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ORIGINAL ARTICLE

Total dietary antioxidant capacity and lung function in an Italian population: a favorable role in premenopausal/never smoker women

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Background/Objectives: Antioxidant-rich foods may favorably influence lung function. We examined possible associations between the total dietary antioxidant capacity (TAC) and pulmonary function in a healthy Italian population.
Subjects/Methods: Until May 2009, 22 300 persons were randomly recruited from the general population in the Moli-sani project. A sample only including healthy women (5824) and men (5848) was analyzed. TAC was measured in foods by three different assays and the ferric reducing-antioxidant power (FRAP) assay was selected as the better indicator of dietary TAC. The European Investigation into Cancer and Nutrition Food Frequency Questionnaire was used for dietary assessment. The association between quintiles of dietary FRAP and pulmonary indexes was assessed using analysis of variance separately for men and women.
Results: After adjustment for confounders, women in the highest quintile of FRAP intake had +39 ml forced expiratory volume in the first second (FEV_1) and +54 ml forced vital capacity, compared with those in the lowest quintile (P for trend $\leqslant 0.006$). Stratified analysis showed that this relationship only occurred in women who were premenopausal/never smokers. In this subgroup, the observed effect of higher FRAP intake on FEV_1 was equivalent to an improvement in pulmonary age of 3.3 years. In men, all significant associations between pulmonary function and TAC were lost after adjustment for confounding.
Conclusions: Dietary TAC may have a favorable role in respiratory health, particularly in premenopausal/never smoker women.
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Introduction

A consistent body of evidence shows a link between consumption of antioxidant vitamins (Schwartz and Weiss, 1994a; Britton *et al.*, 1995; Tabak *et al.*, 1999; Schünemann *et al.*, 2001a, b; Charles *et al.*, 2010), antioxidant-rich foods (Strachan *et al.*, 1991; Cook *et al.*, 1997; Carey *et al.*, 1998; Butland *et al.*, 2000; Kelly *et al.*, 2003; Nettleton *et al.*, 2009), omega-3 polyunsaturated fatty acids and fish (Schwartz and Weiss, 1994b; Shahar *et al.*, 1994; Sharp *et al.*, 1994; Charles *et al.*, 2010) and better pulmonary function.

As foods are eaten in combination, the evaluation of the total antioxidant capacity (TAC) of the diet may be a suitable approach to measure their joint antioxidant effects (Pellegrini *et al.*, 2003). The protective effect on lungs against oxidative stress-induced disease is stronger for fruit intake rather than for its single antioxidant nutrients (Smit *et al.*, 1999; Romieu and Trenga, 2001; Kan *et al.*, 2008), suggesting that bioactive compounds act synergistically in protecting the lungs from oxidative stress (Kan *et al.*, 2008).

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Hence, dietary TAC sums up the free radical scavenging ability of antioxidants in foods (Brighenti *et al*., 2005).

While recent studies have shown negative associations for dietary TAC with C-reactive protein (Brighenti *et al*., 2005; Aronson *et al*., 2006), gastric cancer (Serafini *et al*., 2002) and total mortality (Agudo *et al*., 2007), no study has apparently considered the TAC of the diet in relation to lung function.

The aim of this study was to examine associations between dietary TAC and pulmonary function in a healthy population enrolled in the Moli-sani project.

Subjects and methods

Study subjects

The cohort of the Moli-sani Project (Iacoviello *et al*., 2007; Centritto *et al*., 2009) was recruited in the Molise region, Italy, from city hall registries by a multistage sampling. Thirty percent of randomized subjects refused to participate.

The European Prospective Investigation into Cancer and Nutrition food frequency questionnaire was used for the interview (Pisani *et al*., 1997). The NAF software (Nutritional Analysis of Food Frequency Questionnaires, National Cancer Institute, Milan, Italy) (Pala *et al*., 2003) was used to transform information about food composition into daily intake of food items, energy, macro- and micronutrients and TAC. Nutrient data for specific foods were obtained from the food composition database for epidemiological studies in Italy (Salvini *et al*., 1998) integrated with the TAC values of a number of foods representative of the average Italian diet, such as fruits, vegetables, oils, beverages, spices, dried fruits, sweets, cereals, pulses and nuts (Pellegrini *et al*., 2003, 2006). The TAC database has been widely validated in the Italian population (Brighenti *et al*., 2005; Valtueña *et al*., 2008; Del Rio *et al*., 2011) as well in Spanish (Agudo *et al*., 2007) and Greek populations (Detopoulou *et al*., 2010; Psaltopoulou *et al*., 2011).

TAC was measured in foods by three different assays: the trolox equivalent antioxidant capacity (TEAC) assay measuring the antioxidants' ability to reduce a radical cation in both lipophilic and hydrophilic conditions (Pellegrini *et al*., 1999), the radical-trapping antioxidant parameter (TRAP) and ferric reducing-antioxidant power (FRAP) assays evaluating the chain-breaking antioxidant potential (Ghiselli *et al*., 1995) and the reducing power of the sample (Benzie and Strain, 1999), respectively. The food frequency questionnaire was specifically validated for dietary TAC assessment against TRAP, FRAP and TEAC values estimated by a 3-day weighed food record and plasma TEAC and FRAP in a group of healthy Italian adults (Pellegrini *et al*., 2007).

Pulmonary function was measured, according to the American Thoracic Society criteria (American Thoracic Society, 1995), by V-Max Encore 22D Spirometers equipped with plethysmographyc V62J Autobox and 2 V-Max Encore 20, all with the same Mass Flow Sensor model (Sensormedics Viasys, San Diego, CA, USA).

Daily volume calibration was performed with a 3-l syringe. Volume variability higher than 0.5% from the real value (3 l) was considered unacceptable, and calibration repeated. Tests were performed in the morning, with participants seated and wearing nose-clips. At the end of the test, the acceptability and the reproducibility of the maneuvers were evaluated: participants were included if they had at least three acceptable tests with differences <0.20 l on the best value for forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1).

Percentage of predicted pulmonary indexes were computed using the European Community of Coal and Steel prediction equations that included height and age (Quanjer *et al*., 1993).

A questionnaire about pulmonary symptoms, lung disorders and work exposure was administered by trained monitors.

Exclusion criteria for the spirometric tests were: recent abdominal or ocular surgery, cardiovascular disorders, blood pressure higher than 180/100 mm Hg, untreated glaucoma and ocular lesions or pain during test performance (Miller *et al*., 2005).

Blood pressure and anthropometric measurements

Trained research personnel measured blood pressure by an automatic device (OMRON-HEM-705CP) three times on the non-dominant arm and the average of the last two values was taken as the blood pressure. Body weight and height measurements were made using standardized procedures (Iacoviello *et al*., 2007). Body mass index was calculated as kg/m^2. Waist circumference was measured according to the National Heart, Lung and Blood Institute of the National Institutes of Health guidelines (Iacoviello *et al*., 2007).

Definition of risk factors

Subjects were classified as *never smokers* if they had never smoked cigarettes, *former smokers* if they had smoked cigarettes in the past but had stopped smoking since at least 1 year and *current smokers* if they were currently smoking one or more cigarettes per day on a regular basis. The combination of former and current smokers was considered as 'ever smokers'. Pack-years of cigarette smoking were calculated:

(years smoked $\times$ average number of cigarettes smoked per day)/20 (1 pack contains 20 cigarettes).

Social status was classified as a score based on education, job, income and housing; the higher the score, the higher the social level. Total physical activity (leisure and working time) was classified in metabolic equivalents/day (Centritto *et al*., 2009).

Biochemical measurements

Venous blood samples were obtained between 0700 and 0900 hours. Biochemical analyses were carried out in the centralized Moli-sani laboratory on fresh samples. An automatic analyzer (ILab 350, Instrumentation Laboratories, Lexington, MA, USA) was used to assay serum lipids and glucose. Low-density lipoprotein cholesterol was calculated according to Friedewald.

Population for analysis

Between March 2005 and May 2009, 22 300 persons were recruited in two centres: one at Catholic University in Campobasso, the second at San Timoteo Civil Hospital in Termoli, both in the Molise region. Subjects were excluded from present analysis if they reported a history of cardiovascular disease ($n=1456$), malignancies ($n=724$) or pulmonary disease ($n=1541$), or if pulmonary function tests were missing or not acceptable ($n=6247$). People with extreme energy intake—lower and upper percentiles of the energy-intake distribution—($n=199$), or incomplete medical and dietary questionnaires ($n=172$), or being not born in Italy or not Caucasians ($n=170$) were also excluded. Finally, a sample of 11 672 subjects (5824 women and 5848 men) was analyzed as several exclusion criteria overlapped.

The Moli-sani study was approved by the Catholic University Ethics Committee. All participants provided written informed consent.

Statistics

Normally distributed data are presented as means ± standard error (s.e.). Dietary TAC, assessed as TEAC, TRAP and FRAP was energy-adjusted according to the residual method (Willett and Stampfer, 1986) and categorized into quintiles on the basis of sex-specific distribution or reported as means ± s.e.

The association between quintile of dietary TAC, regressed on total energy intake according to residual methods, and pulmonary indexes was assessed using multivariable analysis of variance separately for men and women. The basic model was adjusted for all covariates associated with both pulmonary function and TAC intake, with a significance level of at least $P<0.1$. The final multivariable models included age and height (with the exception of pulmonary parameters expressed as percentage (%) of predicted), center of recruitment, social status (continuous), physical activity (continuous), smoking status (never, former and current), pack years of smoking, waist circumference, weight, hypertension, dyslipidemia and diabetes (yes or not), diet therapy (yes or not), menopausal status (where appropriate), total energy intake, dietary calcium and sodium intake. Trend tests were calculated on the basis of quintile-based scores used as continuous variables (ordinal variable with values 0–4).

FRAP, TEAC and TRAP are three indicators of dietary TAC, strongly correlated with each other ($r=0.97$; $P<0.0001$). To avoid redundancy in presentation of data, we used in our analyses the indicator that fitted the data at the best. For fitting the data, we intend the selection of the best relationship between the indicator of dietary TAC and indicators of pulmonary function. As test for goodness of fit we used the Akaike Information Criterion (Akaike, 1973; Agudo *et al.*, 2007); the lower the Akaike Information Criterion score, the better the goodness of fit. As FRAP, compared with TEAC and TRAP, showed the lowest Akaike Information Criterion it was selected as the better indicator of dietary TAC. Analyses were stratified by smoking status for men and by menopausal and smoking status for women. Formal tests for interaction were performed between smoking status, menopausal status and smoking/menopausal status and TAC intake by including interaction terms in the fully adjusted model.

To translate into practical term the differences in pulmonary function observed between the fifth and the first quintile of TAC intake, the improvement in pulmonary age (Morris and Thomas, 1985) was calculated from the final multivariable model.

The contribution of single food groups to total dietary TAC was assessed by stepwise multiple regression analysis.

P-values <0.05 indicated statistical significance. However, a level of significance <0.2 was chosen in order to increase power for tests of interaction (Selvin, 1991; Charles *et al.*, 2010). The analyses were performed with SAS 9.1.3 for Windows (SAS Institute, Cary, NC, USA).

Results

TAC intake and pulmonary function in men and women are shown in Table 1. As compared with men, women showed lower levels of dietary TEAC, TRAP and FRAP, total energy,

Table 1 Dietary intake and pulmonary function parameters of men and women from the Moli-sani population[a]

Characteristics	*Men* (n = *5848*)	*Women* (n = *5824*)	P-*value*[b]
TEAC[c]	7.99 ± 0.038[d]	5.24 ± 0.038	<0.0001
TRAP[c]	11.54 ± 0.063	7.85 ± 0.063	<0.0001
FRAP[c]	24.04 ± 0.12	15.83 ± 0.12	<0.0001
Dietary calcium (mg/day)	959.76 ± 5.49	999.28 ± 5.49	<0.0001
Dietary sodium (mg/day)	2624.64 ± 13.81	2204.75 ± 13.80	<0.0001
Total energy intake (kcal/day)	2320.81 ± 8.99	2014.09 ± 8.98	<0.0001
Pulmonary function			
FEV_1 (l)	3.35 ± 0.007	2.86 ± 0.007	<0.0001
FEV_1 (% predicted)	110.33 ± 0.25	111.59 ± 0.25	0.002
FVC (l)	4.35 ± 0.008	3.74 ± 0.008	<0.0001
FVC (% predicted)	115.72 ± 0.25	123.18 ± 0.25	<0.0001
FEV_1:FVC (ratio)	76.97 ± 0.09	76.70 ± 0.09	0.079
FEV_1:FVC ratio (% predicted)	98.89 ± 0.12	96.87 ± 0.12	<0.0001

Abbreviations: FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; FRAP, ferric reducing-antioxidant power; TEAC, trolox equivalent antioxidant capacity; TRAP, radical-trapping antioxidant parameter.
[a]Results are from age- and height-adjusted analysis of variance (except for the % predicted).
[b]*P*-value for the difference between men and women.
[c]Expressed as mmol Trolox/day, mmol Trolox/day and mmol Fe^{2+}/day, respectively, adjusted for total energy according to residual method.
[d]Mean ± s.e. (all such values).

sodium intake and all pulmonary function parameters. Only dietary calcium was significantly higher in women (Table 1).

To explore the relative contribution of food groups to total dietary TAC, we performed stepwise multiple regression analysis with TEAC, TRAP and FRAP intake, respectively, as dependent variable and intake of cereals, vegetables, legumes, oils and nuts, fruit and fruit juices, coffee, tea, chocolate and alcoholic beverages in grams per day as independent variables, while controlling for age and sex. As shown in Table 2, dietary intake of all mentioned food groups explained >80% of the total dietary TEAC, TRAP and FRAP. Coffee, alcoholic beverages, fruit and fruit juices represented the main sources of antioxidants in our population.

As FRAP showed the smallest Akaike Information Criterion, only results for TAC measured as FRAP are reported in further analyses.

In women, regression analysis revealed that FEV1 and FVC were both associated with dietary FRAP ($P=0.015$ and $P=0.018$, respectively) (Table 3). Similar results were observed for dietary TEAC and TRAP (data not shown). In men, in regression analysis, there were no associations between dietary FRAP and pulmonary volumes after adjustment for confounders (Table 4).

Table 2 Contribution of selected food groups to dietary TAC[a]

Food groups	*TEAC (mmol Trolox) (%)*	*TRAP (mmol Trolox) (%)*	*FRAP (mmol Fe^{2+}) (%)*
Coffee	40.5	60.2	50.2
Alcoholic beverages	31.3	22.2	27.9
Fruit and fruit juices	5.96	2.74	4.69
Chocolate	3.38	0.96	1.48
Cereals	0.83	0.66	1.24
Tea	0.53	0.35	0.50
Vegetables, oils, nuts, legumes	0.46	0.20	0.42

Abbreviations: FRAP, ferric reducing-antioxidant power; TAC, total antioxidant capacity; TEAC, trolox equivalent antioxidant capacity; TRAP, radical-trapping antioxidant parameter.
[a]Percentages represent the partial R^2 obtained from a linear regression model (controlled for age and sex) in order to explain the contribution of a variable x_j (food) to the explanation of the variation of a dependent variable y (dietary TEAC, TRAP and FRAP, respectively).

Dietary FRAP, smoking, menopausal status and pulmonary function

The association of dietary FRAP and pulmonary parameters was observed in never, but not in ever smoker women (data not shown), although in the latter group TAC intake was higher (multivariable adjusted FRAP intake: 16.3 ± 0.2 mmol Fe^{2+}/day vs 14.7 ± 0.1 mmol Fe^{2+}/day; $P<0.0001$, ever vs never smoker women, respectively).

Adjusted P-values for FRAP × smoking interaction were as follows: 0.04 for FEV_1, 0.07 for FEV_1 (% of predicted), 0.11 for FVC, 0.21 for FVC (% of predicted), 0.19 and 0.17 for FEV_1:FVC as ratio and as percentage of predicted, respectively.

FEV_1, FVC and percentage of predicted FEV_1 and FVC were significantly associated with FRAP intake in premenopausal, but not in postmenopausal women (adjusted P for interaction $\leqslant 0.16$) (data not shown).

Stratified analyses for smoking and menopausal status showed a positive significant trend only among never smokers premenopausal women for FRAP intake and FEV_1 (+83.4 ml highest vs lowest quintile, $P<0.001$), percentage of predicted FEV_1 (+2.98%, $P<0.001$), FVC (+93.4 ml highest vs lowest quintile, $P=0.002$), percentage of predicted FVC (+2.95%, $P=0.002$), while no significant association was found for FEV_1:FVC or across other strata of smoking and menopausal status (Table 5).

Improvement in pulmonary age

The increment in pulmonary function in the form of FEV_1, observed between the highest and the lowest quintile of dietary FRAP intake, was equivalent to an improvement in pulmonary age of 3.3 years for never smokers/premenopausal women.

Table 3 Multivariable analysis of individual pulmonary parameters according to quintile (Q) of FRAP intake among women

Pulmonary parameters	*Frap intake*					P *for trend*[a]	P *for trend*[b]
	Q1 (n = 1160)	*Q2 (n = 1161)*	*Q3 (n = 1167)*	*Q4 (n = 1168)*	*Q5 (n = 1168)*		
FEV_1 (l)	2.597 ± 0.012[c]	2.619 ± 0.012	2.614 ± 0.012	2.628 ± 0.012	2.636 ± 0.012	0.007	0.006
FEV_1 (% predicted)	112.74 ± 0.55	113.67 ± 0.55	113.57 ± 0.56	114.05 ± 0.55	114.42 ± 0.56	0.900	0.015
FVC (l)	3.343 ± 0.015	3.380 ± 0.015	3.370 ± 0.015	3.397 ± 0.015	3.395 ± 0.015	0.001	0.003
FVC (% predicted)	122.23 ± 0.56	124.58 ± 0.56	124.26 ± 0.57	124.70 ± 0.56	124.97 ± 0.57	0.412	0.018
FEV_1:FVC (ratio)	77.65 ± 0.18	77.51 ± 0.18	77.55 ± 0.19	77.60 ± 0.18	77.58 ± 0.19	0.308	0.839
FEV_1:FVC ratio (% predicted)	97.95 ± 0.24	97.74 ± 0.23	97.84 ± 0.24	97.93 ± 0.24	97.95 ± 0.24	0.030	0.865

Abbreviations: FEV_1, forced expiratory volume in 1 second; FRAP, ferric-reducing antioxidant power forced; FVC, forced vital capacity.
[a]Determined from univariate linear regression models.
[b]Determined from multiple linear regression models ($n=5824$ women) adjusted for age and height (except for the % predicted), weight, waist circumference, center of recruitment, social status, physical activity levels, smoking status (never, former and current), pack-years of smoking, hypertension, diet therapy, menopausal status, total energy intake, dietary calcium and sodium intake.
[c]Mean ± s.e. (all such values).

Table 4 Multivariable analysis of individual pulmonary parameters according to quintile (Q) of FRAP intake among men

Pulmonary parameters	*Frap intake*					P *for trend*[a]	P *for trend*[b]
	Q1 (n = *1168)*	*Q2* (n = *1167)*	*Q3* (n = *1169)*	*Q4* (n = *1172)*	*Q5* (n = *1172)*		
FEV_1 (l)	3.454 ± 0.024[c]	3.438 ± 0.024	3.433 ± 0.024	3.429 ± 0.024	3.468 ± 0.024	0.053	0.805
FEV_1 (% predicted)	104.60 ± 0.74	104.08 ± 0.74	103.85 ± 0.73	103.93 ± 0.73	105.19 ± 0.75	0.005	0.601
FVC (l)	4.483 ± 0.030	4.481 ± 0.030	4.468 ± 0.030	4.474 ± 0.030	4.524 ± 0.031	0.751	0.231
FVC (% predicted)	109.82 ± 0.74	109.85 ± 0.74	109.36 ± 0.73	109.65 ± 0.73	110.83 ± 0.75	0.891	0.268
FEV_1:FVC (ratio)	77.15 ± 0.31	76.75 ± 0.31	76.88 ± 0.30	76.81 ± 0.30	76.77 ± 0.31	< 0.001	0.18
FEV_1:FVC ratio (% predicted)	98.88 ± 0.40	98.33 ± 0.40	98.56 ± 0.40	98.51 ± 0.40	98.66 ± 0.41	< 0.0001	0.656

Abbreviations: FEV_1, forced expiratory volume in 1 second; FRAP, ferric-reducing antioxidant power forced; FVC, forced vital capacity.
[a]Determined from univariate linear regression models.
[b]Determined from multiple linear regression models ($n = 5848$ men) adjusted for age and height (except for the % predicted), weight, waist circumference, center of recruitment, social status (continuous), physical activity levels, smoking habits (never, former and current), hypertension, dyslipidemia, diabetes, diet therapy, total energy intake, dietary calcium and sodium intake.
[c]Mean ± s.e. (all such values).

Table 5 Associations between FRAP intake and levels of pulmonary function according to strata of smoking and menopausal status among women

Smoking, menopausal status and pulmonary parameters	*Frap intake*	P *for trend*[a]
	Δ (Q5–Q1)[b]	
Premenopausal never smokers	($n = 1737$)	
FEV_1 (l)	83.4 ± 0.03	< 0.001
FEV_1 (% predicted)	2.98 ± 1.03	< 0.001
FVC (l)	93.4 ± 0.03	0.002
FVC (% predicted)	2.95 ± 1.12	0.002
FEV_1:FVC (ratio)	0.29 ± 0.38	0.739
Premenopausal ever smokers	($n = 1281$)	
FEV_1 (l)	−0.002 ± 0.03	0.967
FEV_1 (% predicted)	0.15 ± 1.22	0.868
FVC (l)	0.02 ± 0.04	0.381
FVC (% predicted)	1.01 ± 1.28	0.288
FEV_1:FVC (ratio)	−0.70 ± 0.47	0.065
Postmenopausal never smokers	($n = 1728$)	
FEV_1 (l)	0.03 ± 0.03	0.178
FEV_1 (% predicted)	1.48 ± 1.56	0.329
FVC (l)	0.05 ± 0.03	0.199
FVC (% predicted)	1.44 ± 1.55	0.497
FEV_1:FVC (ratio)	−0.08 ± 0.45	0.587
Postmenopausal ever smokers	($n = 1078$)	
FEV_1 (l)	0.01 ± 0.04	0.910
FEV_1 (% predicted)	0.94 ± 1.90	0.807
FVC (l)	−0.0006 ± 0.05	0.630
FVC (% predicted)	0.17 ± 1.85	0.735
FEV_1:FVC (ratio)	0.25 ± 0.64	0.558

[a]Determined from multiple linear regression models adjusted for age and height (except for the % predicted), weight, waist circumference, center of recruitment, social status, physical activity levels, pack-years of smoking (where appropriate), hypertension, diet therapy, total energy intake, dietary calcium and sodium intake.
[b]Differences in ml between the fifth (Q5) and the first (Q1) quintile (all such values).

Discussion

From a sample of 11 672 healthy Italian adults, TAC of the diet was positively associated with pulmonary function among women. Previous studies, mentioned above, had reported positive links between consumption of antioxidant vitamins, antioxidant-rich foods, omega-3 polyunsaturated fatty acids or fish intake and lung function: our results further show that, besides single compounds or foods, the total intake of dietary antioxidants is of a particular relevance. Although other ‘unknown’ bioactive compounds present in foods might act synergistically in protecting the lungs from oxidative stress (Smit *et al*., 1999; Romieu and Trenga, 2001; Kan *et al*., 2008), the influence of the total antioxidant network on pulmonary function has not previously been investigated. TAC might be related to both exogenous and endogenous antioxidant defenses (Zheng *et al*., 2011; Pitsavos *et al*., 2005), further strengthening the hypothesis of a crucial role of redox molecules in improving respiratory health. Associations were statistically significant, independently from confounding factors, in women only, while in men they were lost after adjustment for confounding. Gender differences have previously been observed in respiratory function (Tatsumi, 2009) and oxidative stress (Ochs-Balcom *et al*., 2005). The association between TAC and pulmonary function was stronger in premenopausal women, possibly due to the presence of female hormones and/or the absence of male hormones (Tatsumi, 2009). However, other gender effects unrelated to sex hormones are not excluded (Tatsumi, 2009). On the contrary, the lack of association between TAC intake and pulmonary volumes in men might be attributable to the higher production of whole-body reactive oxygen species observed in healthy men when compared with premenopausal women (Ide *et al*., 2002).

Smoking is another factor influencing the association between TAC and pulmonary function. Smokers had a higher intake of TAC, with foods contributing to TAC intake in our population including coffee consumption, along with alcoholic beverages, fruit and fruit juices and chocolate. However, stratified analysis showed that the association between TAC intake and lung function was only present in never, but not in ever smokers. This is in agreement with a recent study showing a positive association between coffee consumption, and lung function in never and former, but not in current smokers (Nettleton *et al*., 2009). Our results support the deleterious pro-oxidant effects of smoking, which are more potent than the protective effect of the total antioxidant network on lung function (Nettleton *et al*., 2009). Thus, the beneficial effect of TAC could only be detectable in persons who never smoked.

Our finding that a strong significant association between TAC and pulmonary function can be found in premenopausal never smoker women only, reinforces the concept that the influence of dietary antioxidants is better expressed in conditions of low oxidative stress. In premenopausal, never smoking women, where the redox balance should be in an optimal range, dietary antioxidants may inhibit randomly abnormal production of free radicals, supporting endogenous defenses in optimizing pulmonary function.

The extent of the observed association of dietary TAC with pulmonary function, although relatively small, appears to be significant from a public health viewpoint. The differences in pulmonary volumes observed between the fifth and the first quintile of TAC were comparable with those reported by others on antioxidant foods (Strachan *et al*., 1991; Cook *et al*., 1997; Carey *et al*., 1998; Butland *et al*., 2000; Kelly *et al*., 2003; Nettleton *et al*., 2009) or vitamins (Schwartz and Weiss, 1994a; Britton *et al*., 1995; Tabak *et al*., 1999; Schünemann *et al*., 2001a, b; Charles *et al*., 2010) or observed in subjects who developed cardiovascular disease (Schroeder *et al*., 2005). In particular, the +83.4 ml difference in FEV_1 associated with a higher intake of TAC in never smoker premenopausal women was equivalent to an improvement in pulmonary age of 3.3 years. Nevertheless, further studies are needed to clarify the effect of dietary antioxidants on pulmonary function in subjects exposed to different free radical challenges such as smoking. Dietary TAC assessment may help identifying strategies of dietary prevention to avoid pulmonary dysfunction, and reduce the risk of related chronic disease and mortality.

A major strength of this study is that participants were randomly selected from the general population and, therefore, our results can be generalized. Furthermore, we obtained detailed information on several important life-style factors related to pulmonary function, enabling to adjust for their effects in the analysis.

There are, however, some limitations too. First, the cross-sectional nature does not enable determination of causality. Second, TAC is an *in vitro* parameter, therefore some antioxidants contributing to antioxidant activity *in vitro* may be poorly absorbed. Third, we cannot completely rule out the effect of unmeasured (or unknown) confounding factors.

In conclusion, our findings show that dietary TAC may have a favorable role in respiratory health; this is important because reduced pulmonary function is a risk factor for chronic disease and mortality and diets rich in antioxidants may help reducing such a risk.

Conflict of interest

The authors declare no conflict of interest.

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Appendix

Moli-sani Project Investigators

Chairperson: Licia Iacoviello (Campobasso, Italy).

Steering committee: Maria Benedetta Donati (Campobasso, Italy) and Giovanni de Gaetano(Campobasso, Italy) (Chairpersons), Simona Giampaoli (Roma, Italy).

Safety and data monitoring committee: Jos Vermylen (Leuven, Belgio), Chairman, Ignacio De Paula Carrasco (Roma, Italy).

Event adjudicating committee: Deodato Assanelli (Brescia, Italy), Francesco Alessandrini (Campobasso, Italy), Vincenzo Centritto (Campobasso, Italy), Paola Muti (Roma, Italy), Holger Schünemann (Hamilton, Canada)), Pasquale Spagnuolo (Termoli, Italy), Dante Staniscia (Termoli, Italy), Sergio Storti (Campobasso, Italy).

Scientific and organizing secretariat: Francesco Zito (Coordinator, Campobasso and Termoli, Italy), Americo Bonanni (Campobasso, Italy), Chiara Cerletti (Campobasso, Italy), Amalia De Curtis (Campobasso, Italy), Augusto Di Castelnuovo (Campobasso, Italy), Licia Iacoviello (Campobasso, Italy), Antonio Mascioli (Campobasso, Italy), Marco Olivieri (Campobasso, Italy).

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