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G. Riccioni ^a; T. Bucciarelli ^a; B. Mancini ^a; C. Di Ilio ^a; R. Della Vecchia ^b; N. D'Orazio ^a

^a Human Nutrition, Department of Biomedical Sciences, University "G. D'Annunzio", Chieti, Italy ^b "SS Annunziata" Hospital, Respiratory Pathophysiology Center, Chieti, Italy

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ORIGINAL ARTICLE

Plasma Lycopene and Antioxidant Vitamins in Asthma: the PLAVA Study

G. RICCONI, M.D., PH.D.,^{1,*} T. BUCCIARELLI, PROF.,¹ B. MANCINI,¹ C. DI ILIO, PROF.,¹ R. DELLA VECCHIA, M.D.,²
AND N. D'ORAZIO, PROF.¹

¹Human Nutrition, Department of Biomedical Sciences; University "G. D'Annunzio", Chieti, Italy

²Respiratory Pathophysiology Center, "SS Annunziata" Hospital, Chieti, Italy

Objective: Scientific evidence suggests that lycopene and antioxidant vitamins have significant antioxidant and protective effects. **Methods:** This case-control study included 96 subjects (40 asthmatics, 56 healthy control subjects). Baseline blood samples, pulmonary function tests, and clinical and alimentary histories were collected. All subjects were grouped by age, sex, cigarette smoking habit, body mass index, alimentary intake, and atopic status. **Results:** Serum lycopene concentration was significantly lower in asthmatic subjects than in healthy control subjects ($0.10 \pm 0.7 \mu\text{mol/L}$ vs. $0.16 \pm 0.8 \mu\text{mol/L}$ – $p < 0.001$). Serum vitamin A concentration was significantly lower in asthmatics ($2.38 \pm 0.37 \mu\text{mol/L}$) in respect to control subjects ($3.06 \pm 0.56 \mu\text{mol/L}$) ($p < 0.01$). Plasma serum concentration of vitamin E and β -carotene were not found to be different in the two groups. **Conclusions:** Dietary supplementation or adequate intake of lycopene and vitamin A rich foods may be beneficial in asthmatic subjects.

Keywords lycopene, vitamin A, vitamin E, β -carotene, bronchial asthma

INTRODUCTION

Increasing evidence demonstrates that lycopene (lyc), a carotenoid that does not have any provitamin A activity, and antioxidant vitamins present in high concentrations in vegetables and fruits have a significant antioxidant potential effects, suggesting an important role in preventing cardiovascular and cancer disease (1–3). More than 80% of the dietary lyc and antioxidant vitamin intake comes from the consumption of tomato products, including ketchup, tomato juice, and tomato sauce, fruits, and vegetables (4, 5).

A considerable amount of evidence suggests that oxidant stress results in inflammation and tissue damage of the respiratory system and that a lowered cellular reducing capacity increases the risk of developing asthma (6). Dietary, environmental, and genetic factors, which diminish cellular reducing capacity, increase the vulnerability of tissue to oxidative stress and are also likely to increase the risk of asthma (7).

A recent review has evaluated the relationship between serum antioxidant vitamins (A, C, E) and asthma (8), particularly in youth (9), and lower lung function levels in children with an inadequate dietary antioxidant vitamin intake (10).

Regarding bronchial asthma, some published studies view serum concentrations of many antioxidants vitamins, carotenoids, and trace elements among adult populations (11), while others observe the reduction of exercise-induced oxidative stress by a natural antioxidant such as lyc (12, 13). Open questions remain about serum antioxidant plasma concentrations in asthmatic and healthy subjects. The aim of this study was to evaluate plasma concentration of lyc and some types of antioxidant vitamins (A, E, and β -carotene) in asthmatic subjects and control healthy subjects.

METHODOLOGY

Subjects

A total of 96 subjects (56 healthy, 40 asthmatics) were enrolled between January 2005 and September 2006 in the Respiratory Pathophysiology Center ("SS. Annunziata" General Hospital, Chieti, Italy).

Inclusion criteria for the study were the presence of asthma defined by (1) one asthma attack during the previous 12 months and typical asthma symptoms, such as chest tightness, wheezing, and dyspnea or (2) a positive response to methacholine challenge test (MCHt) with provocative cumulative dose causing a 20% reduction in flow expiratory volume at one second ($\text{FEV}_1 \leq 1,600 \mu\text{g/mL}$). The diagnosis was made by a pulmonologist or an internist.

Exclusion criteria for the study were emergency treatment for an asthma exacerbation within 1 month, upper airway infections in the last 4 weeks, hospitalization for asthma during the 3 months before the recruitment, presence of autoimmune disorders, malabsorption, drug or alcohol addiction, pregnancy or lactation, chronic bronchitis, emphysema, cystic fibrosis, bronchiectasis, gastroesophageal reflux disease, diabetes, cancer, and cardiovascular diseases.

Study Design

The **PLAVA** (Plasma Lycopene and Antioxidant Vitamins in Asthma) study is a case-control study including 96 patients: 40 with bronchial asthma and 56 healthy subjects. All patients underwent complete clinical controls, baseline respiratory function test, Methacholine Challenge Test (MCHt), biochemical analyses, and completed the European Prospective Investigation into Cancer and Nutrition (EPIC) alimentary questionnaire (14). The study was approved by the Local Research Ethics Committee of Chieti University, and all subjects provided their informed consent.

*Corresponding author: Graziano Riccioni, MD, PhD, Via G. De Rogatis, 12, 71016 San Severo (FG), Italia; E-mail: griccioni@hotmail.com

Measurements

The subjects came to give blood samples between 8:00 and 10:00 A.M. They were instructed to abstain from ingesting alcohol for 3 days and from smoking and eating for 12 hours before the blood sampling.

All investigators and laboratory personnel were blinded to the subject's case-control status. Blood samples from all subjects were handled identically and blindly through all stages of blood collection, storage, retrieval, and analytic processes. The storage time for plasma in liquid nitrogen freezers was ≈ 12 months until the assays were conducted. Plasma concentrations of vitamin A, vitamin E, lycopene, and β -carotene were measured. In both cases, the controls were assayed in the Biochemical Laboratory of School Medicine in Chieti (Italy) as follows.

A subject was defined as a nonsmoker if he or she had never smoked cigarettes, cigars, or a pipe within the past 30 days. The number of cigarettes, cigars, and pipefuls of tobacco currently smoked daily and the duration of regular smoking in years were recorded on a self-administered questionnaire that was checked by an interviewer.

Body weight was measured by using weighing scales. During the height and weight measurements, the subjects wore light clothing and no shoes. Body mass index (BMI) was computed as the ratio of weight to the square of height (kg/m^2).

Spirometry and Methacholine Challenge Test (MCHt)

Patients had to complete at least three forced vital capacity (FVC) maneuvers to reach the American Thoracic Society (ATS) standards (15). For every group, we assessed FEV₁, peak expiratory flow (PEF), and FVC.

MCHt was performed, following the ATS protocol (16), using a dosimeter (Mefar MB3; Brescia, Italy) and starting by inhaling 6.25 $\mu\text{g}/\text{mL}$ methacholine (lyophilized methacholine 6.4%, Lofarma; Milan, Italy) up to a cumulative dose of 3,200 $\mu\text{g}/\text{mL}$. After basal spirometry (the best among at least three significant tests) and inhalation of a buffered normal saline vehicle solution, patients inhaled increasing doses of methacholine up to a cumulative dose of 3,200 $\mu\text{g}/\text{mL}$; at the end of each inhalation of methacholine, after 90 seconds, a new FEV₁ measurement was performed. MCHt was considered positive when the amount of methacholine, which caused a 20% decrease in FEV₁ (PC₂₀), was $\leq 1,600 \mu\text{g}/\text{mL}$. Basal FEV₁ was obtained from the best among at least three significant tests (differences among the responses $< 5\%$). The test was terminated when FEV₁ had decreased by at least 20% or when the maximum concentration (3,200 $\mu\text{g}/\text{mL}$) had been inhaled. The results were expressed as PC₂₀.

Sample Collection, Storage, and Preparation

Blood samples were collected in polypropylene tubes containing EDTA 1 mM. Samples were stored in a freezer before centrifugation at 3,000 g for 10 minutes at 4°C. 200 μL aliquots of plasma were transferred into foil-wrapped polypropylene tubes. Plasma samples were either used for immediate extraction or stored in the dark at -80°C until the analysis was performed. Sample preparation is as described in Lee et al. (17).

HPLC System and Conditions

Vitamin A, Vitamin E, lycopene, and β -carotene were determined by HPLC. Two water pumps 515 HPLC equipped with Waters auto injector (model 717 plus auto sampler) and Waters 996 photodiode array (PDA) detector were used as HPLC system. Data acquisition and processing were performed using chromatography software, Empower-Pro, by Waters. Analysis was performed by isocratic elution. The flow rate was 1.5 mL/min. The mobile phases used were A-methanol/n-butanol/water (89.5/5/5.5 v/v/v) premixed and vacuum filtered through a 0.45- μm polypropylene membrane filter (Whatman) before use. B-methanol/n-butanol/water (76/19.5/4.5 v/v/v) were premixed and vacuum filtered before use as for A. The elution isocratic profile was as follows 0–7 minutes 100% A; 7–15 minutes 100% B; and 15–20 minutes 100% A for column reequilibration. Auto injections of 20 μL were performed at 5°C. The analytical column used was a replaceable Partisphere 5 C-18 cartridge (110 $\times$ 4.7 mm inner diameter, 5- μm particle size; Whatman) protected by a guard cartridge (C-18, 5 μm) system and maintained at 45°C. PDA wavelength range was from 270 nm to 460 nm, and the chromatograms were extracted at 340 nm for vitamin A, 288 nm for vitamin E, and vitamin E-Acetate (used as internal standard), 441 nm for lycopene and β -carotene. The run time was 20 minutes.

"EPIC" FOOD INTAKE AND NUTRIENTS QUESTIONNAIRE

All subjects completed an alimentary validated questionnaire to determine the alimentary habits and to calculate the contribution of specific nutrients. The EPIC is a validated questionnaire divided into several categories: starters, main dishes, fruit, drinks and cakes. The questions regarding frequency of consumption, subdivided into days, weeks, months and, for some categories, also years, reveal the amount habitually consumed by the individual.

Statistical Analysis

Data were analyzed by using SPSS statistical software (version 10.0 for Windows; SPSS Inc., Chicago). Mean age, BMI, FVC, FEV₁, PEF, PC₂₀, serum lycopene, vitamin A, vitamin E, and β -carotene are reported as mean $\pm$ standard deviation (SD). Cigarette smoking, atopic status, and gender are reported as a percentage. The completed EPIC questionnaire was sent to the National Cancer Institute of Milan (Italy) for data elaboration with an optical reader. The statistical significance of the different concentrations between the two groups was studied using one-way analysis of variance (ANOVA). A p value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the population studied (gender, age, smoking status, BMI, atopic status, FVC, PEF, FEV₁, PC₂₀) included in the study are shown in Table 1.

The mean plasma lycopene concentration in asthmatic subjects (cases) was $0.10 \pm 0.7 \mu\text{mol}/\text{L}$ compared to $0.16 \pm 0.8 \mu\text{mol}/\text{L}$ in the healthy subjects (control group) ($p < 0.001$). The mean plasma vitamin A concentration in asthmatic subjects was $2.38 \pm 0.37 \mu\text{mol}/\text{L}$ compared to $3.06 \pm 0.56 \mu\text{mol}/\text{L}$ in the healthy subjects (control group).

TABLE 1.—Anagraphic details of population study.

Variables	Cases	Control subjects
Subjects (n)	40	56
Gender (M/F)	19/21	27/29
Age (y) [#]	37.1 ± 12.5	36.7 ± 11.8
Smoking (Y/N)*	6/10 (60%)	43/76 (56.57%)
BMI (kg/m ²) [#]	23.42 ± 4.94	24.12 ± 4.21
Atopic status (Y/N)*	21/19	26/30
FVC (% predicted value) [#]	81 ± 12.75	103 ± 13.64
FEV ₁ (% predicted value) [#]	83 ± 10.85	102 ± 11.47
PEF (% predicted value) [#]	80 ± 11.63	101 ± 12.42
PC20 (provocative concentration) [#] μg/mL	875 ± 252	1935 ± 332

*Percentage; [#]mean ± SD.

($p < 0.01$). No appreciable differences in vitamin E ($20.71 \pm 4.51 \mu\text{mol/L}$ vs. 20.93 ± 3.48 and β -carotene ($7.7 \pm 1.5 \mu\text{mol/L}$ vs. $8.1 \pm 1.6 \mu\text{mol/L}$) dietary antioxidant serum concentrations were observed (Table 2).

The daily mean caloric intake of proteins, lipids, carbohydrates, antioxidant vitamins and tomatoes do not show significant differences between the two study groups (Table 3).

DISCUSSION

It is presumed that high-circulating concentrations of lycopene and antioxidant vitamins protect against oxidative stress and its manifestation in bronchial asthma. The main findings of this case-control study are that a low serum lycopene concentration and vitamin A are associated with bronchial asthma.

The Third National Health and Examination Survey (1988–1994) among U.S. adults has examined serum antioxidant concentrations (vitamin A, retinyl esters, vitamin C, vitamin E, vitamin E/cholesterol ratio, vitamin E/triglyceride ratio, α -carotene, β -carotene, β -cryptoxanthin, lutein/zeaxanthin, lycopene, and selenium) in 771 asthmatic subjects and 15,418 subjects without asthma. In this survey, Ford et al. (11) have observed a lower vitamin C concentration in patients with asthma rather than those subjects who have never had asthma.

Wood et al. have reported a lower carotenoid and lycopene concentration in nine asthmatic subjects compared to seven healthy control subjects (18). The authors concluded that asthmatic subjects are relatively deficient in carotenoids, with a consequent increasing susceptibility to oxidative stress; for this reason the authors have hypothesized that dietary carotene supplementation may be beneficial in subjects with bronchial asthma.

This hypothesis has been apparently confirmed by Neumann et al. (12) in a double-blind randomized controlled clinical trial on 20 asthmatic patients with exercise-induced asthma. In this study the daily oral supplementation of lycopene (LYC-O-MATO 30 mg/day) for one week has produced protective effects against exercise-induced asthma in the lycopene-

TABLE 3.—Mean daily intake total Kcal, nutrients, antioxidant vitamins, and tomato in the study population.

Nutrients	Cases	Control	<i>p</i> value
Total Kcal intake	1.693 ± 458	1591 ± 521	<i>ns</i>
Proteins (total g)	91.81	90.12	<i>ns</i>
-Proteins (animal)	60.23	60.37	<i>ns</i>
-Proteins (vegetable)	31.58	30.12	<i>ns</i>
Lipids (total g)	84.09	81.32	<i>ns</i>
-Lipids (animal)	45.59	40.34	<i>ns</i>
-Lipids (vegetable)	38.50	35.41	<i>ns</i>
-Fatty acids (saturated)	29.88	30.12	<i>ns</i>
-Fatty acids (monounsaturated)	43.95	44.58	<i>ns</i>
-Fatty acids (polyunsaturated)	10.26	12.21	<i>ns</i>
Carbohydrates (available – total g)	290.25	300.45	<i>ns</i>
Starch	181.74	178.64	<i>ns</i>
Carbohydrates (soluble)	108.51	102.38	<i>ns</i>
Antioxidant vitamins			
Vitamin A (μ/die)	1012.60	988.24	<i>ns</i>
Vitamin C (μ/die)	142.92	135.21	<i>ns</i>
Vitamin E (μ/die)	8.01	8.15	<i>ns</i>
Tomato			
Raw (g)	18.1	16.8	<i>ns</i>
Cooked (g)	11.5	12.4	<i>ns</i>

supplemented group in respect to placebo. This protective effect was not demonstrated in another randomized controlled trial by Falk et al. (13), who have shown that a daily dose of lycopene (30 mg/day) for one week in 19 young athletes did not affect lung functions after exercise and did not provide any protective effect against clinical difficulty in breathing in young athletes.

Regarding vitamin A, Arora et al. (19) in a case-control study involving 35 asthmatic children and 29 control subjects have observed that the deficiency of vitamin A was four times more common in children with severe persistent asthma, as compared with mild intermittent asthma. A cross-sectional study conducted in Thailand by Bloem et al. (20) on 1,772 children (1–8 years of age) have documented that children with deficient serum retinol had a fourfold greater risk of respiratory disease. In the same study an interventional supplementation trial of oral vitamin A (200,000 IU/die) on 166 children (1–5 years of age) for 2 months documented a reduced incidence of respiratory symptoms when compared to the control group.

There are many hypotheses to explain the altered state of vitamin A in asthma. Children with asthma are known to have frequent exacerbation because of airway inflammation, infection, exertion, or stress. All these factors have been shown to decrease the serum retinol levels by increasing cellular demand for retinol and increasing urinary loss (20–22).

Picado et al. (23) in a case-control study have investigated dietary intake (estimated from a food frequency questionnaire) and plasma/serum level of vitamin A in a group of 118 asthmatic patients with various degrees of severity and compared the results with 121 healthy subjects. They found no difference between patients and healthy subjects both in daily micronutrient/antioxidant intake than in plasma/serum level of vitamin A.

Tomatoes are the most commonly consumed fresh vegetable in the Mediterranean diet. There are emerging data that support the evidence that tomato consumption reduces risk of cardiovascular disease and cancer (1–3). This

TABLE 2.—Serum concentration of antioxidant vitamins and lycopene.

Variables	Cases	Control subjects	<i>p</i>
Serum lycopene (μmol/L)*	0.10 ± 0.7	0.16 ± 0.8	< 0.001
Serum vitamin A (μmol/L)*	2.38 ± 0.37	3.06 ± 0.41	< 0.01
Serum vitamin E (μmol/L)*	20.71 ± 4.51	20.93 ± 3.48	<i>Ns</i>
Serum β -carotene (μmol/L)*	7.7 ± 1.5	8.1 ± 1.6	<i>Ns</i>

*Mean ± SD.

evidence is present even in bronchial asthma, and for this reason tomatoes play an important role in a healthy diet (24).

Carotenoids comprise a class of natural fat-soluble pigments that are found in numerous fruits and vegetables. The consumption of a diet rich in carotenoids and vitamin A has been epidemiologically correlated with a lower risk for several diseases, in particular bronchial asthma (25).

The protective effect of lyc is due to ability of carotenoids to quench singlet oxygen, a potential inhibitor of lipid peroxidation (26). In particular, lyc exhibits the highest physical quenching rate of all carotenoids (27). It has also been shown that serum concentrations of lyc are inversely correlated with serum thiobarbituric acid-reactive substances (TBARS) (28), an indicator of lipid peroxidation.

The results of the present study show that low serum concentrations of lyc and vitamin A are associated with bronchial asthma. Lyc, a biomarker of tomato-rich-food intake, may play a role in the prevention of bronchial asthma; for this reason lyc may have a public health relevance. Asthmatics are relatively deficient in carotenoids, increasing susceptibility to oxidative stress. Dietary supplementation with carotenoids may be beneficial in asthma.

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