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Dietary modulation of oxidative stress in chronic obstructive pulmonary disease patients

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Abstract

A total of 267 clinically stable chronic obstructive pulmonary disease (COPD) patients provided complete data about diet and oxidative stress markers in order to assess the relationship between antioxidant rich food groups and nutrients, and serum markers of oxidative stress in COPD. Dietary data of the last 2 years was assessed using a validated food frequency questionnaire (122 items). Levels of carbonyls, nitrotyrosine, malondialdehyde and reduced glutathione (GSH) were measured in serum. Vitamin E intake was inversely associated with levels of carbonyls ($p = 0.05$) and olive oil was positively associated with GSH levels ($p = 0.01$), in active smokers. Intake of vegetables was related to a decrease of malondialdehyde levels ($p = 0.04$) in former smokers. No statistically significant associations were found between remaining dietary antioxidants and serum oxidative stress markers. These results provide new data for a potential dietary modulation of systemic oxidative stress in COPD patients, particularly in those that continue smoking.

Keywords: *Antioxidants, food intake, pulmonary disease, chronic obstructive, oxidative stress, public health, smoking*

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide, and it is expected to become the fourth leading cause of mortality by 2030 [1]. COPD is characterized by a complex chronic inflammatory condition usually associated with smoking-induced inflammation and oxidative stress [2]. Increased oxidative burden plays an important role in the pathogenesis of COPD, causing direct injury to the lung cells, increase of proteolytic activity due to inactivation of anti-proteases, mucus hypersecretion, and activation of transcription factors such as NF-κB, leading to cytokine release and neutrophil recruitment, further increasing inflammation and oxidative stress [3,4]. Moreover, free radical stress appears to extend beyond the lung in COPD patients, contributing to systemic manifestations of the disease [4]. Given the importance of oxidative stress in COPD, it has been hypothesized that targeting oxidative stress with antioxidants or boosting endogenous levels of antioxidants is likely to have beneficial effects in COPD [5]. However,

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although antioxidant rich foods, such as fruits and vegetables, have been associated with better lung function or symptoms in COPD patients [6–9], to our knowledge no studies have evaluated the potential impact of dietary habits on oxidative stress markers in COPD patients.

The present study aims to assess the association between antioxidant components of patients' diet and several oxidative stress markers, according to smoking status, in the frame of the 'Phenotype and Course of COPD Project (PAC-COPD)' [10]. This information could help to partially understand the underlying mechanisms of the association between foods and nutrients and COPD.

Subjects and methods

Study population

This study is a cross-sectional analysis in the 'Phenotype and Course of COPD Project (PAC-COPD)'. Briefly, the PAC-COPD study includes COPD patients recruited during their first hospital admission at nine university hospitals in Spain between January 2004 and March 2006, with a confirmed diagnosis of COPD (post-bronchodilator forced expiratory volume in the first second to forced vital capacity ratio (FEV_1/FVC) $\leq$ 0.70) [11] in a clinically stable condition, at least 3 months after discharge. Detailed information on PAC-COPD recruitment, methods and results is available elsewhere [12]. The protocol was approved by the Ethics Committees of all the participating hospitals and written informed consent was obtained from all the COPD patients.

From the 342 patients included in the PAC-COPD cohort, a total of 267 had available information on diet and oxidative stress markers. No differences regarding socio-demographic characteristics, comorbidities, dyspnea or lung function parameters were found between PAC-COPD patients who provided dietary information and those who did not, as previously published [13]. All epidemiological and clinical measures were performed at clinical stability at least 3 months after the recruitment, with the exception of dietary assessment, that was performed 9–12 months after the recruitment.

Dietary assessment

A previously validated 122-item food frequency questionnaire (FFQ) [14] was used asking for dietary habits in the last 2 years, thus including the time period when blood measurements were done. The questionnaire was administered by two trained interviewers that ensured no missing data in any item. The questionnaire included specific Spanish foods and gathered information on seasonality of food consumption. Reported information was converted into a daily intake frequency of each food, which was in turn converted into the daily intake in grams per day for each food. A Spanish food composition table of the Centre for Superior Studies in Nutrition and Dietetics (CESNID) was used to estimate nutrient intake from each food in the questionnaire [15]. Antioxidant dietary intake was assessed through the following selected foods and nutrients: fruits, vegetables, olive oil, beta-carotene, vitamin C and vitamin E. More details about the creation and validation of the FFQ have been previously published [14]. Additionally, our group tested the reproducibility of the FFQ when telephonically administered. Briefly, moderate-to-high correlations were found between the first and second questionnaire administration and no statistically significant differences in means of intakes of most food groups, macro- and micronutrients were found [13].

Clinical and functional assessment

Information regarding socio-demographic characteristics, pharmacological treatment, respiratory symptoms and lifestyle was obtained using a standardized epidemiological questionnaire. Nutritional status was assessed through body mass index (BMI) and fat free mass index (FFMI) (measured using bioimpedance). Additionally, post-bronchodilator spirometry (FEV_1, FVC and FEV_1/FVC ratio) and arterial oxygen and carbon dioxide partial pressures (PaO_2, $PaCO_2$) were also measured. The Charlson index of comorbidity [16] was obtained by an expert pulmonologist from medical records and personal anamnesis and exploration. Detailed information on the methods is described elsewhere [10,12].

Markers of oxidative stress

Blood samples were obtained at clinical stability and immediately centrifuged at 2000–3000 rpm for 10 min. Serum was extracted and stored in cryotubes at −80°C. Serum oxidative stress markers (carbonyls, nitrotyrosine, malondialdehyde and reduced glutathione) were determined from all the participating COPD patients from the different centres in the same laboratory at IMIM-Hospital del Mar (Barcelona, Spain).

Protein carbonyls were measured using an enzyme immuno-assay kit (Northwest Life Sciences Specialties, LLC, Vancouver, WA) based on an enzyme-linked immunosorbent assay (ELISA). Absorbances were read at 450 nm using a 655 nm filter as a reference. Intra-assay and inter-assay coefficients of variation for blood protein carbonyl formation ranged from 4.5–5.0% in both cases. The minimum detectable concentration of protein carbonyls in plasma was set to be 0.1 nmol/mg. Malondialdehyde (MDA) levels were determined using the MDA Assay (Northwest

Life Sciences Specialties), based on the reaction of MDA with thiobarbituric acid (TBA), which forms an MDA-TBA adduct that absorbs strongly at 532 nm. Spectra from 400–700 nm were recorded in a spectrophotometer and third derivative analyses were performed in order to quantify sample MDA levels in the assay. Intra-assay and inter-assay coefficients of variation for blood MDA quantification ranged from 5.0–6.0% in both cases. The minimum detectable concentration of MDA in plasma was set to be 0.08 μM (nmol/ml). Nitrotyrosine levels were measured using the Nitrotyrosine ELISA kit (Northwest Life Sciences Specialties). The absorbance in each sample was measured at 450 nm. Intra-assay and inter-assay coefficients of variation for blood nitrotyrosine quantification ranged from 2.5–3.0% in both cases. The minimum detectable concentration of nitrotyrosine in plasma was set to be 2 nM (nmol/l). Reduced glutathione (GSH) (one of the strongest endogenous antioxidants) was measured using the Glutathione Assay (Northwest Life Sciences Specialties). The absorbance in each sample was read in a microplate reader with kinetics capability at 405 nm. Intra-assay and inter-assay coefficients of variation for blood GSH quantification ranged from 4.5–5.0% in both cases. The minimum detectable concentration of GSH in plasma was set to be 0.1 μM (nmol/ml). All measures were done following the precise manufacturer's instructions and a standard curve was always generated with each assay run.

Statistical analysis

Socio-demographic and clinical characteristics, intakes of main antioxidant-rich foods and nutrients, treatment with antioxidant mucolytics and oxidative stress markers were described by mean (SD), median (P25–P75) or number (%), as appropriate according to each variable distribution. Comparisons between antioxidant intake levels and oxidative stress markers levels were assessed using several approaches. First, both groups of variables were used as continuous and their relationship assessed using bivariate linear regression. Secondly, we compared, using Students' *t* test, the level of oxidative stress markers between low and high food and nutrient intake. Low and high intakes were defined as being below or above the median consumption of the food or nutrient: fruits 280 g/day, vegetables 242 g/day, olive oil 15 g/day, beta-carotene 2578 μg/day, vitamin C 148 mg/day and vitamin E 10 mg/day. This comparison was then represented graphically. To test for the possibility of differences only in extreme intakes, we also compared the level of oxidative stress markers between first quartile and fourth quartile of foods and nutrients intake, using Students' *t*-test.

Prior to multivariate linear regression both exposure and outcome variables were transformed using Box-Cox [17] when necessary to fulfill the assumption of normality. Multivariate linear regression models were used to estimate the association between antioxidant-rich foods and nutrients and oxidative stress markers, after adjusting for potential confounders (age, gender, BMI, FFMI, antioxidant mucolytic treatment, statins treatment, smoking status, COPD severity and self-reported traffic density in the patients' street address). The potential confounding factors were included in the final models only if they were related to both the exposure and the outcome. Possible effect modification by smoking status was assessed through stratification of the final models, given the key role of cigarette smoke as a source of oxidants. Goodness of fit of all linear regression models was assessed through graphical study of the residuals [18]. As sensitivity analyses, all analyses were repeated: (i) excluding women (7% of total subjects), (ii) excluding patients with asthma (29% of total subjects) (self-reported asthma or positive bronchodilator test—defined as an increase of post-bronchodilator $FEV_1 > 200$ ml or 12% of pre-bronchodilator FEV_1 value - post-bronchodilator) [2] and (iii) including all anti-oxidant foods or nutrients in the multivariate models. Data analysis was conducted using Stata 8.2 (StataCorp, College Station, TX).

Results

Table I shows the main characteristics of the patients. Ninety-three per cent of participants were males with a mean age of 68 years. Most subjects had moderate-to-severe COPD (distribution in COPD severity stages: 5% mild, 52% moderate, 37% severe and 6% very severe). Mean consumption of antioxidant foods and nutrients was moderate-to-high, with most mean intakes above the Spanish recommendations [13] and higher than those in other developed countries such as the US [19]. As expected, current smokers presented significantly higher values of carbonyls (0.22 nmol/mg) and nitrotyrosine (7.61 nmol/l) than former smokers (0.17 nmol/mg and 6.18 nmol/l, respectively), despite no differences in FEV_1 or FEV_1/FVC being observed.

A 0.004 nmol/mg reduction of carbonyl levels for each 1 mg increase of vitamin E intake ($p = 0.05$) was found in bivariate linear regression analysis of antioxidant intake and oxidative stress markers. No other statistically significant associations were found. Figure 1 shows graphically that serum mean concentrations of carbonyls, nitrotyrosine, malondialdehyde and reduced glutathione (GSH) were indeed very similar between subjects with low and high intake of fruits, vegetables, olive oil, beta-carotene, vitamin C and vitamin E. No differences

Table I. Description of sociodemographic and clinical data, antioxidant food and nutrient intake and levels of serum oxidative stress markers in 267 COPD patients, according to smoking status.

	All (n = 267*) M (SD)	Former smokers (n = 178) M (SD)	Current smokers (n = 80) M (SD)	p-value**
Male [n(%)]	249 (93)	173 (97)	68 (85)	< 0.001
Age (years)	68 (8)	71 (7)	62 (9)	< 0.001
BMI (kg/m^2) [n(%)]				
< 20	6 (2)	2 (1)	3 (4)	0.162
≥ 20 & < 25	50 (19)	30 (17)	18 (23)	
≥ 25 & < 30	113 (42)	82 (46)	27 (34)	
≥ 30	98 (37)	64 (36)	32 (40)	
FFMI (kg/m^2)	19.8 (3.1)	20 (2.9)	19.4 (3.3)	0.088
Primary or higher education [n(%)]	153 (59)	102 (57)	49 (39)	0.414
Active worker [n(%)]	44 (16)	14 (8)	30 (37)	< 0.001
Low socioeconomic status*** [n(%)]	203 (82)	140 (84)	57 (78)	0.285
Age when started smoking (years)	16 (4)	16 (4)	16 (5)	0.799
Age when quit smoking (years) (n=178)	59 (11)	59 (11)	—	—
Smoking duration (years)	45 (12)	44 (12)	46 (10)	0.472
Smoking intensity (packs/day)	1.3 (0.9)	1.5 (0.9)	0.7 (0.7)	< 0.001
Pack-years	68 (40)	69 (41)	69 (36)	0.760
> 1 Comorbidities (Charlson index) [n(%)]	146 (55)	106 (60)	34 (43)	0.011
Dyspnea score (MMRC) [n(%)]				
0 (none)	21 (8)	12 (7)	8 (10)	0.403
1 (slight)	14 (5)	10 (6)	4 (5)	
2 (moderate)	116 (44)	76 (43)	37 (46)	
3 (moderately severe)	63 (24)	40 (22)	20 (25)	
4 (severe)	14 (5)	13 (7)	1 (1)	
5 (very severe)	38 (14)	27 (15)	10 (13)	
Post-bronchodilator FEV_1 (% predicted)	53 (16)	53 (16)	52 (15)	0.802
Post-bronchodilator FEV_1/FVC	54 (12)	55 (12)	54 (12)	0.487
PaO_2 (mmHg)	75 (11)	75.4 (10.9)	73.6 (10.7)	0.268
$PaCO_2$ (mmHg)	42 (5)	41 (5.1)	43.1 (4.5)	0.005
Antioxidant mucolytic treatment [n(%)]	6 (2)	5 (3)	1 (1)	0.442
Fruits (g/d)	270 (153)	282 (155)	249 (144)	0.098
Vegetables (g/d)	296 (128)	295 (127)	303 (127)	0.468
Olive oil (g/d)	17 (12)	17 (12)	18 (13)	0.464
Beta-carotene (μg/d)	2514 (1663)	2511 (1681)	2541 (1692)	0.871
Vitamin C (mg/d)	160 (74)	162 (74)	157 (76)	0.684
Vitamin E (mg/d)	11 (4)	10 (3)	12 (5)	0.061
Carbonyls (nmol/mg)	0.19 (0.09)	0.17 (0.07)	0.22 (0.12)	0.009
Nitrotyrosine (nmol/l)	6.6 (4.4)	6.18 (4.36)	7.61 (4.47)	0.003
Malondialdehyde (nmol/ml)	9.3 (5.8)	8.78 (5.1)	10.43 (6.9)	0.111
Reduced glutathione (nmol/ml)	3.2 (0.64)	3.19 (0.66)	3.11 (0.61)	0.170

*Former and current smokers do not add to the total because of two never smokers and seven subjects with missing data about current smoking status when serum samples were obtained.
**p-value for comparison of former vs current smokers.
***Skilled or unskilled manual workers classified as low socioeconomic status.

were found between the first and fourth quartile of food group and nutrient intake.

Multivariate linear regression models for the association between transformed dietary antioxidants and oxidative stress markers were built, adjusting for age, gender, BMI, FFMI, antioxidant mucolytic treatment, statins treatment, smoking status, COPD severity and self-reported traffic density in the patients' street address. However, since none of these variables exhibited a significant relationship with the outcome nor altered the relationship between dietary antioxidants and the outcomes, they were removed from the final models, so the presented final models were all bivariate. Intake of vitamin E was associated with a decrease in serum carbonyl levels in current smoker COPD patients (p=0.05) (Table II). Similarly, olive oil intake was associated with an increase in GSH serum levels in current smoker COPD patients (p=0.01). Intake of vegetables was associated with a decrease in malondialdehyde serum levels only in former smokers. No other statistically significant associations were found. Sensitivity analyses excluding women and excluding asthmatics yielded very similar results. The inclusion of all anti-oxidant foods or nutrients in the multivariate models yielded very similar estimates for all the described associations, although the association between vitamin E and carbonyls lost statistical significance (p-value = 0.14 instead of 0.05).

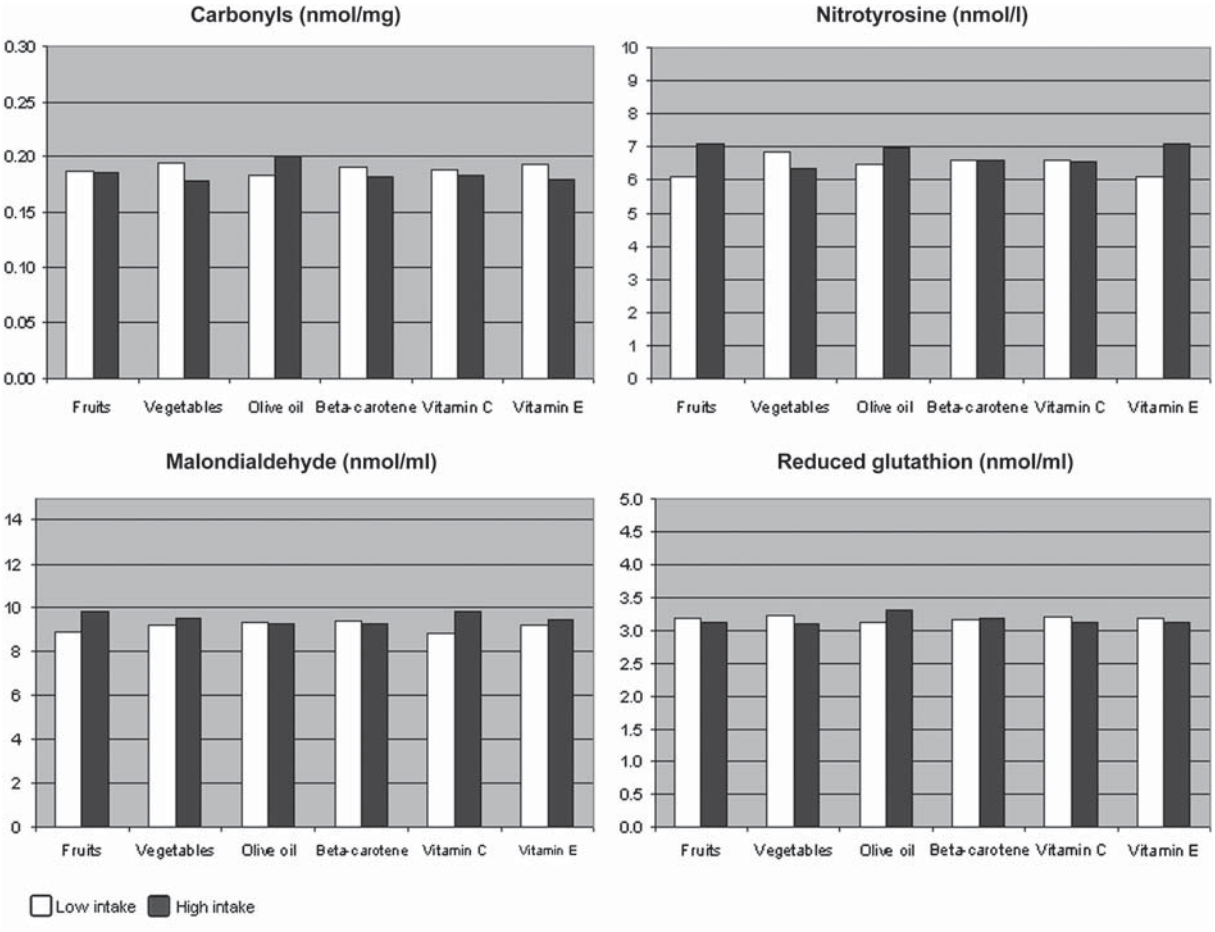

Figure 1. Mean serum oxidative stress markers levels according to low or high antioxidant food or nutrient intake in 267 COPD patients.

Discussion

This is the first study assessing the association between daily dietary antioxidant food and nutrient intake and levels of serum oxidative stress markers in COPD patients. Vitamin E and olive oil were, respectively, associated with reduced carbonyl levels and increased GSH levels in current smoker COPD patients. Intake of vegetables was associated with a slight reduction in malondialdehyde levels in former smokers. No other statistically significant associations were found.

This study has several limitations that could make the interpretation of the data speculative to some extent. First of all, dietary intake is solely estimated based on interviews. Therefore, there could be some degree of misclassification, inherent to the use of food frequency questionnaires (FFQ), and this could be partially responsible for our null associations with some of the dietary antioxidants. However, FFQs are the standard tool to assess food and nutrient intake in epidemiological studies, since other potentially more valid instruments are not feasible in this context. Secondly, the cross-sectional study design makes a causal link impossible to be established, as such studies give no information on the temporal ordering of possibly causal events. However, we consider unlikely that dietary habits change as a result of oxidative stress levels, being more likely that oxidative stress levels are modulated according to diet. Finally, the fact that our findings were mostly restricted to active smokers is biologically consistent with the increased demand for antioxidants due to the increase of reactive oxygen species coming from tobacco smoke inhalation [20]. In contrast, given the current knowledge, the authors could not find an explanation for the fact that the association between vegetables and MDA was restricted to former smokers.

An association was found between dietary vitamin E intake and carbonyl serum levels in COPD patients that are still smokers. This result is in agreement with a previous small clinical trial ($n=35$) that showed a reduction in oxidative-induced DNA breaks in the vitamin E (400 mg/day) and C (250 mg/day) supplemented group vs the placebo group, after a 12 week supplementation period in COPD patients [21]. However, another clinical trial ($n=24$) found no significant differences in biochemical parameters of oxidant–antioxidant status in plasma, leukocytes and red cells between vitamin E and placebo groups, after an 8-week vitamin E supplementation (800 mg/day) in COPD patients [22]. A trial in the general

Table II. Associations* between vegetables, fruits, olive oil, beta-carotene, vitamin C and vitamin E intakes and serum reduced glutathione (GSH), carbonyls, nitrotyrosine and malondialdehyde, using linear regression models according to smoking status, after appropriate transformations** of variables using Box-Cox.

	All subjects (n = 267)		Former smokers (n = 178)		Current smokers (n = 80)	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
Carbonyls (nmol/mg$^{0.46}$)						
Fruits (g/d)	–0.00006	0.12	–0.00002	0.61	–0.0001	0.32
Vegetables (g/d)	–0.00006	0.22	–0.00008	0.10	–0.00008	0.48
Olive oil (g/d$^{0.34}$)	0.007	0.40	–0.001	0.90	0.02	0.28
Beta-carotene (μg/d$^{0.45}$)	–0.00022	0.72	–0.00027	0.67	–0.00019	0.89
Vitamin C (mg/d)	–0.0001	0.22	< –0.00001	0.96	–0.00028	0.12
Vitamin E (mg/d$^{0.61}$)	–0.01	0.14	–0.006	0.43	–0.025	0.05
Nitrotyrosine (nmol/l$^{-0.02}$)						
Fruits (g/d)	0.00001	0.32	0.00001	0.32	< 0.00001	0.80
Vegetables (g/d)	< –0.00001	0.57	–0.00001	0.46	0.00001	0.61
Olive oil (g/d$^{0.34}$)	–0.0003	0.77	0.0005	0.72	–0.0018	0.29
Beta-carotene (μg/d$^{0.45}$)	–0.00003	0.70	–0.00001	0.89	0.00001	0.93
Vitamin C (mg/d)	< 0.00001	0.68	0.00001	0.69	0.00001	0.70
Vitamin E (mg/d$^{0.61}$)	–0.0004	0.67	0.0006	0.57	–0.0006	0.61
Malondialdehyde (nmol/ml$^{-0.08}$)						
Fruits (g/d)	–0.00001	0.74	–0.00001	0.66	–0.00001	0.85
Vegetables (g/d)	–0.00002	0.22	–0.00005	0.04	–0.00001	0.85
Olive oil (g/d$^{0.34}$)	0.0011	0.76	0.0002	0.95	0.0031	0.63
Beta-carotene (μg/d$^{0.45}$)	0.00011	0.67	0.0001	0.75	–0.00006	0.90
Vitamin C (mg/d)	< –0.00001	0.99	–0.00003	0.48	0.00002	0.70
Vitamin E (mg/d$^{0.61}$)	–0.001	0.66	0.001	0.88	–0.005	0.29
GSH (nmol/ml)						
Fruits (g/d)	–0.00017	0.52	–0.00035	0.28	0.00005	0.92
Vegetables (g/d)	–0.00001	0.97	–0.0001	0.81	0.00016	0.78
Olive oil (g/d$^{0.34}$)	0.089	0.10	0.033	0.63	0.231	0.01
Beta-carotene (μg/d$^{0.45}$)	–0.0003	0.94	–0.0001	0.98	–0.00272	0.70
Vitamin C (mg/d)	–0.00003	0.96	–0.0004	0.55	0.00033	0.72
Vitamin E (mg/d$^{0.61}$)	0.021	0.62	–0.014	0.81	0.076	0.23

*Reported associations are all bivariate. All the potential confounding factors were not independently related to the outcome or to the exposure, nor modified the estimates for the exposure.

**According to Box-Cox methodology [17], some of the variables were transformed (as detailed in the table) to approach a normal distribution. Therefore, coefficients where the exposure (the food/nutrient) or the outcome (the oxidative stress marker) were transformed cannot be interpreted as the increase in 1 unit of the outcome for an increase in 1 unit in the exposure.

population (n=39) administering daily an antioxidant-supplemented drink (including 600 mg of vitamin C, 400mg of vitamin E and 30 mg of beta-carotene) to non-COPD smoker subjects during a 4-week period [23] showed a reduction both in lipid peroxidation (assessed by breath pentane) and susceptibility of LDL to oxidation. Finally, clinical trials assessing the effect of non-dietary antioxidant supplements such as nebulized N-acetylcysteine (NAC) showed different results depending on the length of the supplementation period. For instance, Kasielski and Nowack [24] showed a 2-fold reduction in exhaled air H_2O_2 in subjects receiving nebulized NAC during a 9-month treatment compared with subjects receiving placebo, while a single dose nebulized NAC administration study showed transient increases in exhaled H_2O_2, suggesting pro-oxidant effects of nebulized NAC [25]. Our study assessing the role of the last 2-years self-reported dietary antioxidants intake in a non-intervened sample of COPD patients concurs with evidence obtained from the above-mentioned clinical trials with long-term antioxidants supplementation.

Laboratory research also supports the biological plausibility of this finding. Vitamin E is known to be one of the most potent lipophilic antioxidants. Reactive oxygen species react with polyunsaturated lipids forming aldehydes such as malondialdehyde and proteins forming carbonyls and both are defined as oxidative stress markers [26]. Garibaldi et al. [27] evaluated the relationship between retinol and tocopherols (vitamin E) levels in plasma and protein carbonyls, concluding that both could at least partially prevent oxidative damage of circulating proteins. The lack of association between vitamin E and malondialdehyde in our study could be explained by the fact that oxidative degradation of the polyunsaturated fatty acids in LDL only occurs when vitamin E is largely depleted [28]. Altogether, both clinical and laboratory research data are consistent with our finding of an association between dietary vitamin E and carbonyls (as markers of oxidative stress).

A second relevant result is the association, also found in COPD patients that continue to smoke, between olive oil consumption and increased GSH serum levels, an association not previously reported in COPD patients. This result is supported by current knowledge about olive oil components. Briefly, olive oil is an important source of phenolic antioxidants, vitamin E and other potentially relevant antioxidant substances [29], thus making plausible that its consumption could increase endogenous antioxidant levels. Moreover, experimental studies have suggested that even low concentrations of flavonoids, which are present in olive oil, could stimulate the transcription of a critical gene for GSH synthesis [30].

We found an inverse association between vegetable intake and malondialdehyde serum levels in former smokers, which is plausible given that vegetables are important sources of antioxidant vitamins [15]. The fact that this result was found with a food group rather than with specific nutrients could be attributed to the phenomenon known as food synergy, that is the additive or more than additive influence of foods and food constituents on health [31]. In our case, an association is found with a food group rather than with the individual foods that constitute the specific food group.

In contrast to what we expected according to previous literature [6–9], we found no associations for other antioxidant-rich foods or nutrients. A potential explanation for this partial lack of findings could be the relatively high intake of antioxidants in our subjects. As an example, the mean consumption of fruits and vegetables in our sample (566 g/day) was close to the 4th quartile ($>$ 588 g/day) in the large multicentric European Prospective Investigation into Cancer and Nutrition (EPIC study) [32]. Therefore, in our study, few subjects were below the threshold level where bigger and more easily detectable effects may occur. Another explanation is the difficulty in relating 2-years average dietary habits and precise levels of oxidative stress markers (at a given point of time), when both daily variability in any-nutrient intake and differences in mean lifetime of the markers really exist. In this context, the finding of some associations is (i) clinically relevant even when a higher number of associations were tested and (ii) probably more important than would have been in a short-term effects study.

There is compelling evidence that a healthy diet is a beneficial factor in improving and/or preventing multiple chronic diseases, including chronic lung diseases [9]. However, most influential COPD guidelines, such as that produced by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) or the consensus between the American Thoracic Society and the European Respiratory Society [2,11] do not include any specific dietary recommendation to COPD patients, beyond the increase of caloric intake for the prevention of weight loss. This study adds new evidence to what other previous studies regarding antioxidant rich foods and COPD [6–9] had pointed out. Therefore, the authors' opinion is that a healthy antioxidant-rich diet, including vitamin E sources such as vegetable oils, nuts and seeds, should be considered in COPD international guidelines, as well as in recommendations to COPD patients in the clinical setting.

In conclusion, this is the first comprehensive study so far assessing the association between antioxidant food and nutrient intake and levels of serum oxidative stress markers in COPD patients. Vitamin E and olive oil are associated with reduced oxidative stress levels (lower carbonyls and higher GSH, respectively) in current smoker COPD patients. These results, if confirmed, provide new evidence on dietary modulation of systemic oxidative stress in COPD patients that continue to smoke and so on the potential underlying mechanisms behind the association of diet with COPD. Furthermore, our results suggest that, beyond the importance of reinforcing anti-smoking initiatives at all levels of the healthcare process, the recommendation of increasing the dietary antioxidant intake in COPD patients may be especially important in active smokers.

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References

[1] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006;3: 2011–2030.

[2] Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2008. Available online at: http://www.goldcopd.com, accessed 15 July 2009.

[3] MacNee W. Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2005;2:50–60.

[4] Langen RC, Korn SH, Wouters EF. ROS in the local and systemic pathogenesis of COPD. Free Radic Biol Med 2003; 35:226–235.

[5] Rahman I. Antioxidant therapeutic advances in COPD. Ther Adv Respir Dis 2008;2:351–374.

[6] Smit HA, Grievink L, Tabak C. Dietary influences on chronic obstructive lung disease and asthma: a review of the epidemiological evidence. Proc Nutr Soc 1999;58:309–319.

[7] Smit HA. Chronic obstructive pulmonary disease, asthma and protective effects of food intake: from hypothesis to evidence? Respir Res 2001;2:261–264.

[8] Ferreira I, Brooks D, Lacasse Y, Goldstein R. Nutritional intervention in COPD: a systematic overview. Chest 2001; 119:353–363.

[9] Romieu I, Trenga C. Diet and obstructive lung diseases. Epidemiol Rev 2001;23:268–287.

[10] Garcia-Aymerich J, Gómez FP, Antó JM, on behalf of the PAC-COPD Research Study Group. [Phenotypic characterization and course of chronic obstructive pulmonary disease (COPD) in the PAC-COPD Study: design and methods]. Arch Bronconeumol 2009; 45:4–11.

[11] Celli BR, MacNee W, ATS/ERS Task Force. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J 2004;23: 932–946.

[12] Balcells E, Antó JM, Gea J, Gómez FP, Rodríguez E, Marin A, Ferrer A, de Batlle J, Farrero E, Benet M, Orozco-Levi M, Ferrer J, Agustí AG, Gáldiz JB, Belda J, Garcia-Aymerich J; PAC-COPD Study Group. Characteristics of patients admitted for the first time for COPD exacerbation. Respir Med 2009;103:1293–1302.

[13] de Batlle J, Romieu I, Antó JM, Mendez M, Rodríguez E, Balcells E, Ferrer A, Gea J, Rodriguez-Roisin R, Garcia-Aymerich J; The PAC-COPD Study Group. Dietary habits of firstly admitted Spanish COPD patients. Respir Med 2009; 103:1904–1910.

[14] Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC, Salvini S, Willett WC. Development and validation of a food frequency questionnaire in Spain. Int J Epidemiol 1993;22:512–519.

[15] [CESNID Food composition tables]. Centre d'Ensenyament Superior de Nutrició i Dietètica (CESNID). Madrid: McGraw-Hill Interamericana; 2003.

[16] Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol 1994;47: 1245–1251.

[17] Box GEP, Cox DR. An analysis of transformations. J R Stat Soc Series B Stat Methodol 1964;26:211–234.

[18] Armitage P, Berry G. Multiple measurements. In: Statistical Methods in Medical Research, Blackwell Scientific Publications, Oxford, U.K., 1987;308–312.

[19] Centers for Disease Control and Prevention (CDC). Prevalence of fruit and vegetable consumption and physical activity by race/ethnicity–United States, 2005. MMWR Morb Mortal Wkly Rep 2007;56:301–304.

[20] Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. Am J Epidemiol 1988;127:283–296.

[21] Wu TC, Huang YC, Hsu SY, Wang YC, Yeh SL. Vitamin E and vitamin C supplementation in patients with chronic obstructive pulmonary disease. Int J Vitam Nutr Res 2007; 77:272–279.

[22] Nadeem A, Raj HG, Chhabra SK. Effect of vitamin E supplementation with standard treatment on oxidant-antioxidant status in chronic obstructive pulmonary disease. Indian J Med Res 2008;128:705–711.

[23] Steinberg FM, Chait A. Antioxidant vitamin supplementation and lipid peroxidation in smokers. Am J Clin Nutr 1998; 68:319–327.

[24] Kasielski M, Nowak D. Long-term administration of N-acetylcysteine decreases hydrogen peroxide exhalation in subjects with chronic obstructive pulmonary disease. Respir Med 2001;95:448–456.

[25] Rysz J, Stolarek RA, Luczynski R, Sarniak A, Wlodarczyk A, Kasielski M, Nowak D. Increased hydrogen peroxide concentration in the exhaled breath condensate of stable COPD patients after nebulized N-acetylcysteine. Pulm Pharmacol Ther 2007;20:281–289.

[26] Mayne ST. Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 2003;133:933s–940s.

[27] Garibaldi S, Aragno I, Odetti P, Marinari UM. Relationships between protein carbonyls, retinol and tocopherols level in human plasma. Biochem Mol Biol Int 1994;34: 729–736.

[28] Esterbauer H, Jürgens G, Quehenberger O, Koller E. Autoxidation of human low density lipoprotein: loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes. J Lipid Res 1987;28:495–509.

[29] Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci F, Selvaggini R, Montedoro GF. Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure. Inflammopharmacology 2009;17:76–84.

[30] Moskaug JØ, Carlsen H, Myhrstad MC, Blomhoff R. Polyphenols and glutathione synthesis regulation. Am J Clin Nutr 2005;81:277s–283s.

[31] Jacobs DR, Jr, Steffen LM. Nutrients, foods, and dietary patterns as exposures in research: a framework for food synergy. Am J Clin Nutr 2003;78:508s–513s.

[32] Vrieling A, Verhage BA, van Duijnhoven FJ, Jenab M, Overvad K, Tjønneland A, Olsen A, Clavel-Chapelon F, Boutron-Ruault MC, Kaaks R, Rohrmann S, Boeing H, Nöthlings U, Trichopoulou A, John T, Dimosthenes Z, Palli D, Sieri S, Mattiello A, Tumino R, Vineis P, van Gils CH, Peeters PH, Engeset D, Lund E, Rodríguez Suárez L, Jakszyn P, Larrañaga N, Sánchez MJ, Chirlaque MD, Ardanaz E, Manjer J, Lindkvist B, Hallmans G, Ye W, Bingham S, Khaw KT, Roddam A, Key T, Boffetta P, Duell EJ, Michaud DS, Riboli E, Bueno-de-Mesquita HB. Fruit and vegetable consumption and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer 2009;124:1926–1934.

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