [20,21]. We observed that PON1 activity was not related to FV intake in the present study participants. In addition, plasma levels of ox-LDL were not correlated with PON1 activity and sex-related differences were not observed. Interventional studies have reported that polyphenols and anthocyanin-rich diets can positively modulate PON1 activity or expression [20,21]. Other authors failed to confirm these results. The contradictory results are likely related to the complex interconnections between FV chemical composition, lifestyle, and genetic PON1 isoforms.

The protective effect exerted by PON1 against lipid peroxidation of LDL and biological membranes has been widely demonstrated *in vitro* [18,19]. In the present study, plasma levels of ox-LDL were negatively correlated with HDL-C but not with PON1 activity and sex-related differences were not observed. The correlation between PON1 activity and levels of circulating ox-LDL *in vivo* is debated. PON1 is not the only extracellular antioxidant enzyme in the blood. Other components associated to HDL such as platelet acetyl factor-hydrolase, lecithin-cholesterol acyltransferase, and ApoA1 might have a role in preventing formation of ox-LDL in the circulation [18].

Conclusions

Based on the data obtained, it is possible to confirm that a diet rich in FV antioxidants may improve plasma levels of carotenoids and antioxidant status. Increased consumption of FV increases the plasma antioxidant capacity in both sexes. However, sex-specific differences in oxidative stress markers of lipid peroxidation have been observed and the protective effect exerted by dietary carotenoids can be found to a higher extent in women than in men. Sex differences are present in many chronic diseases. Dietary recommendations toward a higher consumption of antioxidants should be highlighted in prevention or treatment of diseases in which sex-related differences in oxidative stress play a considerable role.

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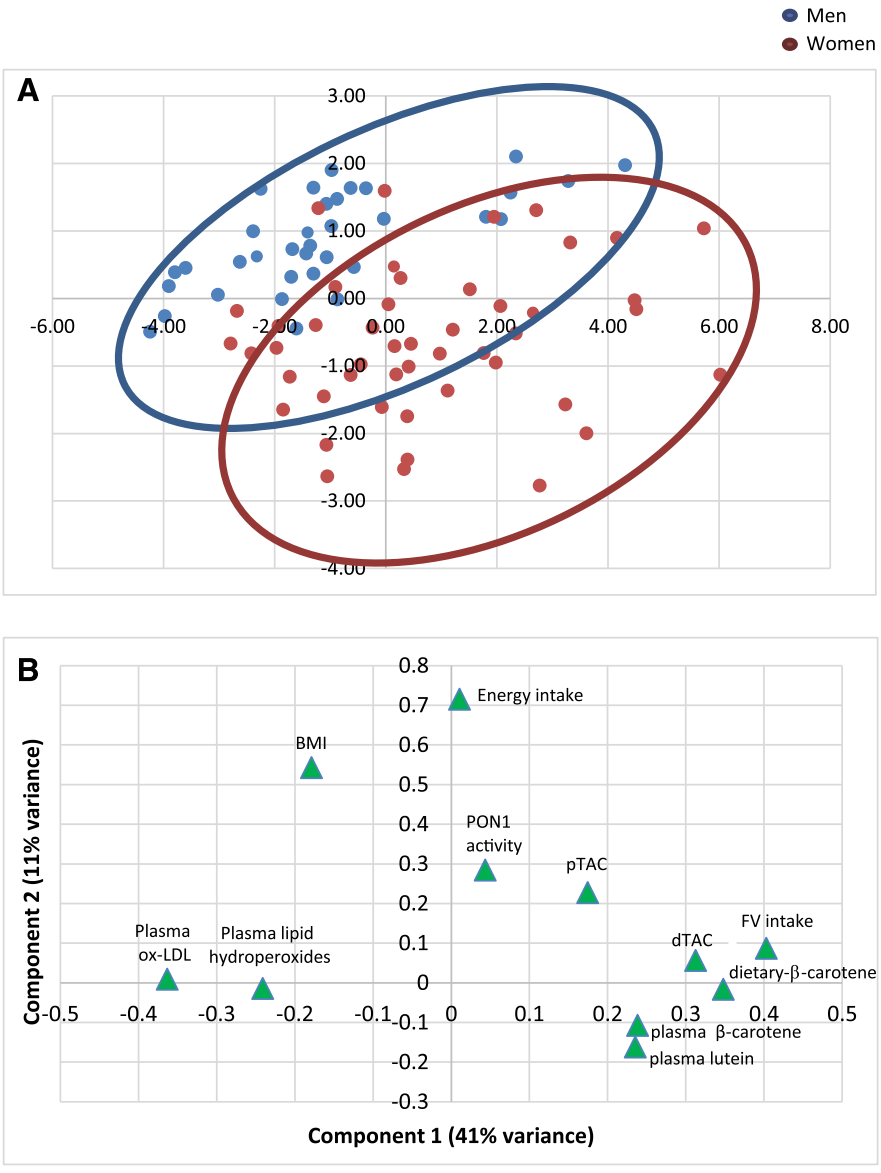

Fig. 5. Principal component analysis: (**A**) Scatter plot of scores and loadings and (**B**) considering the first two components. BMI, body mass index; dTAC, dietary total antioxidant capacity; FV, fruits and vegetables; ox-LDL, oxidized low-density lipoprotein; PON1, paraoxonase-1; pTAC, plasma total antioxidant capacity.

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