Original Article

Comparison of plasma and intake levels of antioxidant nutrients in patients with chronic obstructive pulmonary disease and healthy people in Taiwan: a case-control study

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The imbalance of oxidant/antioxidant plays an important role in the development of chronic obstructive pulmonary disease (COPD). There is increasing evidence that individuals with high antioxidative nutrient levels in the diet or in blood tend to maintain better lung function. This study was conducted to determine whether COPD patients in Taiwan have lower plasma concentrations of antioxidative nutrients than do healthy people, and whether the dietary habits of COPD patients in Taiwan affect their intake of vitamin C and carotenoids. Thirty-four COPD patients and 43 healthy persons (with normal lung function) aged 50 years or older were recruited. Fasting venous blood was collected to measure concentrations of vitamins A, C, and E and carotenoids. Endogenous and H_2O_2-induced additional DNA damage (markers of oxidative stress) in white blood cells were assayed. Dietary intakes of vitamin C and carotenoids were assessed by a food-frequency questionnaire. Compare to the healthy controls, COPD patients had significantly lower plasma concentrations of vitamins A, C, and E; α- and β-carotene; and total carotenoids but significantly higher endogenous and H_2O_2-induced white blood cell DNA damage. Intakes of vitamin C and several carotenoids were lower in the COPD group, and COPD patients consumed significantly fewer vegetables and fruits than did the healthy controls. In conclusion, COPD patients in Taiwan have lower levels of antioxidative nutrients in their plasma and diet than do healthy people. Intakes of vitamin C and carotenoids are correlated with dietary habits.

Key Words: COPD, antioxidants, Taiwan, fruit, vegetable

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), a chronic inflammatory disease of the lung that is characterized by progressive and irreversible airflow limitation, is a major health problem worldwide.[1] Exposure to oxidants, a decrease in antioxidant capacity, and gene polymorphism may all contribute to the development of COPD through various mechanisms, such as inactivation of antiproteases, alveolar epithelial injury, and increased sequestration of neutrophils in the pulmonary microvasculature.[1-3] Accumulating evidence suggests that the levels of antioxidative nutrients, such as vitamins A, C, and E and carotenoids, in the diet or in blood are positively associated with lung function.[4-7] For example, Schünemann *et al.*[6] found significant associations of dietary vitamins C, E and carotenoids with lung function as assessed by forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) as the percentage of the predicted value (FEV_1% and FVC%, respectively) in 1616 men and women in New York state. Recently, a multivariate analysis of data from the US National Health and Nutrition Examination Survey revealed that higher serum levels of vitamins A, C, and E and β-cryptoxanthin are independently associated with higher values of FEV_1.[4] However, studies of antioxidative nutrient status in plasma in COPD patients in other countries are limited.

The major food sources of vitamin C and carotenoids are vegetables and fruits. Vitamin A may also be obtained from consumption of vegetables and fruit that are rich in carotenoids. People in Chinese societies believe that food can be categorized into three groups: cooling, neutral, and heating. The consumption of cooling foods is thought to exacerbate the symptoms of COPD, such as cough, phlegm, and even breathlessness; in contrast, heating foods may exacerbate the symptoms of inflammation.[8,9] COPD patients in Taiwan may therefore decrease their consumption of cooling or heating foods to avoid suffering

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from disturbing conditions. However, because many kinds of fruits and vegetables are categorized as cooling foods, whether this dietary habit affects the consumption of vegetables and fruit or the intake of some antioxidants in COPD patients in Taiwan is unclear.

The aims of the current study were therefore to investigate (1) the differences in oxidative stress and in the plasma levels of vitamins A, C, and E and carotenoids between COPD patients and healthy controls in Taiwan, and (2) the association between intakes of vitamin C and carotenoids and dietary habits.

MATERIALS AND METHODS

Subjects

This case-control study was conducted from 2005 to 2006 for 1.5 years. Thirty-four physician-diagnosed COPD patients with FEV_1/FVC <70% and without diabetes mellitus, chronic liver cirrhosis, renal failure, or other chronic inflammatory diseases were recruited from the chest clinic of Chung-Shan Medical University Hospital. Another 43 healthy controls without a history of COPD or the other chronic diseases mentioned above were recruited from the health examination center in the same hospital or from local elderly centers in the same city. Both COPD patients and healthy controls were 50 years or older. Informed consent was obtained from all subjects, and the study was approved by the Institutional Review Board at Chung Shan Medical University.

Questionnaire Interview

Information on demographics, smoking, drinking, and the consumption of energy, vegetables, and fruit was obtained by personal interview. A 24-hour diet recall was performed to estimate the intakes of total energy, carbohydrate, protein, and lipids. Habitual consumption of 68 vegetable or fruit items, including 27 cooling foods (12 vegetables and 15 fruits), 32 neutral foods (24 vegetables and 8 fruits), and 9 heating foods (5 vegetables and 4 fruits) (Appendix 1), was estimated by a semi-quantitative food-frequency questionnaire (FFQ). The expert validity of the questionnaire had been established by 5 nutritional professionals. Intakes of vitamin C and carotenoids were also estimated by the same FFQ, and the data base "USDA National Nutrient Database for Standard Reference (SR), release 20" was used to analyze the nutrient composition. Preformed vitamin A is found only in foods of animal origin, whereas provitamin A carotenoids are found in many plants.[10] Thus, in the current study the intakes of various carotenoids, including α- and β-carotenes, from vegetables and fruit rather than the intake of total vitamin A, as well as vitamin E, were estimated, because vegetables and fruit are not major sources of these two vitamins.[10]

Measurement of Antioxidative Nutrients in Blood

Fasting blood samples were collected in tubes containing heparin to determine concentrations of retinol, ascorbic acid, α-tocopherol, α- and β-carotenes, lutein, and lycopene by HPLC methods. The plasma ascorbic acid level was measured within 4 h after sampling by the methods described by Ching *et al*.[11] and Chevion *et al*.[12] Plasma α-tocopherol was determined by reversed-phase HPLC with detection at 295 nm.[13] The retinol and individual carotenoid levels in plasma were measured by using the HPLC method described by Talwar *et al*.[14] The intra- and inter-assay precisions (as percentages of the coefficient of variation) for the HPLC assays were all 10-15%. Total carotenoids were calculated as the sum of α-carotene, β-carotene, lutein, and lycopene in μmol/L.

The Comet Assay in White Blood Cells

Whole blood (100 μL) was added to 100 μL of cold RPMI 1640 medium containing 10% DMSO as described previously to cryopreserve the white blood cells (WBCs).[15] The mixture was immediately frozen at -80°C and was

Table 1. Characteristics of COPD patients and healthy controls†

Variable	COPD patients	Healthy controls	*p* value‡
Sex (male/female)	21/13	13/30	0.011
Age (year)	70.4 ± 9.6	63.9 ± 5.9	0.002
Height (cm)	157.5 ± 9.2	156.2 ± 9.8	0.599
Weight (kg)	56.9 ± 10.4	59.8 ± 9.2	0.244
BMI (kg/m^2)	22.8 ± 3.2	24.6 ± 2.9	0.029
FEV_1	54.9 ± 21.5	86.6 ± 14.1	0.003
FEV_1/FVC	57.1 ± 13.1	75.4 ± 6.5	0.005
Smoking status (%)			<0.001
Current smoker	3 (9%)	2 (5%)	
Former smoker	13 (41%)	0 (0%)	
Never smoker	16 (50%)	41 (95%)	
Plasma concentration of nutrients			
Vitamin A (μg/mL)	1.8 ± 2.1	2.7 ± 1.9	0.047
Vitamin C (μg/mL)	6.9 ± 0.8	9.4 ± 1.0	0.042
Vitamin E (μg/mL)	5.5 ± 1.6	8.2 ± 2.7	<0.001
α-Carotene (μg/mL)	0.07 ± 0.83	0.44 ± 0.76	0.05
β-Carotene (μg/mL)	0.35 ± 0.14	0.62 ± 0.58	0.006
Lutein (μg/mL)	0.05 ± 0.48	0.07 ± 0.04	0.107
Lycopene (μg/mL)	0.02 ± 0.01	0.04 ± 0.08	0.206
Total carotenoids (μg/mL)	0.49 ± 0.20	1.12 ± 0.98	<0.001

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity. †Values are mean ± SD. ‡The difference between groups in sex was compared by χ^2 test; the difference in smoking status was compared by Fisher's exact test; differences in the other characteristics were compared by Student's *t*-tests.

thawed in a water bath (37°C, 1-2 min) just before the comet assay. The comet assay was adapted from the method of Singh *et al.*[16] In this assay, 60 μL of the mixture of whole blood was mixed with 450 μL of low-melting-point agarose, and 140 μL of the mixture was applied to a frosted microscopic glass slide that was precoated with a layer of 1% normal-melting-point agarose (150 μL). After application of a third layer of 1% normal-melting-point agarose, 150 μL of H_2O_2 (100 mM) was added to the top of two slides for each sample for 5 min at 25 °C to determine the resistance of DNA in whole-blood WBCs against oxidative challenge.[17] Because whole-blood samples are more resistant to oxidative challenge than are isolated lymphocytes,[15] the concentration of H_2O_2 used here is higher than in some studies.[17] The control slides, which were used to measure endogenous DNA damage, were kept on ice. After washing with PBS, all slides were then immersed in cold-lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium lauryl sarcosinate, 1% Triton X-100) for 1 h at 4°C. The slides were then placed in an electrophoresis tank, allowing the DNA to unwind for 15 min in the alkaline solution (300 mM NaOH and 1 mM EDTA). Electrophoresis was then performed at 300 mA for 20 min in the same alkaline solution at room temperature. The slides were then neutralized with 0.4 M Tris-HCl buffer (pH 7.4) and stained with ethidium bromide. For each subject, two slides for endogenous and another two for H_2O_2-induced DNA damage were assayed, with 50 comets on each slide. The images were analyzed by computer with Comet Assay III software (Perceptive Instruments Ltd, UK) as % DNA in the tail position.

Statistical Analyses

Statistical analyses were performed using SPSS for Windows (version 12.0; SPSS, Inc., Chicago, IL). Except for categorical variables, data are expressed as means with SD. The differences between the two groups were compared by Student's *t*-tests. Categorical variables were compared by chi-square tests or by Fisher's exact tests. Multiple linear regression analysis was used to assess the association of antioxidative nutrients with endogenous and H_2O_2-induced additional DNA damage. Correlation analysis was used to determine the correlation between the intake levels of vitamin C or carotenoids and the consumption of different kinds of vegetables and fruits. Logistic regression analysis was used to estimate the association of nutrients with the risk of COPD. Statistical results were considered significant at $p \leq 0.05$.

Table 2. Relationships between concentrations of plasma nutrients and DNA damage by multiple regression analysis†

	β (*p* value)	R^2
Endogenous DNA damage		
Model 1‡		
Vitamin A	-0.55±1.54 (0.725)	0.012
Vitamin C	-1.86±2.01 (0.36)	0.012
Vitmain E	-10.9±4.01(0.008)	0.095
α-Carotene	-4.54±2.37 (0.061)	0.072
β-Carotene	-1.42±2.88 (0.623)	0.004
Lutein	-4.83±2.69 (0.077)	0.045
Lycopene	-6.30±4.60 (0.173)	0.035
Total carotenoids	-4.08±2.54 (0.112)	0.036
Model 2§		0.332
Vitmain E	-5.75±5.80 (0.328)	
α-Carotene	-3.08±2.51 (0.228)	
Lutein	-7.11±3.64 (0.058)	
Model 3¶		0.426
Vitmain E	-1.26±5.60 (0.823)	
α-Carotene	-0.79±2.49 (0.753)	
Lutein	-4.24±3.53 (0.237)	
H_2O_2 -lesion		
Model 1‡		
Vitamin A	-0.37±3.74 (0.922)	<0.001
Vitamin C	-9.27±4.68 (0.052)	0.054
Vitmain E	3.34±10.2 (0.744)	0.002
α-Carotene	-4.83±5.42 (0.378)	0.017
β-Carotene	-7.17±6.84 (0.299)	0.016
Lutein	-4.76±6.64 (0.476)	0.007
Lycopene	0.34±11.3 (0.976)	<0.001
Total carotenoids	-11.6±6.06 (0.060)	0.05
Model 2§		0.132
Vitmain C	-9.00±5.15 (0.086)	
Total carotenoids	-11.2±7.05(0.119)	
Model 3¶		0.176
Vitmain C	-8.71±5.01 (0.087)	
Total carotenoids	-5.79±7.42 (0.439)	

†Concentrations of plasma nutrients were converted to log concentrations. ‡Model 1: without adjustment for any variables. § Model 2: adjusted for sex, age, BMI, smoking status, carbohydrate intake, protein intake, and those variables that were significantly ($p<0.05$) or borderline significantly ($0.05<p<0.1$) associated with DNA damage in model 1. ¶Model 3: adjusted for the variables listed in model 2 except that smoking status was replaced by group.

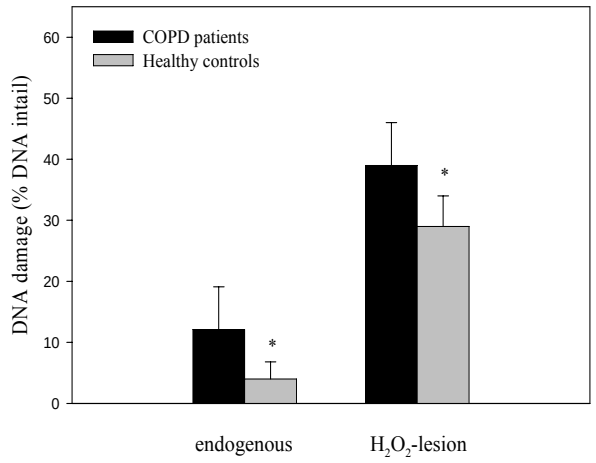

Figure 1. Endogenous and H_2O_2-induced additional DNA damage (H_2O_2-lesion) of whole white blood cells as assessed by comet assays in COPD patients and healthy controls. *Values (mean±SD) are significantly different from the COPD group (p<0.001)

RESULTS

The characteristics of the subjects with COPD and the healthy controls are shown in Table 1. Sex, age, body mass index (BMI), and smoking status differed significantly between the two groups. The COPD patients were older than the healthy individuals, whereas the mean BMI of the COPD patients was lower than that of the healthy controls. Although the percentages of current smokers were similar in the two groups, 41% of the COPD patients were former smokers and 95% of the control group had never smoked, which resulted in a significant difference in smoking status between the two groups (p<0.001). The plasma levels of vitamins A, C, and E, α- and β-carotenes, and total carotenoids were significantly lower in the COPD group than in the healthy group (Table 1). Concentrations of lutein and lycopene, however, did not differ significantly between the two groups. In addition, the results of one-way analysis of variance and the multiple comparison by Tukey's method revealed that no significant differences were observed in the plasma levels of each individual antioxidant among current, former, and never smokers (data not shown).

Endogenous and H_2O_2-induced additional DNA damage to WBCs were determined by the comet assays as markers of oxidative stress. The comet assay, which detects strand breaks in DNA, is a sensitive method for analyzing oxidative DNA damage and has been used to assess oxidative stress in COPD patients.[18,19] The results showed that endogenous WBC DNA damage in COPD patients was significantly greater than that in the healthy controls (Figure 1). To investigate the oxidative resistance of WBCs, we further incubated the WBCs with H_2O_2 before performing the comet assays. The results showed that H_2O_2 markedly increased the DNA damage to WBCs in both groups. Furthermore, the H_2O_2-induced additional DNA damage, that is, the difference between endogenous and hydrogen peroxide induced DNA damage of each sample (refer to as H_2O_2-lesion) was significantly higher in the COPD group than in the healthy controls (39±7 vs. 29±5, p<0.05), implying that the oxidative resistance of WBCs was lower in the COPD patients than in the healthy controls.

The relationships between plasma levels of antioxidative nutrients and endogenous DNA damage and H_2O_2-lesion were evaluated by multiple regression analyses as

Table 3. Daily nutrient intakes of the COPD patient and healthy controls†

	COPD patients	Healthy controls	p value‡
Energy (kcal/day)	1573 ± 445	1709 ± 277	0.148
Carbohydrate (g/day)	181 ± 52	214 ± 46	0.009
Protein (g/day)	57 ± 16	72 ± 13	<0.001
Fat (g/day)	71 ± 38	65 ± 18	0.438
Vitamin C (mg/day)	110 ± 63	153 ± 69	0.013
Diet§	(76 ± 53)	(104 ± 68)	0.06
Diet + supplement¶	114 ± 81	191 ± 140	0.009
α-Carotene (μg/day)	221 ± 361	333 ± 422	0.240
Diet§	(136 ± 242)	(230 ± 305)	0.162
Diet + supplement¶	221 ± 361	333 ± 422	0.240
β-Carotene (μg/day)	3210 ± 1904	4291 ± 2208	0.032
Diet§	(2223 ± 1174)	(2834 ± 1597)	0.075
Diet + supplement¶	3393 ± 1829	4388 ± 2233	0.460
Lutein (μg/day)	2278 ± 1843	3370 ± 2370	0.036
Diet§	(1471 ± 1134)	(2189 ±1620)	0.037
Diet + supplement¶	2323 ± 1833	3394 ± 2382	0.040
Lycopene (μg/day)	525 ± 669	1281 ± 1144	0.001
Diet§	(377 ± 438)	(860 ± 751)	0.001
Diet + supplement¶	580 ± 662	1309 ± 1136	0.010
Total carotenoids (μg/day)	6234 ± 3480	9271 ± 4838	0.004
Diet§	(4053 ± 2447)	(5988 ± 3451)	0.009
Diet + supplement¶	6517 ± 3737	9423 ± 4883	0.006

†Values are mean ± SD. The intakes of total energy and the macronutrients were calculated from 24-hour diet recall; the intakes of individual antioxidant were estimated from FFQ. ‡Differences between groups were compared by Student's t-tests. §Values were calculated from diet only. The values shown in parentheses are relative intakes (the intake of each nutrient calculated from diet /1000 kcal total energy). ¶Values were calculated from diet plus vitamin supplements.

shown in Table 2. Plasma levels of vitamin E were significantly related to endogenous DNA damage to WBCs. We also found negative associations between the plasma levels of α-carotene or lutein and endogenous DNA damage, although with less significance ($0.05< p <0.10$). Only the inverse association between the plasma level of lutein and endogenous DNA damage remained of borderline significance when the analyses were adjusted for confounders, including sex, age, BMI, smoking status, and intakes of carbohydrate and protein, and for the other nutrient levels in plasma (model 2). When the effect of smoking status was replaced by group in the multivariate model, however, none of the plasma levels of antioxidative nutrients was significantly related to endogenous DNA damage (model 3). In the analyses of the relations between antioxidant status and H_2O_2-lesion, vitamin C and total carotenoids were each inversely associated with H_2O_2-lesion with borderline significance ($0.05< p <0.10$, model 1). The inverse association for vitamin C, but not for total carotenoids, remained of borderline significance after adjustment in models 2 and 3 for the abovementioned confounders.

As shown in Table 3, intakes of total energy were not significantly different between the two groups. Except for α-carotene, the mean absolute intakes of vitamin C, all individual carotenoids, and total carotenoids were significantly lower in the COPD group than in the healthy group. When the use of supplementation was also considered, however, the difference in the absolute intake of β-carotene became insignificant. When the intake from diet was adjusted for total energy intake, the relative intakes of lutein, lycopene, and total carotenoids, respectively, in the COPD group remained significantly lower than those in the healthy controls; whereas the intakes of vitamin C and β-carotene in COPD group became borderline significantly lower than those in the healthy controls (p=0.06 and 0.075, respectively). Significant positive correlations between plasma and dietary levels of α-carotene (r=0.382, p=0.008) and total carotenoids (r=0.242, p=0.006) were observed when all the subjects were pooled together, while there were no significant correlations for vitamin C and other carotenoids (data not shown). However, analyzed by groups separately, we found no significant correlations between plasma and dietary levels in the healthy controls except for α-carotene (r=0.516, p=0.07) (data not shown).

Table 4. Distribution of frequency of three types of vegetables plus fruit in COPD patients and in healthy controls

Type	COPD patients	Healthy controls	p value
Cooling			
Portions/month†	23 ± 13	69 ± 29	<0.001
Percentage ‡	20%	35%	<0.001
Relative consumption§	0.49 ± 0.31	1.31 ± 0.72	<0.001
Neutral			
Portions/month†	83 ± 36	117 ± 40	<0.001
Percentage‡	74%	59%	<0.001
Relative consumption§	1.83 ± 0.81	2.42 ± 0.95	0.07
Heating			
Portions/month†	7 ± 6	11 ± 7	0.025
Percentage‡	6%	6%	0.630
Relative consumption§	0.20 ± 0.20	0.21 ± 0.20	0.597
Total			
Portions/month†	119 ± 56	187 ± 63	<0.001
Relative consumption§	2.52 ± 1.05	3.93 ± 2.06	<0.001

†Values are portions/month of vegetables plus fruit (mean ± SD). ‡he Values are the percentages of cooling, neutral, or heating vegetables and fruit to total intake of vegetables and fruit. §The values are relative consumptions, that is, the consumption of each type of vegetables and fruit/1000 kcal total energy.

Table 5. Correlation of antioxidative nutrients and frequency of vegetables plus fruit in COPD patients and in healthy controls

	r (p value)			
	Cooling	Neutral	Heating	Total
COPD patients				
Vitamin C	0.42(0.016)	0.11(0.563)	0.28(0.123)	0.25(0.166)
α-Carotene	0.16(0.394)	0.40(0.024)	0.29(0.113)	0.37(0.036)
β-Carotene	0.37(0.036)	0.78(<0.001)	0.68(<0.001)	0.77(<0.001)
Lutein	0.15(0.413)	0.706(<0.001)	0.42(0.017)	0.60(<0.001)
Lycopene	0.29(0.111)	0.20(0.262)	0.05(0.776)	0.23(0.199)
Total carotenoids	0.32(0.073)	0.80(<0.001)	0.58(0.001)	0.75(<0.001)
Healthy controls				
Vitamin C	0.02(0.909)	0.31(0.053)	0.36(0.024)	0.27(0.099)
α-Carotene	0.10(0.553)	0.28(0.08)	-0.12(0.463)	0.21(0.203)
β-Carotene	0.34(0.031)	0.59(<0.001)	-0.17(0.291)	0.51(0.001)
Lutein	0.20(0.217)	0.53(<0.001)	0.06(0.715)	0.43(0.006)
Lycopene	0.16(0.321)	0.12(0.111)	-0.12(0.481)	0.13(0.410)
Total carotenoids	0.30(0.061)	0.58(<0.001)	-0.14(0.374)	0.49(0.001)

Table 6. Adjusted odds ratios for COPD according to selected factors by logistic regression

Factor	Odds Ratio	95% CI
Model 1		
Male	1.97	0.30-13.10
Age	1.12	1.01-1.23
BMI	0.83	0.62-1.09
Smoking status (current+former)	157	5.62-4400
Daily intake of carbohydrate	0.98	0.96-0.99
Daily intake of protein	1.02	0.97-1.08
Frequency of vegetables and fruit intake†	0.97	0.96-0.99
Model 2		
Male	3.38	0.47-24.2
Age	1.11	0.99-1.24
BMI	0.78	0.59-1.03
Smoking status (current +former)	96.3	4.26-2174
Daily intake of carbohydrate	0.82	0.66-1.01
Daily intake of protein	1.17	0.66-2.07
Vitamin A	0.99	0.94-1.04
Vitamin C	0.99	0.90-1.09
Vitamin E	0.88	0.69-1.11
Total carotenoids	0.99	0.99-1.00

BMI, body mass index. †Summed frequency of vegetables and fruit intake in one month.

Comparing to the healthy group, the habitual consumption of vegetables and fruits (servings/month) of cooling, neutral, and the sum of vegetables and fruits were significantly lower in the COPD group (Table 4). The percentage consumption of cooling vegetables and fruits was also significantly lower in COPD patients, whereas the percentage consumption of heating vegetables and fruits did not differ significantly between the two groups. The relative intakes of different categories of vegetables and fruit were calculated, expressed as portion/1000 kcal, as the consumption of vegetables and fruit were positively correlated with the intake of total energy (data not shown). Similar results were also observed when the comparison was made based on the relative consumption of cooling, neutral, and total vegetables plus fruit (Table 4). As shown in Table 5, in COPD patients, the intake of vitamin C was significantly positively associated with the consumption of cooling foods. In contrast, the intake of vitamin C was significantly positively associated with the consumption of neutral and heating rather than cooling foods in the healthy subjects. The intake of β-carotene was significantly associated with all types of vegetables and fruits in the COPD group. Except for lycopene, the intake of all other carotenoids was significantly correlated with total consumption of vegetables and fruits in the COPD group.

The results of the logistic regression analysis revealed that age and smoking experience (former plus current smoking) independently increased the risk of COPD (Table 6, model 1), whereas the overall consumption of vegetables and fruits independently decreased the risk of COPD (OR=0.96; 95% CI=0.96-0.99). The intake of individual nutrients, however, did not independently affect the risk of COPD (Table 6, model 2).

DISCUSSION

Oxidative stress is considered to play a role in the pathogenesis of COPD,[20] and a deficiency of antioxidants could be a risk factor for a decline in lung function. A recent multivariate analysis showed that higher serum concentrations of vitamins A, C, and E and β-cryptoxanthin were independently associated with higher levels of FEV_1.[4] Consistent with these findings, in the study we demonstrated that the levels of vitamins A, C, and E and of α- and β-carotenes in plasma were significantly lower in COPD patients than in healthy people. Furthermore, consistent with the study by Ceylan *et al*,[18] we also demonstrated that the levels of endogenous- and H_2O_2-induced additional DNA damage were significantly higher in COPD patients than in healthy people. These findings consistently suggest that COPD patients in Taiwan may have greater oxidative stress and lower oxidative resistance than do healthy people.

The mean concentrations of fasting plasma vitamins A, C and E in both COPD patients and healthy controls in our study were >0.2 μg/mL, >3 μg/mL, and >5 μg/mL, respectively, which were considered within acceptable range in healthy persons.[21] Nevertheless, Ochs-Balcom *et al*. reported that the serum levels of β-carotene, lutein/zeaxanthin and lycopene in American subjects with chronic airflow limitation were about 0.178, 0.439, and 0.132 μg/mL, respectively.[5] Chiu *et al*. found that the mean plasma levels of α-carotene, β-carotene, and lycopene in smokers in Taiwan were 0.033, 0.12 and 0.043 μg/mL, respectively; whereas the values in nonsmokers were 0.067, 0.35 and 0.063 μg/mL, respectively.[22] In general, our findings are comparable with the abovementioned studies. However, some discrepancies existing among studies may be due to the different detecting methods and the characteristics of subjects.

In the multiple linear regression analysis, we observed that after adjustment for the group effect, none of the antioxidant levels in plasma were significantly associated with endogenous DNA damage. This may have been because the chronic inflammation in COPD patients results in modest baseline DNA damage, such that suboptimal plasma levels of antioxidants would not confer beneficial effects. In contrast, the negative association between the plasma level of vitamin C and H_2O_2-induced additional DNA damage remained of borderline significance after

adjustment for the confounders, including smoking status or group, implying that a high level of vitamin C in plasma may prevent the extra oxidative stress such as that resulting from inhaling air pollutants or exercise.[23] These findings agree with our previous study showing that vitamin C supplementation in COPD patients increases the resistance of DNA in WBCs to the challenge of H_2O_2 in vitro.[24]

The intakes of antioxidative nutrients may be beneficial to lung function.[5-7] McKeever *et al.* suggested that high intakes of vitamin C reduce the decline in FEV_1 and may help to prevent COPD.[7] In the present study, we also found that absolute and relative intakes of vitamin C and several carotenoids in COPD patients in Taiwan were lower than those in healthy people. Furthermore, our results showed that in COPD patients, the intakes of vitamin C, β-carotene, α-carotene, and lutein were significantly correlated with the consumption of cooling or the sum of vegetables and fruits. These findings imply that dietary habits may affect the intake of antioxidative nutrients in COPD patients, who consumed less of all kinds of vegetables and fruits than healthy people, especially cooling vegetables and fruits. A recent study also showed that elderly people in Taiwan tend to avoid eating foods that are categorized as cooling or heating by traditional Chinese medicine to avoid suffering from unfavorable conditions.[25] Because many fruits and vegetable are sorted to cooling foods,[8] we hypothesize that the low consumption of total vegetables and fruits in COPD patients is associated with this concept. In fact, in our current study we found that 91% of COPD patients hardly ate any cooling fruit (<1 portion/year) except for oranges, starfruits, and pears which are rich in vitamin C; among them, 75% ate only ≤5 items (out of 12 items listed) of cooling vegetables. Asparagus, cucumber, and bitter gourds, which are rich in vitamin C or β-carotene are the top 3 items chosen by COPD patients. However, only 28% of the healthy subjects did not eat half the items of cooling vegetables and fruit. The different patterns of food choices might also explain why vitamin C correlated to cooling food consumption in the COPD patients but not in the healthy controls. An unexpected finding of our study was the low correlation between plasma levels and dietary intakes of vitamin C and carotenoids in our subjects. Because dietary intake was estimated by a FFQ, however, measurement errors in estimating the consumption of vitamin C and carotenoids may have contributed to the low correlation.

Consistent with previous studies,[26,27] we found that the total intake of vegetables and fruits, rather than of individual vitamin C or carotenoids, independently decreased the risk of COPD. This may be explained by the fact that in addition to vitamin C and carotenoids, vegetables and fruits also contain many other beneficial phytochemicals, such as flavonoids, that ubiquitously exist in plant foods and have been observed to possess many bioactivities including anti-inflammatory and antioxidative effects.[28,29] In a cohort study in the Netherlands, Tabak *et al.* found that total intake of catechin, flavonol, and flavone is positively associated with FEV_1.[30] However, it is possible that we did not find independent inverse associations between individual antioxidative nutrients and lung function because of the sample size of our study.

Although the differences in the age and gender distribution among the groups may be a limitation of this study, our data did not show that male sex is a significant risk factor in the logistic regression analyses (Table 6). It has been suggested that male sex was considered a risk factor for COPD in some studies, possibly because of the higher smoking and occupational exposures than that of women.[31] Regrading age, there is growing evidence of higher prevalence of COPD in the elderly,[32] suggesting that aging may be an independent risk factor for COPD. In addition, the cross-sectional design and the subsequent uncertainty about cause-effect relations is a weakness of our study. The sample size may also have limited the power of the results. However, in the current study, we observed that age and smoking experience were apparent risk factors for COPD, whereas the overall consumption of vegetables and fruits may have an independent effect in decreasing the risk for COPD. To our knowledge, this is the first study to explore the dietary habits of COPD patients in Taiwan, specifically in relation to the consumption of vegetables and fruits. The findings of the current study may provide important information regarding dietary patterns and nutritional education in COPD patients in Chinese society.

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Appendix 1. The categories of vegetable and fruit

Neutral foods	Sweet Potato Leaves	Chinese Date/ Jujube	Cucumber	Yellow Watermelon
Yellow or Red Pepper	Okra	Cumquat	Loofah	Pear
Chayote Leaves	Amaranth	Cherry	Alfalfa Sprouts	Mangosteen/Mangostan
Broccoli	Gynura	Banana	Bamboo Shoots	Coconut
White Cabbage Sprout	Water Spinach	Grape	Orange, Sunkist	
Red Cabbage	Lettuce		Cherry Tomato	**Heating foods**
Pakchoi	Kidney Bean	**Cooling foods**	Tankan	Chilli Pepper
Celery	Spinach	Bean Sprouts	Orange	Green Onion
White Cabbage	Eggplant	Wax Gourd/ White Gourd	Grapefruit	Chinese Leek
Cabbage	Carrot	Bottle Gourd/ Calabash	Tangerine	Onion
Broccoli	Apple	Chinese Heading Cabbage	Starfruit	Ginger
Bok Coy	Garland Chrysanthemum	Bitter Gourd	Tomato	Longan
Lima Bean	Guava	Mooli / Chinese Radish	Honeymelon/ Cantaloupe	Durian
Edible Rape/ Rapeseed	Papaya	Oriental Pickling Melon	Muskmelon	Litchi
		Asparagus	Watermelon	Mango

AUTHOR DISCLOSURES

The author(s) declare that they have no competing interests.

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Original Article

Comparison of plasma and intake levels of antioxidant nutrients in patients with chronic obstructive pulmonary disease and healthy people in Taiwan: a case-control study

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探討台灣慢性阻塞性肺病患者血液及飲食中的抗氧化營養素含量：病例對照研究

氧化物/抗氧化物間的不平衡在慢性阻塞性肺病(COPD)的疾病發展上扮演著重要的角色。越來越多的證據顯示，血液或膳食中抗氧化營養素濃度較高的個體，可能維持有較好的肺功能。本研究的目的是探討台灣 COPD 病患血液及飲食中的抗氧化營養素含量是否較健康人低，以及 COPD 患者的飲食習慣是否影響其維生素 C 及各種類胡蘿蔔素的攝取量。研究中招募了 34 名 COPD 病患及 43 名肺功能正常的健康人(年齡≧50 歲)參與試驗。收集受試者血液樣本，分析血漿維生素 A、C、E 以及類胡蘿蔔素濃度，並且分析白血球的內生性及 H_2O_2 誘發的 DNA 傷害（氧化壓力指標）。受試者維生素 C 及類胡蘿蔔素之膳食攝取量則以食物頻率問卷來分析。結果顯示，與健康對照組相比，COPD 患者血漿中維生素 A、C、E、α-和 β-胡蘿蔔素及總類胡蘿蔔素的濃度均顯著較低，但白血球內生性及 H_2O_2 誘發的 DNA 傷害則顯著較高。COPD 患者維生素 C 及數種類胡蘿蔔素的攝取量較健康者低，此外 COPD 患者蔬菜水果攝取量亦顯著較低。總結而言，本研究結果顯示，台灣 COPD 患者血漿及膳食中抗氧化營養素含量比健康人低，其維生素 C 及類胡蘿蔔素攝取量與飲食習慣具相關性。

關鍵字：慢性阻塞性肺病、抗氧化物質、台灣、水果、蔬菜