

Ordinary article

Dietary micronutrients/antioxidants and their relationship with bronchial asthma severity

Background: Because little is known about micronutrient/antioxidant intake and asthma severity, we investigated dietary intake and plasma/serum levels of micronutrients/antioxidants in a group of asthma patients with various degrees of severity, and compared the results with healthy subjects.

Methods: A case control study was carried out on 118 asthma patients and 121 healthy subjects. The severity of the disease was classified by division of patients into four groups. Normal dietary micronutrient/antioxidant intake was estimated from a food frequency questionnaire. Plasma/serum levels of vitamins C, E, and A, selenium, magnesium, zinc, and platelet glutathione peroxidase (GSH-Px) activity were also determined.

Results: No differences in daily micronutrient/antioxidant intake were seen between patients and healthy subjects. The severity of the disease showed no significant relationship with micronutrient/antioxidant intake. There were no differences in plasma/serum levels in any of the micronutrients/antioxidants between healthy subjects and asthmatics. Nor were any differences found between asthma groups in severity in the biochemical measures, except in platelet GSH-Px activity, which was significantly lower in the most severe groups.

Conclusions: In this study, we found no evidence of any association between micronutrient/antioxidant intake or plasma/serum levels of micronutrients/antioxidants and asthma. Reduction of platelet GSH-Px activity in the most severe patients suggests that these patients have a diminished capacity to restore part of the antioxidant defences.

**C. Picado¹, R. Deulofeu²,
R. Lleónart³, M. Agustí³, J. Mullol³,
L. Quintó³, M. Torra⁴**

¹Servei de Pneumologia i Allergia Respiratoria, ²Servei de Bioquímica Clínica, ³Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), ⁴Servei de Toxicologia, Hospital Clínic, Departament de Medicina, Universitat de Barcelona, Barcelona, Spain

Key words: antioxidants; bronchial asthma; diet; micronutrients.

César Picado
Servei de Pneumologia
Hospital Clínic
Villarroel 170
08036 Barcelona
Spain

Accepted for publication 14 July 2000

Recent studies suggest that an association may exist between a low intake of certain micronutrients and asthma (1). It has also been hypothesized that a deficient antioxidant capacity may also play a role in the pathogenesis of asthma (2).

Human antioxidant defences include ascorbic acid (vitamin C), α -tocopherol (vitamin E), vitamin A, enzymes such as glutathione peroxidase, and trace elements including selenium and zinc.

Low intake of vitamin C has been associated with wheezing (3, 4), increased risk of bronchial hyperresponsiveness (5), and reduced levels of FEV₁ (6, 7). Dietary intake of vitamin E has a positive influence on wheezing (8) and lung function (8). Low dietary intake of vitamin A has been shown to be associated with airflow limitation (9).

Selenium is an essential component of glutathione peroxidase (GSH-Px). It has been suggested that lowered GSH-Px activity due to a low intake of selenium may play a role in asthma (10–13).

Many studies have evaluated the effect of the dietary intake of micronutrients and antioxidants on wheeze (3, 4, 8), lung function (6–9), and bronchial hyperreactivity (5), as assessed by challenge tests. However, neither the presence of wheeze, the demonstration of bronchial hyperresponsiveness, nor low lung function can be used as a substitute for the diagnosis of asthma.

In epidemiologic studies the potential impact of both the severity of the disease and its treatment on the characteristics of asthma patients' diet should also be considered. One example of the possible influence of asthma therapy on the diet is the severe corticosteroid-dependent patient who modifies his/her diet to reduce caloric intake in order to prevent weight gain resulting from the use of systemic corticosteroids. Reduction or modification of dietary intake in these patients can be accompanied by a low intake of micronutrients and antioxidants. Therefore, in order to elucidate the role of dietary factors in asthma, it is important to perform studies on patients with a clearly defined diagnosis of

asthma. In addition, only patients with diets not influenced by food supplementation or avoidance should be included in the study. In a recent study, we reported that asthma is associated with a decrease in energy intake (14). We also found severe asthma with regular oral corticosteroid therapy to be associated with reduced plasma protein and albumin levels (14).

Although a number of studies have evaluated the possible role of dietary micronutrients/antioxidants in asthma, little is known about the influence of either the severity of the disease and/or its treatment on intake and on the plasma/serum levels of these micronutrients.

The objective of our study was to investigate whether a relationship exists between the dietary intake of micronutrients/antioxidants and asthma. We also studied the effects of asthma severity on plasma/serum levels of vitamins, selenium, magnesium, and zinc, and platelet GSH-Px activity.

Material and methods

Study subjects

A total of 150 consecutive asthmatic patients attending the outpatient clinic were asked to take part in the study. They all presented a history of intermittent wheezing, shortness of breath, and chest tightness; they all had a diagnosis of asthma and were taking asthma medication. The severity of the disease was characterized in four groups of patients by a method similar to the one proposed in the Global Initiative for Asthma (GINA) (15). This method was modified in order to include the characteristics of therapy in the classification of the severity of disease. The four groups were as follows: intermittent (group 1), mild persistent (group 2), moderate persistent (group 3), and severe (group 4). Group 1 comprised patients who were on β_2 -adrenergic agents on demand. Group 2 comprised patients who regularly used β_2 -adrenergic agents, with or without low doses of inhaled corticosteroids. Group 3 comprised patients with a continuing history of episodic asthma, most of whom were on regular inhaled corticosteroid therapy, and group 4 comprised patients with a current history of chronic unremitting asthma requiring high doses of inhaled corticosteroids and regular oral corticosteroid therapy, or frequent short courses of oral corticosteroids.

Aspirin-intolerant asthma was deduced from the patient's history. In patients with only one attack precipitated by aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs), aspirin intolerance was confirmed by an oral challenge test with aspirin. In patients with two or more asthma attacks precipitated by aspirin or NSAIDs, the oral test was not carried out.

A total of 150 healthy volunteers were selected as a control population from various sources, including neighbors of patients ($n=112$), relatives of the staff members ($n=10$), and the blood donor population ($n=28$). The control subjects had never had any episode of breathlessness and/or wheezing and had never used asthma medication.

All subjects lived in the area surrounding the hospital with a very homogeneous middle-class population.

Only subjects (patients and healthy volunteers) from the native population were included in the study. Smokers, subjects receiving vitamin supplements, or those who were on an exclusion diet were excluded.

A total of 118 patients and 121 subjects met all the inclusion

criteria and agreed to participate in the study. The subjects gave informed consent to the study, which was approved by the ethics committee of the institution.

The skin prick test was performed with common allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, cat, dog, grass-pollen mixture, tree-pollen mixture, *Parietaria judaica*, *Aspergillus fumigatus*, *Alternaria tenuis*, and cockroach) (Ifidesa-Aristegui, Bilbao, Spain). Histamine (10 mg/ml) and glycerol were used as positive and negative controls. A skin prick reaction was regarded as positive if the wheal size was over 3 mm. Subjects were considered to be atopic if they had a positive reaction to any of the allergens in the testing panel.

Food frequency questionnaire

All subjects completed a food frequency questionnaire (FFQ). We used a 150-item semiquantitative FFQ to assess usual dietary intake over the previous 6 months. A trained dietitian who was unaware of the subjects' characteristics administered the FFQ to all the subjects. Micronutrient/antioxidant intake was computed from the reported frequency of consumption of each specified unit of food or beverage, and from published data on the micronutrient/antioxidant content of the specified portions. To help the subjects to quantify food consumption, the dietitian used photographs of servings with six progressive portions of the reported consumed foods.

Biochemical measurements

A fasting 100-ml sample of venous blood was taken between 8 and 9 a.m. Serum α -tocopherol (vitamin E) was measured by high-performance liquid chromatography (HPLC), by the method of Shearer (16). Serum retinol (vitamin A) was measured by HPLC by the method of Catigiani & Bieri (17). Whole-blood total ascorbic acid (vitamin C), which includes ascorbic and dehydroascorbic acid, was measured by HPLC by the method of Speek et al. (18). Methods for vitamin measurements were initially calibrated with the standard reference material 968b for fat-soluble vitamins, from the National Institute for Standards and Technology (NIST) (Gaithersburg, MD, USA), and were periodically controlled by participation in the Micronutrients Measurement Quality Assurance Program, also from the NIST.

Serum selenium concentration was determined by the direct electrothermal atomic absorption spectrophotometric method with palladium as matrix modifier. We used a Perkin-Elmer 3030 spectrometer, HGA-600 furnace and AS-60 automatic sampler. The L'vov platform, Zeeman background correction, and other specifications of the STPF (stabilised temperature platform furnace) concept were followed (19). Within-day precision, between-day precision, and the accuracy of the method were confirmed by the analysis of Standard Reference Material SERONORM^{TR} (selenium certified value = 86 $\mu\text{g/l}$).

Zinc was measured by atomic absorption spectrophotometry.

Platelet GSH-Px activity was determined by a spectrophotometric assay based on the oxidation of NADPH, by a method previously described in detail elsewhere (20).

Statistics

Dietary intake of selenium, vitamin A, vitamin C, and magnesium was skewed; therefore, a logarithmic transformation was applied to the data before formal analysis. However, summary statistics are reported in the original scale in the text and the tables. Serum vitamin E values were adjusted for total cholesterol (μM vitamin E: mM total cholesterol). Dietary information was analyzed by the method of Willett (21). Correlation between dietary and serum vitamin levels was tested by simple Pearson correlation analyses with crude values. Means of dietary intake and biochemical measurements (adjusted to

Table 1. Demographic data from patients and controls expressed as mean $\pm$ SEM (range), *P* value

	Asthma	Controls	<i>P</i>
<i>n</i>	118	121	
Sex (M/F)	48/70	45/76	NS
Age (years)	41.6 $\pm$ 1.4 (16–72)	38.8 $\pm$ 1.3 (17–74)	NS
Weight (kg)	65.8 $\pm$ 1.2 (40–101)	66.2 $\pm$ 1.3 (45–98)	NS
Height (cm)	164.0 $\pm$ 0.8 (146–186)	164.8 $\pm$ 1.0 (150–188)	NS

total energy intake) were compared between patients and controls, and between the four groups of patients, by an ANOVA model adjusting for age and sex. Results were considered statistically significant if the observed two-sided significance level (*P* value) was not greater than 0.05. Values in the test and tables are means $\pm$ SEM. Statistical analysis was carried out using SPSSWIN 6.1.3 statistical software (SPSS, Inc., 1989–95).

We calculated the empirical power of the study, defined as the percentage of significant tests over 1000 samples, by bootstrapping (22).

Results

Demographic and clinical characteristics

Demographic characteristics were similar in patients with asthma and control subjects (Table 1). The distribution of patients as regards severity is shown in Table 2. Patients from group 1 were significantly (ANOVA, $P < 0.01$) younger than those with moderate (groups 2 and 3) and severe asthma (group 4).

In group 1, there were no patients on inhaled corticosteroids. In group 2, 22 out of 40 patients were on inhaled corticosteroids (180 $\pm$ 100 mg/day, range 0–400 for 11 $\pm$ 19 months). Seventeen out of 24 patients in group 3 were on inhaled corticosteroid therapy (380 $\pm$ 260 mg/day, range 0–800 for 10 $\pm$ 15 months), and 19 out of 24 patients in group 4 were on this therapy (1060 $\pm$ 380 mg/day, range 800–2000, for 9 $\pm$ 16 months). The mean dose of inhaled corticosteroids was significantly higher ($P < 0.05$) in groups 3 and 4 than in group 2. The difference was also significantly different ($P < 0.05$) between groups 3 and 4. Patients from group 4 had a significantly lower FEV₁ (ANOVA, $P < 0.001$) and FVC (ANOVA, $P < 0.01$) than those from groups 1 and 2 (Table 2).

The prevalence of atopy defined according to the results of the prick test was significantly higher in groups 1 (67%) and 2 (61%) with respect to group 4 (39%) (Table 2).

Eighteen patients were aspirin-intolerant. They all belonged to groups 3 (14 patients) and 4 (four patients).

Only patients from group 4 were on regular oral corticosteroid therapy (mean 11.5 mg/day, range 5–20 mg/day) or were receiving frequent short courses of oral steroids.

Food frequency questionnaire

The daily micronutrient/antioxidant intakes are given

by asthma and control groups in Table 3. No differences in daily micronutrient/antioxidant intake were seen between patients and healthy subjects.

No differences in micronutrient/antioxidant intake were found between the four groups of asthma patients (Table 4).

No differences in the characteristics of micronutrient/antioxidant intake were found between atopic and nonatopic subjects after adjusting by age and sex (data not shown).

The empirical power of the study calculated by bootstrapping ranged from low levels for vitamin E (12%, 95% confidence interval 9–16) to moderate levels for vitamin C (42%, 95% confidence interval 36–46).

Biochemical measurements

There were no differences in plasma/serum levels in any of the micronutrients/antioxidants between healthy subjects and asthmatics (Tables 5 and 6). Nor were any differences found between asthma groups as regards severity in the biochemical measurements, except in platelet GSH-Px activity (ANOVA, $P < 0.05$), which was significantly lower in the most severe groups (groups 3 and 4).

Aspirin-intolerant patients did not show any significant difference in micronutrients/antioxidants, either in dietary intake or biochemical measurements, in comparison with aspirin-tolerant patients.

Correlations between food frequency questionnaire and biochemical measures

Correlation between vitamin C intake and blood levels was statistically significant between crude values ($r = 0.47$, $P < 0.001$). After adjustment by total energy intake, the correlation coefficient between vitamin C intake and blood levels was 0.099 (95% confidence intervals, 0.067–0.131). This means that the relationship between vitamin C intake and blood levels was 1/100 (for each 100 units of ingested vitamin C, the blood level increased by 1 unit). No correlation was found between dietary values (crude and total energy adjusted) and biochemical measures of α -tocopherol, retinol, selenium, magnesium, and zinc.

Discussion

We investigated differences in dietary micronutrient/

Table 2. Demographic data from asthmatic patients according to severity expressed as mean $\pm$ SEM (range), *P* value, ANOVA

Severity	1	2	3	4	<i>P</i>
<i>n</i>	30	40	24	24	
Age (years)	29.5 $\pm$ 2.2 (16–63)	40.8 $\pm$ 2.2 (19–72)	47.2 $\pm$ 2.9 (19–67)	50.4 $\pm$ 2.3 (44–99)	<0.05*
Weight (kg)	63.0 $\pm$ 2.6 (44–99)	64.8 $\pm$ 2 (47–101)	65.9 $\pm$ 1.7 (48–79)	70.2 $\pm$ 3 (40–96)	NS
Height (cm)	165.8 $\pm$ 1.6 (150–182)	163.7 $\pm$ 1.6 (148–186)	161.7 $\pm$ 1.7 (146–180)	165.0 $\pm$ 1.9 (148–186)	NS
FEV ₁ (%)	86.0 $\pm$ 1.1 (80–93)	83.0 $\pm$ 1.01 (77–91)	74.0 $\pm$ 1.2 (62–86)	62.0 $\pm$ 2.1 (46–75)	<0.05*
FVC (%)	91.0 $\pm$ 1.3 (82–101)	87.0 $\pm$ 0.9 (81–95)	84.0 $\pm$ 1.2 (75–94)	78.0 $\pm$ 1.9 (68–88)	<0.05*
Atopy (%)	67	61	59	39	<0.05*

* Groups 1, 2 vs 4.

antioxidant intake between asthmatics and nonasthmatics. Only patients with clearly defined asthma and with diets not compromised by food supplementation or avoidance were included. The usual dietary intake was measured by an FFQ. FFQs have been found to relate well to more detailed methods of dietary evaluation (21).

The FFQ-estimated intake of vitamin C was correlated with blood concentration ($r=0.47$). However, we did not find any correlation between dietary nutrient intake and biochemical measurements with the other tested micronutrients/antioxidants. This is in keeping with previous studies, which have generally shown little or no correlation between dietary intake evaluation and biochemical quantification of these micronutrients/antioxidants (21, 23). Significant correlations are more often found in studies in which at least some of the recruited subjects are on supplemented diets (23). However, in our study, these subjects were excluded. Moreover, there are two reasons to explain why plasma/serum levels of micronutrients/antioxidants may not be correlated with dietary intake:

- 1) a single plasma/serum measurement of a micronutrient may be a poor marker of long-term intake detected by FFQ
- 2) plasma/serum levels of some micronutrients/antioxidants do not always reflect the level of their stores (liver, skeleton, and kidney).

We found no evidence of any association between either dietary intake or plasma/serum levels of micronutrients/antioxidants and asthma. Nor did we find evidence that the severity of the disease has any influence on the plasma/serum levels of these substances.

According to our results, no relationship exists between asthma and retinol intake. Troisi et al. (24) found that vitamin E may have a modest effect on the incidence of asthma. We did not find any difference in either vitamin E intake or serum levels between asthma patients and nonasthmatic controls.

Some studies have reported short-term effects of vitamin C in the bronchoprovocation test and improvements in the lung-function test (25), but a beneficial effect of vitamin C was not detected in other studies (26). Olusi et al. (27) and Aderole et al. (28) found significantly higher plasma concentrations of vitamin C in controls than in asthma patients. However, no relationship was detected between vitamin C levels and asthma severity. In contrast, Troisi et al. (24) found no relationship between vitamin C intake and the subsequent development of asthma in women. Nor could Cook et al. (29) find any relationship between plasma vitamin C levels and wheezing.

Table 3. Daily micronutrient/antioxidant intake (crude values) for patients and controls, mean $\pm$ SEM. ANOVA adjusted for total energy intake, sex, and age

	Patients	Controls	<i>P</i>
Magnesium (mg/day)	330.0 $\pm$ 120	363.0 $\pm$ 168	NS
Zinc (mg/day)	10.0 $\pm$ 3	11.0 $\pm$ 3.3	NS
Selenium (μ g/day)	73.0 $\pm$ 20	78.0 $\pm$ 35	NS
Vitamin A (μ g/day)	882.0 $\pm$ 685	827.0 $\pm$ 704	NS
Vitamin C (mg/day)	159.0 $\pm$ 75	165.0 $\pm$ 98	NS
Vitamin E (mg/day)	6.7 $\pm$ 2	6.7 $\pm$ 2.4	NS

Table 4. Daily micronutrient/antioxidant intake (crude values) for patients according to severity. Mean $\pm$ SEM. ANOVA adjusted for total energy intake, sex, and age

	Severity				<i>P</i>
	1	2	3	4	
Patients (<i>n</i>)	30	40	24	24	
Magnesium (mg/dl)	358.0 $\pm$ 155	321.0 $\pm$ 78	336.0 $\pm$ 153	328.0 $\pm$ 69	NS
Selenium (mg/day)	79.0 $\pm$ 17	68.0 $\pm$ 16	67.0 $\pm$ 16	75.5 $\pm$ 27	NS
Zinc (mg/day)	11.3 $\pm$ 3.6	9.9 $\pm$ 2.4	9.6 $\pm$ 2.2	10.3 $\pm$ 3.4	NS
Retinol (vitamin A) (μ g/day)	1005.0 $\pm$ 981	673.2 $\pm$ 419	990.0 $\pm$ 581	968.0 $\pm$ 658	NS
Vitamin C (mg/day)	177.0 $\pm$ 76	147.0 $\pm$ 69.9	162.0 $\pm$ 82	152.0 $\pm$ 74	NS
Vitamin E (mg/day)	7.6 $\pm$ 1.8	6.4 $\pm$ 1.8	6.5 $\pm$ 2.0	6.4 $\pm$ 2.1	NS

Table 5. Plasma/serum values in patients and controls. Results are presented as mean $\pm$ SEM. ANOVA adjusted for sex and age

	Patients	Controls	P
Magnesium (mg/dl)	2.0 $\pm$ 1.2	2.0 $\pm$ 1.1	NS
Zinc (mg/dl)	78 $\pm$ 16	80 $\pm$ 13	NS
Selenium (μ g/dl)	79.0 $\pm$ 1.1	77.5 $\pm$ 2.7	NS
Vitamin A (μ g/dl)	73 $\pm$ 25	72 $\pm$ 24	NS
Vitamin C (μ mol/l)	54 $\pm$ 17	58 $\pm$ 19	NS
Vitamin E (μ mol/l)	28 $\pm$ 7	29 $\pm$ 7	NS
Vitamin E/Chol (mmol/mg)	0.13 $\pm$ 0.01	0.14 $\pm$ 0.01	NS
GSH-Px (mU/10 ⁹ platelets)	156.9 $\pm$ 5.2	145.4 $\pm$ 6.2	NS

Selenium is an essential component of glutathione peroxidase (GSH-Px), which reduces hydrogen peroxidase and other organic peroxides to nontoxic substances. Studies performed to determine a possible relationship between selenium levels and asthma have yielded contradictory results. Stone et al. (13) found that patients with asthma have lower concentrations of selenium in plasma and whole blood, but not in platelets, than controls. However, there was no concomitant reduction in GSH-Px activity in whole blood or platelets. In contrast, Flatt et al. (10) found that in whole blood, but not in plasma, selenium concentration and GSH-Px activity were lower in asthmatics than in healthy subjects. Similarly, reduced platelet GSH-Px activity was found by Misso et al. (11) in patients with asthma. Pearson et al. (12) found that aspirin-tolerant asthmatics had higher serum selenium concentrations than either aspirin-intolerant patients or control subjects. However, only aspirin-intolerant patients with asthma were found to have reduced platelet GSH-Px activity. In contrast, Plaza et al. (20) could not find any significant difference between platelet GSH-Px activity in aspirin-intolerant asthmatics and that in either aspirin-tolerant patients or healthy subjects. It has been suggested that GSH-Px levels may reflect the intensity of the inflammatory activity in asthma. Bibi et al. (30) demonstrated a close correlation between asthma severity and erythrocyte GSH-Px activity. Similarly, Pearson & Suarez-Mendez (31) also observed that platelet GSH-Px activity was lower in patients with severe asthma than in those with mild asthma. In

keeping with this study, we found that platelet GSH-Px activity was significantly lower in patients with the most severe asthma. Since all these studies were cross-sectional, they could not determine whether the low platelet GSH-Px activity is responsible for asthma severity or is merely the consequence of an increased consumption of antioxidants in patients with a more active inflammatory process. In any case, the restoration of normal GSH-Px activity by increasing selenium intake might be a therapeutic alternative in asthma. Hasselmark et al. (32) found that selenium supplementation improved clinical symptoms in asthma patients, suggesting that the restoration of GSH-Px may improve control of bronchial inflammation.

Although Britton et al. (33) found that dietary intake of magnesium was related to lung function, airway hyperreactivity, and self-reported wheezing in the general population, we could not find any difference, either in dietary intake or magnesium serum levels, between patients and healthy subjects. Like us, de Valk et al. (34), and Falker et al. (35) did not find any magnesium deficiency in asthmatics with respect to nonasthmatics, nor did the severity of the disease correlate with serum magnesium levels (35).

The statistical power of our study was low to moderate (20–40%). Therefore, the lack of statistically significant differences in micronutrient/antioxidant intake between asthma and controls may have resulted from the study's being underpowered, resulting in a type 1 error.

The possible relationship between asthma and dietary intake of micronutrients has been deduced from studies which investigated the prevalence of wheezing (3, 4, 8, 33) or the presence of bronchial hyperresponsiveness (5). However, up to 10% of normal subjects are hyper-responsive to bronchoconstrictor stimuli, and wheezing is more prevalent than asthma in the general population (36).

A reduced intake of vitamins A, E, or C is associated with an increased level of airflow obstruction (6–9). Since subjects with nonasthmatic airflow limitation demonstrate histamine or methacholine airway hyper-responsiveness (37), it may well be that a reduced vita-

Table 6. Plasma/serum values in patients according to disease severity. Mean $\pm$ SEM

	Severity				P
	1	2	3	4	
n	30	40	24	24	
Magnesium (mg/dl)	2.0 $\pm$ 0.2	2.0 $\pm$ 0.2	2.0 $\pm$ 0.1	2.0 $\pm$ 0.2	NS
Zinc (mg/dl)	84.0 $\pm$ 14	77.0 $\pm$ 18	77.0 $\pm$ 16	75.0 $\pm$ 11	NS
Selenium (μ g/dl)	77.5 $\pm$ 2.7	76.5 $\pm$ 2.2	77.1 $\pm$ 3.1	79.9 $\pm$ 3.1	NS
Retinol (vitamin A) (μ g/dl)	82.0 $\pm$ 23	76.0 $\pm$ 19	77.0 $\pm$ 27	75.0 $\pm$ 33	NS
Vitamin C (mmol/l)	53.0 $\pm$ 16	53.0 $\pm$ 6	55.0 $\pm$ 17	50.0 $\pm$ 25	NS
Vitamin E (mmol/l)	26.0 $\pm$ 7	28.0 $\pm$ 6	29.0 $\pm$ 9	30.0 $\pm$ 8	NS
GSH-Px (mU/10 ⁹ platelets)	162.5 $\pm$ 9.3	152.7 $\pm$ 11.1	125.0 $\pm$ 13.4	122.5 $\pm$ 16	0.03*

* Groups 1 and 2 vs 3 and 4. ANOVA adjusted for sex and age.

mins A, E, or C intake may predispose to bronchial hyperresponsiveness, simply by reducing airway diameter rather than by inducing asthma.

If the important question is to know whether or not changes in the diet are associated with asthma, it seems more logical to investigate the relationship of diet and asthma than the association of dietary intake and indicators of asthma such as wheezing and hyperresponsiveness.

In summary, we could not find any association between micronutrient/antioxidant intake or plasma/serum levels of micronutrients/antioxidants and asthma. Re-

duction of platelet GSH-Px activity in the most severe patients suggests that their capacity to restore part of the antioxidant defences is diminished.

Acknowledgments

This study was supported by grants from Fondo de Investigaciones Sanitarias (FIS-92/698 and 94/337), Sociedad Española de Neumología y Cirugía Torácica (SEPAR), and CIRIT (1998GR-00112). J.M. was supported in part by a grant from Ministerio de Educación y Ciencia (Spain).

References

- MONTELEONE CA, SHERMAN AR. Nutrition and asthma. *Arch Intern Med* 1997;**157**:23–34.
- HATCH GE. Asthma, inhaled oxidants, and dietary antioxidants. *Am J Clin Nutr* 1995;**61** Suppl 3:625S–630S.
- SCHWARTZ J, WEISS ST. Dietary factors and their relationship to respiratory symptoms: the second National Health and Nutrition Examination Survey. *Am J Epidemiol* 1990;**132**:67–76.
- BODNER G, GODDEN D, BROWN K, LITTLE J, ROSS S, SEATON A. Antioxidant intake and adult-onset wheeze: a case-control study. *Eur Respir J* 1999;**13**:22–30.
- SOUTAR A, SEATON A, BROWN K. Bronchial reactivity and dietary antioxidants. *Thorax* 1997;**52**:166–170.
- BRITTON JR, PAVORD ID, RICHARDS HA, et al. Dietary antioxidant vitamin intake and lung function in the general population. *Am J Respir Crit Care Med* 1995;**151**:1383–1387.
- SCHWARTZ J, WEISS ST. The relationship of dietary vitamin C intake to level of pulmonary function in the First National Health and Nutrition Survey (NHANES I). *Am J Clin Nutr* 1994;**59**:110–114.
- DOW L, TRACEY M, VILLAR A, et al. Does dietary intake of vitamins C and E influence lung function in older people? *Am J Respir Crit Care Med* 1996;**154**:1401–1404.
- MORABIA A, SERENSON A, KUMANYIKI S, et al. Vitamin A, cigarette smoking and airway obstruction. *Am J Respir Crit Care Med* 1989;**140**:1312–1316.
- FLATT A, PEARCE N, THOMSON CD, SEARS MR, ROBINSON MF, BEASLEY R. Reduced selenium in asthmatic subjects in New Zealand. *Thorax* 1990;**45**:95–99.
- MISSO NL, POWERS KA, GILLON RL, STUART GA, THOMPSON PJ. Reduced platelet glutathione peroxidase activity and serum selenium concentration in atopic asthmatic patients. *Clin Exp Allergy* 1996;**26**:838–847.
- PEARSON DJ, SUAREZ-MENDEZ VJ, DAY JP, MILLER PF. Selenium status in relation to reduced glutathione peroxidase activity in aspirin-sensitive asthma. *Clin Exp Allergy* 1991;**21**:203–208.
- STONE J, HINKS LJ, BEASLEY R, HOLGATE ST, CLAYTON BA. Reduced selenium status of patients with asthma. *Clin Sci* 1989;**77**:495–500.
- PICADO C, DEULOFEU R, LLEONART R, et al. Lipid and protein metabolism in asthma. Effects of diet and corticosteroid therapy. *Allergy* 1999;**55**:569–575.
- Global Initiative for Asthma. Global strategy for asthma management and prevention. NHLBI/WHO workshop. National Institutes of Health, National Heart, Lung and Blood Institute 1995, Publication no. 95–3659:1–176.
- SHEARER MJ. Vitamin E. In: CK LIM, editor. HPLC of small molecules. London: Oxford University Press, 1986:177–182.
- CATIGIANI GL, BIERI JG. Measurement of serum retinol by high performance liquid chromatography. *Clin Chem* 1983;**29**:708–712.
- SPEEK AJ, SCHRIJVER J, SCHREURS WHP. Measurement of total ascorbic acid in biological fluids. *J Chromatogr* 1984;**305**:53–59.
- VAN CAUWENBERG R, ROBBERECHT H, VAN DAEL P, DEELSTRA H. Direct selenium determination in human whole blood by graphite furnace atomic absorption spectrometry with deuterium correction using a L'vov platform, a Pd/Mg matrix modification and appropriate dilution. *J Trace Elem Electrolytes Health Dis* 1990;**4**:127–131.
- PLAZA V, PRAT J, ROSSELLO J, et al. *In vitro* release of arachidonic acid metabolites, glutathione peroxidase, and oxygen-free radicals from platelets of asthmatic patients with and without aspirin intolerance. *Thorax* 1995;**50**:490–496.
- WILLETT WC. Nutritional epidemiology. New York: Oxford University Press, 1990.
- BRADLY E, TIBSHIRANI RJ. An introduction to Bootstrap. New York: Chapman & Hall, 1993.
- JACQUES PF, SULSKY SI, SADOWSKI JA, PHILLIPS JCC, RUSH D, WILLETT WC. Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. *Am J Clin Nutr* 1993;**57**:182–189.
- TROISI RJ, WILLETT WC, WEISS ST, TRICHOPOULOS D, ROSNER B, SPEIZER FE. A prospective study of diet and adult-onset asthma. *Am J Respir Crit Care Med* 1995;**151**:1401–1408.
- COHEN HA, NEUMAN I, NAHUM H. Blocking effect of vitamin C in exercise-induced asthma. *Arch Pediatr Adolesc Med* 1997;**151**:367–370.
- MALO JL, CARTIER A. Lack of acute effects of ascorbic acid on spirometry and airway responsiveness to histamine in subjects with asthma. *J Allergy Clin Immunol* 1986;**78**:1153–1158.
- OLUSI SO, OJUTIKU OO, JESSOP WJE, IBOKO MI. Plasma and white blood cell ascorbic acid concentrations in patients with bronchial asthma. *Clin Chim Acta* 1979;**92**:161–166.
- ADERELE WR, ETTIE SI, ODUWOULE O, IKPEME SJ. Plasma vitamin C (ascorbic acid) levels in asthmatic children. *Afr J Med Sci* 1985;**14**:115–120.
- COOK DG, CAREY IM, WHINCUP PH, et al. Effect of fresh fruit consumption on lung function and wheeze in children. *Thorax* 1997;**52**:628–633.
- BIBI H, SCHLESINGER M, TABACHNIK E, SCHWARTZ Y, ISCOVITZ H, IAINA A. Erythrocyte glutathione peroxidase activity in asthmatic children. *Ann Allergy* 1988;**61**:339–340.
- PEARSON J, SUAREZ-MENDEZ J. Abnormal platelet hydrogen peroxide metabolism in aspirin hypersensitivity. *Clin Exp Allergy* 1990;**20**:157–163.

32. HASSELMARK L, MALMGREN R, ZETTERSTRÖM O, UNGE G. Selenium supplementation in intrinsic asthma. *Allergy* 1993;**48**:30–36.
33. BRITTON J, PAVORD I, WISNIEWSKI A, et al. Dietary magnesium, lung function, wheezing, and airway hyperreactivity in a random adult population. *Lancet* 1994;**344**:357–362.
34. DE VALK HW, KOK PT, STRUYVENBERG A, et al. Extracellular and intracellular magnesium concentrations in asthmatic patients. *Eur Respir J* 1993;**6**: 1122–1125.
35. FALKER D, GLAUSER J, ALLEN M. Serum magnesium levels in asthmatic patients during acute exacerbations of asthma. *Am J Emerg Med* 1992;**10**:1–3.
36. COCKCROFT DW. Airway hyper-responsiveness. Relevance of random population data to clinical usefulness. *Am Rev Respir Dis* 1990;**142**:497–500.
37. RAMSDALE EH, MORRIS MM, ROBERTS RS, HARGRAVE FE. Bronchial responsiveness to methacholine in chronic bronchitis: relationship to airflow obstruction and cold air responsiveness. *Thorax* 1984;**39**: 912–918.