Antioxidant Supplementation and Lung Functions among Children with Asthma Exposed to High Levels of Air Pollutants

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To evaluate whether acute effects of ozone, nitrogen dioxide, and particulates with mass median diameter less than 10 μm could be attenuated by antioxidant vitamin supplementation, we conducted a randomized trial using a double-blinded design. Children with asthma (n = 158) who were residents of Mexico City were randomly given a daily supplement of vitamins (50 mg/day of vitamin E and 250 mg/day of vitamin C) or a placebo and were followed from October 1998 to April 2000. Pulmonary function tests were carried out twice a week in the morning. During the follow-up observation period, the mean 1-hour maximum ozone level was 102 ppb (SD = 47), and the mean 24-hour average PM_{10} level was 56.7 μg/m³ (SD = 27.4). In children with moderate and severe asthma, ozone levels 1 day before spirometry were inversely associated significantly with forced expiratory flow (FEF_{25-75}) (−13.32 ml/second/10 ppb; p = 0.000), FEV_1 (−4.59 ml/10 ppb; p = 0.036), and peak expiratory flow (PEF) (−15.01 ml/second/10 ppb; p = 0.04) in the placebo group after adjusting for potential confounding factors. No association between ozone and lung functions was observed in the supplement group. We observed significant differences in lung function decrements between groups for FEF_{25-75} and PEF. Our results suggest that supplementation with antioxidants might modulate the impact of ozone exposure on the small airways of children with moderate to severe asthma.

Keywords: childhood asthma; antioxidants; air pollution; Mexico

Exposure to oxidants from different sources including cigarette smoke, air pollutants, and endogenous sources has been related to asthma incidence or severity (1, 2), and experimental and epidemiologic studies have suggested that antioxidant supplementation could modulate the acute change in lung functions observed among people exposed to photo-oxidants (3–10). It is therefore reasonable to state that antioxidants could play an important role in asthma.

The role of antioxidant supplementation on air pollutant toxicity has been studied both in animals and humans. The results suggest that increased intake of antioxidants modulates the pulmonary response to exposure to photo-oxidants such as ozone or nitrogen dioxide (NO_2) (11, 12). Studies on ozone inhalation toxicology show that vitamin E supplementation protects against lipid peroxidation and that levels of vitamin E in the lung tissue of animals receiving vitamin supplements increased significantly after the animals were exposed to ozone, whereas vitamin E levels in the lung tissue of animals that did not receive supplements decreased. This observation suggests that vitamin E is mobilized toward the lung tissue in response to oxidative stress (13). Controlled studies in humans, on both healthy subjects (5, 9) and individuals with asthma (6), have also suggested that antioxidant supplementation (vitamin C and vitamin E) may protect against the acute effects of ozone on lung functioning. Vitamin E and vitamin C are known to inhibit lipid peroxidation and may decrease the production of prostaglandin E_2, a metabolite of arachidonic acid produced by lipid peroxidation of lung cells after ozone exposure (14, 15). New evidence from randomized supplementation studies conducted in healthy, free-living adults in the Netherlands (7, 8) and Mexico (10) strongly supports the hypothesis that antioxidant supplementation protects against the acute effects of ozone on lung function. In addition, the lining fluid of patients with asthma has been reported to be low in vitamin C and vitamin E, and the presence of increased amounts of oxidized glutathione indicates that patients with asthma are subject to increased oxidative stress (16).

The Metropolitan Area of Mexico City experiences significant air pollution problems because of high levels of ozone, with the 1-hour daily maximum average ozone frequently exceeding 110 ppb (Mexican norm) (17). Studies conducted among children with asthma residing in Mexico City have shown a decrement in pulmonary functions and an increase in respiratory symptoms (18–20). We therefore conducted a study among children with asthma residing in Mexico City to determine if antioxidant supplementation could modulate the adverse effect of exposure to air pollutants on lung function of these children.

METHODS

Study Population

Children were recruited through the allergy clinic of the Hospital Infantil de Mexico Federico Gómez. All children were observed over a period of time for their asthmatic condition at this clinic and were classified according to the National Heart, Lung, and Blood Institute (21) as intermittent, mild persistent, moderate persistent, or severe. One hundred and sixty children were recruited and followed for 12 weeks in groups of 40 in a sequential manner (from October 1998 to April 2000). Parents were informed about the study and asked to sign

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a consent form to allow the participation of their child. The ethical committees of the Hospital Infantil de Mexico and the Instituto Nacional de Salud Pública reviewed the protocol.

For baseline information, parents filled out a general-purpose questionnaire on sociodemographic, health, and risk factors for asthma. In addition, parents filled out a food-frequency questionnaire regarding their child's dietary intake. Parents were also provided with a journal and were instructed to record daily symptoms and peak flow measurement twice a day and to note an asthma attack as well as the medication used. Children performed a spirometric test, and blood samples were obtained. Randomization to receive the supplement (250 mg/day of vitamin C and 50 mg/day of vitamin E, or 50 IU of DL-α-tocopherol-acetate) or placebo was conducted in a double-blind manner. None of the health personnel at the clinic or the patients knew the assignment code. Placebos and supplements were similar in appearance and aftertaste (provided by Roche Laboratory, Mexico City, Mexico). Parents received a supply for 1 week and were instructed to give a pill to their child once daily. On the following week, parents returned the empty container and were provided with a new one.

During the 12-week follow-up period, children performed two spirometric tests per week. Before each spirometry, mothers were asked whether their children had had respiratory symptoms and/or had received any medication on that day. If at the time of the spirometry the child presented respiratory symptoms, then he or she was evaluated by a physician who decided if the spirometry could be performed. Each week the study team reviewed the diaries with the mother at the time of her visit for the spirometry, and a new diary was provided. Lung functions were measured with a spirometer (Medifacts Pneumotachograph, San Clemente, CA) according to the specification of the American Thoracic Society (22). All spirometric tests were conducted in the morning and at around the same time every week for each subject to minimize the effect of diurnal variation on pulmonary functions. Before testing, the volume reading of each spirometer was calibrated with a 3-L syringe. The spirometric test was conducted in a room under conditions of stable temperature and relative humidity. All lung function tests were examined by the same pneumologist, and the best of three technically acceptable maneuvers was selected for each test. Additional blood samples were obtained after 6 weeks of follow-up and at the end of the follow-up to check compliance with the supplementation. Two participants did not complete the follow-up because their mothers found it too cumbersome to visit the asthmatic clinic twice a week, and so they were excluded from the analysis.

Exposure Assessment

We obtained measurements of nitrogen dioxide (NO_2), sulfur dioxide (SO_2), particulates (with mass median diameter less than 10 μm [PM_{10}]), ambient ozone, and climatic variables (relative humidity, and minimum, maximum, and daily average temperatures) from the Mexican government's air monitoring stations. SO_2 was measured with an ultraviolet fluorescent analyzer (analyzer-model 100, Advanced Pollution Instrumentation [API], San Diego, CA), and NO_2 was measured using chemiluminescence (analyzer-model 200 MCA, API). Ozone levels were measured via UV photometry (analyzer-model 400, API). For measuring PM_{10}, the particle Mass Monitor (Series 1,400 Sensor Unit of Rupprecht and PataSchnick Co., Inc., Albany, NY) was used. The residence of each child who participated in this study was located using a map, and each child was assigned to the monitoring station that was closest to his or her residence. None of the monitoring stations was located more than 5 km distant from a child's residence. Given the low concentrations observed for SO_2, we decided to drop this pollutant from further analysis.

Biochemical Analysis

Blood samples were collected on ethylenediamine tetraacetic acid vacutainer tubes, immediately covered with aluminum foil, and centrifuged to obtain plasma. Aliquots were stored at −70°C and shipped on dry ice to the laboratory of Dr. Gary Hatch at the U.S. Environmental Protectoin Agency (EPA) for analysis. To determine α-tocopherol, the samples were minimally thawed and diluted with an equal volume of absolute ethanol containing 1 μg/ml butylated hydroxyanisole. One milliliter of heptane was added and the sample vortexed. This sample was centrifuged at 1,000 rpm (5 minutes) at 4°C. The heptane layer was removed and another 1 ml of fresh heptane added. The sample was again vortexed and centrifuged as before until three volumes of heptane had been added. These volumes were combined and dried under nitrogen. The residue was dissolved in 0.2 ml of methanol and analyzed by high-performance liquid chromatography with electrochemical detection (23).

Statistical Analysis

Baseline data on lung functions, collected before supplementation started, were not used in the analysis to avoid learning effects. We studied the effects of ozone, NO_2, and PM_{10} exposure on changes in the pulmonary function parameters on days of the spirometry and on the previous days. We used different averages of ozone exposure (1-hour max, 24-hour average, 8-hour moving average) but used the 1-hour max as exposure to ozone in the final analysis because of the high correlation between these measurements. For NO_2 and PM_{10} we also used different averages (1-hour max, 24-hour average for NO_2, and 1-hour max, 24-hour average, and 8-hour moving average for PM_{10}). Pearson correlation was determined between levels of air contaminants and various climatic variables (24). Data from the food-frequency questionnaire were transformed into nutrient intake using the program Scorenut developed by the National Institute of Public Health for Mexican diet (25).

Baseline characteristics were compared between the two groups; for continuous variables we used a *t* test with previous verification of the normality distribution assumption, and for dichotomous variables we used a chi-square test (24).

We analyzed data using generalized estimating equation models. These models have been described by Liang and Zeger (26). A key feature of this approach is that it models the marginal expectation of the response variable as a function of explanatory variables, taking into account the within-child correlation and allowing for the use of time-dependent covariates. This gives the regression coefficients the usual interpretation.

We first generated bivariate generalized estimating equation models by studying the association between air pollutant exposure and the results of the pulmonary function tests, FEV_1, FVC, forced expiratory flow (FEF_{25-75}), and peak expiratory flow (PEF). We adjusted models for potential confounding factors including participants' age, age^2, height, height^2, and exposure to environmental tobacco smoke, temperature (minimum, mean, and maximum), and maximum relative humidity. In the final models, we only adjusted for participants' height, minimum temperature, the use of inhaled corticoids and bronchodilators (BD), and the presence of an asthma attack or dyspnea on the day of the spirometry because the inclusion of other variables did not modify the results. In the second model, the analysis was restricted to children with moderate and severe asthma (excluding children with mild asthma), and in the third model, ozone, NO_2, and PM_{10} were included. We compared regression coefficients using a *t* test (14) to estimate the effect of the supplementation and conducted all analyses using Stata software, Ver. 70 (Stata Corporation, College Station, TX) (27).

RESULTS

Study Population

Table 1 presents the characteristics of the population according to the assigned groups: placebo or supplement group. There was no significant difference in the major characteristics between the two groups. The mean age of the participants was 8.6 years (range 6–16 years) in the supplement group and 8.9 years in the placebo group (range 6–17 years).

Of the participants, 59% were classified as suffering from moderate (n = 43) or severe (n = 4) asthma in the supplement group, whereas in the placebo group 45% were classified as suffering from moderate (n = 33) or severe (n = 2) asthma. Only three participants were nonatopic. Skin tests showed positive results most frequently for dermatophagoides, cat, *Blatella germanica*, and histamin. Baseline pulmonary tests were also similar between the two groups: 2.15 L (SE = 0.95) for FVC in the placebo group and 1.97 L (SE = 0.52) in the supplement group (p = 0.18); 1.74 L (SE = 0.70) for FEV_1 in the placebo

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION OF CHILDREN WITH ASTHMA RESIDING IN MEXICO CITY, 1998–2000

Characteristics	Supplement (*n* = 80)	Placebo (*n* = 78)	p Value
Sex, % male	66.7	63.3	0.66
Age, yr	8.6	8.9	0.38
Time residing in Mexico City, yr	8.8	9.1	0.47
Weight, kg	3.2	3.2	0.79
Age at asthma diagnosis, yr	3.3	3.3	0.97
Number of crises of asthma in the last 12 mo	2.6	2.6	0.94
Asthma severity			
Mild, %	41	55	0.07
Moderate and severe, %	59	45	0.07
Chronic cough in the last 12 mo, %	81	74	0.29
Wheezing in the last 12 mo without a cold, %	47	54	0.63
Rhinitis, %	36	45	0.27
Antibiotics used in last 12 mo, %	58	43	0.07
Use of inhaled medicine ever, %	95	88	0.14
Smoking at home			
Mother, %	21	14	0.26
Father, %	46	46	0.99
Others, %	18	23	0.44
Pets at home*, %	53	53	0.99
Dog, %	43	36	0.39
Cat, %	4	13	0.04
Birds, %	18	19	0.78
Cockroach*, %	18	28	0.14

* Proportion of homes for which parents reported having pets or pest.

TABLE 2. AIR POLLUTION LEVELS DURING THE STUDY FROM THE MEXICO CITY MONITORING NETWORK, 1998–2000

	Mean	SD	Minimum	Maximum
PM_{10}, μg/m³				
24-h average	56.68	27.36	9.92	249.06
8-h moving average, max	81.08	39.22	11.25	447.25
1-h maximum	137.42	78.67	17	594
O_3, ppb				
24-h average	32	12	7	80
8-h moving average, max	69	31	9.5	184
1-h maximum	102	47	12	309
NO_2, ppb				
24-h average	30	15	5	110
1-h maximum	66	39	8	298
SO_2, ppb				
24-h average	33	31	1	367
1-h maximum	15	10	1	115
Minimum temperature, °C	10.06	3.38	0.6	18.8
Maximum humidity	53.58	17.02	0.1	100
Minimum humidity	18.32	13.97	0.1	73

Definition of abbreviations: NO_2 = nitrogen dioxide; O_3 = ozone; PM_{10} = particulates with mass median diameter less than 10 μm; SO_2 = sulfur dioxide.

group and 1.65 L (SE = 0.52) in the supplement group ($p = 0.34$); 1.84 L/second (SE = 0.79) for $FEF_{25–75}$ in both groups; and 3.97 L/second (SE = 1.69) for PEF in the placebo group and 3.64 L/second (SE = 1.29) in the supplement group. The number of spirometric tests per child was similar between groups and ranged from 12 to 28 with a mean of 22 tests.

Baseline medians (interquartile range) of reported total caloric intake (kcal) of participants were 1,457 (716) in the placebo group and 1,566 (767) in the supplement group. Similarly, there was no difference in vitamin C and α-tocopherol intake between the two groups. The estimates of vitamin C and vitamin E intakes (mg) were, respectively, 72.7 (52.2) and 4.6 (2.5) in the placebo group, and 73.8 (80.9) and 4.7 (3.8) in the supplement group.

Exposure Data

Table 2 presents the levels of the air pollutants during the study period. The ozone 1-hour maximum ranged from 12 to 309 ppb, with a mean of 102 ppb. The 24-hour average of NO_2 ranged from 5 to 110 ppb, with a mean of 30 ppb. The 24-hour average of PM_{10} ranged from 9.92 to 249.06 μg/m³, with a mean of 56.68 μg/m³. The ozone 1-hour maximum was positively correlated with NO_2 and PM_{10} ($r = 0.58$, $p < 0.001$; $r = 0.53$, $p < 0.001$, respectively). The mean of the minimum temperature during the study period was 10.06°C (SD = 3.38°C). Ozone, NO_2, and PM_{10} were negatively correlated with minimum temperature ($r = -0.24$, $p < 0.001$; $r = -0.60$, $p < 0.001$; and $r = -0.51$, $p < 0.001$, respectively).

Blood Data

Blood samples were collected from the participants at the beginning of the study, after follow-up at 6 weeks, and at the end of the study. Baseline plasma α-tocopherol levels were similar between the placebo and supplement groups. Plasma α-tocopherol levels were significantly higher in the group receiving supplement than those in the placebo group after follow-up at 6 and 12 weeks. Plasma α-tocopherol levels increased significantly in the supplement group between the beginning of the study and follow-up at 6 and 12 weeks ($p < 0.01$). No significant change was observed in the placebo group (Table 3).

Lung Function Measurements and Air Pollutant Exposure

Table 4 presents the results of the statistical analyses of lung function parameters and ozone concentrations (1-hour max) 1 day before the spirometric test for the supplement and placebo groups. After adjustments for age, height, minimum temperature, use of inhaled corticoids and BD, as well as the presence of symptoms on the day of the spirometry and time since the beginning of the study, ozone levels were negatively correlated with lung function parameters (FEV_1, $FEF_{25–75}$) in the placebo group (Model 1). These decrements were statistically significant only for $FEF_{25–75}$. The amplitudes of the decrements were larger when the analysis was restricted to children with moderate and severe asthma and were significant for FEV_1, $FEF_{25–75}$, and PEF

TABLE 3. α-TOCOPHEROL CONCENTRATIONS, COMPARISON BETWEEN PLACEBO AND SUPPLEMENT GROUPS AT FIRST, SIXTH, AND TWELFTH WEEK OF THE FOLLOW-UP IN CHILDREN WITH ASTHMA IN MEXICO CITY, 1998–2000

	Alpha-tocopherol (*ng/ml*)				
Subgroup	*n*	Mean	± SD	Range	p Value*
Baseline†					
Supplement group	77	2,998.5	1,020.6	1,194–5,383	
Placebo group	77	3,125.8	1,086.4	1,057–6,564	0.455
Week 6					
Supplement group	80	4,120.7	1,361.8	696–6,850	
Placebo group	78	2,801.7	1,131.0	508–6,737	< 0.001
Week 12†					
Supplement group	73	4,198.1	1,751.4	1,679–10,233	
Placebo group	76	2,883.2	855.3	525–5,617	< 0.001

* p Value was obtained by *t* test comparing supplement and placebo groups.
† Baseline blood samples were unavailable for three children in the supplement group and one child in the placebo group. At Week 12, blood samples were not obtained for seven children in the supplement group and two children in the placebo group.

TABLE 4. CHANGE IN LUNG FUNCTION RELATED TO OZONE (10 PPB) CONCENTRATIONS 1 d BEFORE SPIROMETRY (P VALUE WAS OBTAINED BY *t* TEST COMPARING THE COEFFICIENTS OF SUPPLEMENT AND PLACEBO GROUPS)

Lung Function	Subgroup	Model 1* β (*SE*)	Model 2† β (*SE*)	Model 3‡ β (*SE*)
FVC, ml/10 ppb	Supplement	0.67 (1.55)	0.10 (2.17)	0.05 (2.32)
	Placebo	0.54 (1.64)	−3.20 (3.30)	−1.91 (3.02)
	Effect of supplement	0.49	2.61	1.96
FEV_1, ml/10 ppb	Supplement	0.19 (1.35)	−0.18 (1.84)	−0.06 (1.96)
	Placebo	−0.90 (1.26)	−4.70‖ (1.97)	−4.59‖ (2.18)
	Effect of supplement	1.09	4.52¶	4.5¶
$FEF_{25–75}$, ml-s/10 ppb	Supplement	1.01 (2.63)	−0.44 (3.34)	−0.93 (3.53)
	Placebo	−5.75‖ (2.36)	−12.03§ (3.44)	−13.32§ (3.73)
	Effect of supplement	6.76‖	11.58‖	12.39‖
PEF, ml-s/10 ppb	Supplement	5.13 (4.36)	5.70 (5.58)	6.89 (5.97)
	Placebo	2.40 (4.65)	−13.25‖ (6.60)	−15.01‖ (7.30)
	Effect of supplement	2.73	16.67‖	21.90‖

Definition of abbreviations: $FEF_{25–75}$ = forced expiratory flow between 25% and 75%; GEE = generalized estimating equation model; PEF = peak expiratory flow.

* Model 1 GEE includes all children adjusted for age, height, minimum temperature, use of inhaled corticoids and bronchodilator, respiratory symptoms on the day of the spirometric test, and time since the beginning of the study. The model includes 80 subjects and 1,770 observations in the supplement group, and 78 subjects and 1,719 observations in the placebo group.

† Model 2 GEE includes only moderate and severe asthmatics, adjusted for height, minimum temperature, use of inhaled corticoids and bronchodilator, respiratory symptoms on the day of the spirometric test, and time since the beginning of the study. The model includes 47 subjects and 1,056 observations in the supplement group, and 35 subjects and 799 observations in the placebo group.

‡ Model 3 is similar to model but adjusted for NO_2 and PM_{10} (24-hour average). The model includes 47 subjects and 1,051 observations in the supplement group and 35 subjects and 796 observations in the placebo group.

§ $p \leq 0.01$.

‖ $0.01 \leq p < 0.05$.

¶ $0.05 \leq p < 0.10$.

(Model 2). In contrast, in the supplement group, none of the lung function parameters was associated with ozone concentrations. The difference between placebo and supplement groups was statistically significant for $FEF_{25–75}$ and PEF ($p < 0.001$) and only marginally significant for FEV_1 ($p < 0.10$). As observed for ozone, NO_2 levels (24-hour average of the previous day) were negatively correlated with $FEF_{25–75}$ and FEV_1 in the placebo group but not in the supplement group; however, none of these associations reach statistical significance (change for 1 ppb of NO_2 in children with moderate and severe asthma corresponding to Model 2 presented in Table 4 for ozone: $FEF_{25–75}$, −1.63 ml/second, $p = 0.17$ in placebo group and −0.11 ml/second, $p = 0.97$ in the supplement group; FEV_1, −1.00 ml, $p = 0.15$ in the placebo group and −0.29 ml, $p = 0.81$ in the supplement group; FVC, −1.26 ml, $p = 0.21$ in the placebo group and −0.03 ml, $p = 0.97$ in the supplement group; PEF, −0.93, $p = 0.70$ in the placebo group and −0.34, $p = 0.88$ in the supplement group). For PM_{10} levels, results were less consistent, and we did not observe significant changes in lung function parameters in either the placebo or the supplement groups.

During the follow-up period, 82.9% of the children with moderate and severe asthma in the supplement group and 80% in the placebo group reported using inhaled corticoids. In both groups, use of inhaled corticoid was positively correlated with lung function; we did not observe evidence of interaction between corticoid use and vitamin supplementation. Similarly, we did not observe an interactive effect between the use of a BD and vitamin supplementation (data not shown).

In multipollutant models including ozone, NO_2, and PM_{10} levels, we observed significant decrements in lung function related to ozone exposure in the placebo group, but no such significant changes were observed in the supplement group (Model 3) (Figure 1). As can be seen in Model 2, the difference between the two groups was significant for $FEF_{25–75}$ and PEF. The amplitude of the modulating effect was very similar to that observed in the monopollutant model (Model 2).

We also investigate a potential synergic effect between ozone and PM_{10} by stratifying the data into quartiles on the basis of the levels of PM_{10} and comparing the results of the models that included ozone and PM_{10} with those results restricted to the upper quartile of the PM_{10} distribution ($PM_{10} > 70\ \mu g/m^3$), including the same children in each analysis. On days following high PM_{10} levels, the decrements in $FEF_{25–75}$ and FEV_1 in the placebo group were larger and the modulating effect of the supplement was higher than that observed in the models including all days; however, there was no significant difference between the estimates of these models, in part, because of the small sample size (data not shown).

We repeated the analyses, exploring the impact of several days of ozone exposure on changes in lung functions. The impact in the placebo group was slightly larger than that observed with a single day exposure and increased with the number of days included in the cumulative average up to 6 days before the spirometry. No significant change was observed in the supplement group.

DISCUSSION

The results of this study suggest that vitamin supplementation might protect against the acute effect of ozone on lung function. This protective effect appeared to be greater among children with moderate asthma than among those with mild asthma. Ozone levels were significantly related to a decrement in $FEF_{25–75}$, FEV_1, and PEF in the placebo group but not in the group receiv-

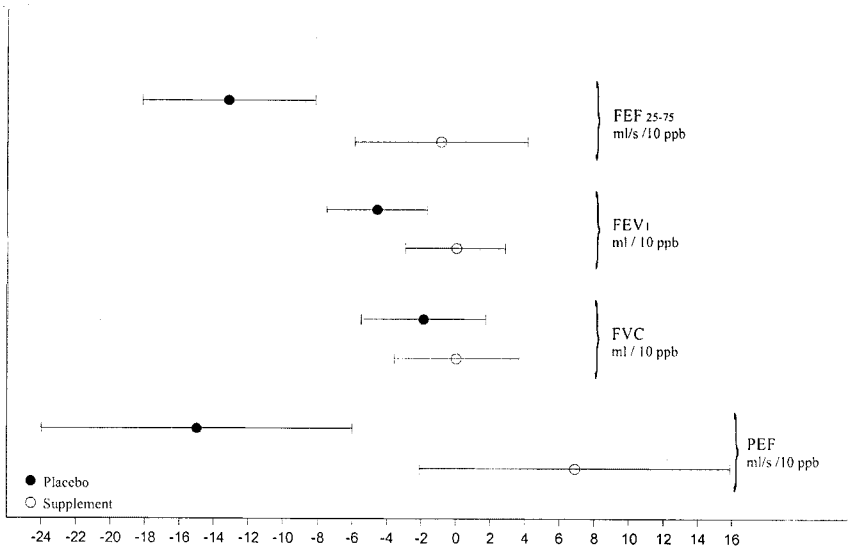

Figure 1. Change in lung functions of patients with moderate and severe asthma per 10 ppb change in maximum hourly levels of ozone, 1 day before spirometry. We present the point estimates as well as the 95% confidence interval around the estimate (on the basis of the pooled variance from the *t* test comparing the supplement and placebo groups) for FVC, FEV_1, $FEF_{25–75}$, and PEF. The difference in impact of ozone exposure on lung functions between the supplement and placebo groups was significant for $FEF_{25–75}$ and PEF (*t* test, $p < 0.05$). *The dashed line* corresponds to no change in lung function (zero change). On the basis of a model, including 47 subjects and 1,051 observations in the supplement group and 35 subjects and 796 observations in the placebo group (Model 3 in Table 4).

ing supplement. NO_2 levels were also related to decrement in lung functions in the placebo group but not significantly so. No clear pattern was observed in relation to PM_{10} levels. In multipollutant models, ozone levels remained negatively correlated with $FEF_{25–75}$, FEV_1, and PEF in the placebo group, and vitamin supplementation significantly attenuated the lung function decrement for $FEF_{25–75}$ and PEF related to ozone exposure. The attenuation for FEV_1 was only marginally significant. Because our measurements of air pollutant concentrations were based on monitoring network data, exposure is likely to be misclassified, leading to an underestimation of the protective effect of the supplement.

Ozone imposes an oxidative burden on the lung in two ways: directly, as a consequence of its oxidizing character during exposure, and indirectly, by engendering inflammation resulting in lung function impairment and exacerbation of respiratory symptoms in susceptible individuals such as asthmatics (28). Antioxidants are present in the lung-lining fluid on the surface of the respiratory tract and react rapidly with ozone. Ascorbate (vitamin C) is an excellent reducing agent that scavenges a variety of free radicals and oxidants. In addition, ascorbate prevents lipid peroxidation through its reaction with membrane α-tocopherol (29) and reduces the concentration of the tocopherol radical by regenerating its nonradical form, thereby restoring its scavenging activity (30). α-Tocopherol (vitamin E) is present in the lining fluid but at relatively low concentrations. It is thought to be secreted by type II cells. α-Tocopherol is a powerful antioxidant, both in terms of its direct free-radical scavenging activity and its ability to terminate lipid peroxidation (29). Several studies have shown depletion of antioxidants in the fluid lining the respiratory tract as a consequence of ozone exposure (28), suggesting that antioxidants, in particular ascorbate, play a critical role against inflammatory oxidative stress induced by ozone. In addition, patients with asthma have been found to have low concentrations of vitamin C and vitamin E in their lung-lining fluid and to be subject to increased oxidative stress, as expressed by an increased amount of oxidized glutathione in their airways (16).

The role of antioxidant supplementation in reducing the acute effects of ozone exposure has been investigated in controlled studies with animals and humans (4); however, there is little information on the impact of antioxidant supplementation on the acute effects of photo-oxidant exposure in free-living populations. Studies on animals have provided information about the impact of dietary deficiencies on the predisposition of animals to ozone injury and have shown that exposure to a high ozone concentration led to lipid peroxidation that was greater in animals that did not receive vitamin E supplements and produced more severe lesions in vitamin E–deficient animals (31). Elsayed and coworkers (32) have also shown that levels of vitamin E in the lung tissue of animals receiving vitamin E supplements increased significantly after the animals were exposed to ozone (0.5 ppm for 5 days), whereas vitamin E levels in the lung tissue of nonsupplemented animals decreased. This suggests that vitamin E is mobilized toward the lung tissue in response to oxidative stress. In a controlled study of humans, supplementation with vitamin C (1 g before ozone exposure) and vitamin E (800 IU daily) protected against the acute effects of ozone (600 $\mu g/m^3$ equivalent to 300 ppb) on FEV_1 and FVC (5). In another study conducted among asthmatics, the results suggest that vitamin C (500 mg) and vitamin E (400 IU) had a protective effect against PEF decrements after exposure to ozone (240 $\mu g/m^3$ equivalent to 120 ppb) (6). More recently, Samet and coworkers reported that in healthy volunteers with a dietary restriction of vitamin C intake, antioxidant supplementation might confer some protection against ozone-induced pulmonary function decrement but not against inflammatory response. These subjects received 250 mg vitamin C, 50 IU α-tocopherol, and 12 oz of vegetable cocktail and were exposed to 400 ppb for 2 hours. Ozone-induced reduction in FEV_1 and FVC were 30 and 24% less, respectively, in the supplemented cohort (9). Two studies conducted in the Netherlands suggest that antioxidant supplementation protects against the acute effect of ozone on lung functions of unconstrained individuals. A partial protective effect of supplementation was observed for FEV_1 and FVC (8). In a study conducted in Mexico among shoe shiners, we also observed that antioxidant supplements protected against lung function decrements after ozone exposure. Significant effects were observed for FVC, FEV_1, and $FEFV_{25–75}$ (10). To our knowledge, no study to date has been conducted among children with asthma in an unconstrained setting.

Several factors need to be considered in the interpretation of our results. The major baseline characteristics of the two groups participating in our randomized trial were similar. Furthermore, by using a placebo, we were able to conduct a double-blinded intervention in which neither the participants nor the spirometer technicians knew to which group participants were

assigned. Therefore, information bias could not explain our results. However, if subjects in the supplement groups had used BD more often and inhaled cortocoids before the spirometry; this could have led to a bias in our results. We observed no difference between the supplement and the placebo groups in the use of these medications before the spirometric tests during the follow-up period. Among children with mild asthma, 78.1% in the supplement group and 76.7% in the placebo group report using BD ($p = 0.88$) at least once during the follow-up before the spirometry. Among the children who used BD, the average proportion of BD use during the follow-up over all spirometric tests was 45.8% in the supplement group and 46.2% in the placebo group ($p = 0.97$). Among children with moderate and severe asthma, 93.6% in the supplement group and 91.4% in the placebo group report using BD at least once during the follow-up before the spirometry ($p = 0.8$). Among these children, the average proportion of BD use during the follow-up was 46.0% in the supplement group and 42.5% in the placebo group ($p = 0.76$). Regarding inhaled corticoid use, among mild asthmatics, 73.4% in the supplement group and 76.6% in the placebo group reported using inhaled corticoids ($p = 0.75$), and among moderate and severe asthmatics, 82.9% reported using inhaled corticoids in the supplement group and 80% in the placebo group ($p = 0.7$). Therefore, differences in the use of medication before the spirometry cannot explain our results. In addition, dosage of medication was in accordance with the severity of the disease for inhaled corticoids and with the age and the severity of the disease for BD; therefore, it was similar in both groups.

Because the exposure estimation of participating children was based on the monitoring network and not on personal measurement and/or activity pattern of the children, some misclassification in exposure is probable. This will tend to underestimate the effect of the air pollutant on lung function, and potentially the protective effect of antioxidant supplementation. The greater effect of ozone exposure on lung function parameters was observed with a 1-day lag period. We observed a larger decrement in the placebo group when we considered ozone exposure over several days than when considering a single day up to 6 days, but no significant changes were observed in the supplemented group. This suggests that the supplement would modulate the subchronic effects of ozone on children's lungs.

We observed significant decrements in the lung functions of children assigned to the placebo in relation to ozone exposure for FEV_1, $FEF_{25–75}$, and PEF. However, the larger modulating effect of the supplementation was observed for $FEF_{25–75}$. In experimental studies, repeated exposure to ozone has been linked to alteration in small airway functions, whereas persistent depression of the FVC and FEV_1 baselines has generally been uncommon (33). Recently, Frank and coworkers reported spirometric evidence of persistent change in small airways during 4 consecutive days of ozone exposure concomitant with inflammatory response, with marked increase in neutrophils and kinins in bronchoalveolar lavage (33). It is not clear if this dysfunction might be linked with permanent loss in the event of chronic exposure to ozone. However, in free-living populations long-term exposure to ozone has been linked with impaired small airway functions (34). Ozone has been shown to directly increase the flow resistance of the small airways, and small airway effects have been demonstrated in subjects exposed to ozone after controlling for any effect related to concomitant decrease in FVC (35, 36). In addition, dosimetric and histologic evidences suggest that small airways receive the greatest dose of ozone and are most sensitive to injury (37, 38), and increase in protective enzyme and glutathione (a cosubstrate for two antioxidant enzymes) is significantly increased only in the distal airway of monkeys after long-term ozone exposure (39, 40). A number of mechanisms may contribute to the narrowing of the distal airway, including increased smooth muscle tone, edema, localized inflammation, and mucus hypersecretion (28). Although a weak correlation has been observed between inflammatory response and persistent functional changes (33, 40), the fact that functional and inflammatory responses follow different time courses might obscure an association (33). By disrupting the cascade of chemical reactions associated with lipid peroxidation and by scavenging reactive oxygen species, antioxidants could modulate the effect of ozone on pulmonary function decrements (41) and inflammatory responses (35).

Our study population was composed of children with mild, moderate, and severe asthma. However, the modulating effect of antioxidants was observed mainly among moderate to severe asthmatics. This may suggest that children with more advanced asthma are more susceptible to the impact of ozone exposure because of a decrease in antioxidant defenses in the lung. Lower levels of antioxidants and erythrocyte antioxidant enzymes (e.g., SOD) have been observed in the sera of children with asthma (42, 43). In addition, higher levels of F2-isoprostanes, markers of oxidative stress, have been observed in the plasma of asthmatics in comparison with control subjects (44). The baseline antioxidant intake and plasma concentrations of vitamin E were similar in the supplement and placebo groups. Participants reported intake of vitamin C close to the recommended dietary daily allowance (RDA); however, vitamin E intake was well below the RDA for the age range of children in the study (almost half of the RDA) (45). This was reflected in the low level of vitamin E observed in the serum as compared with other populations (46). During the course of the study, plasma vitamin E levels rose by 45.6% in the supplemented group, which is comparable to that observed in other supplementation studies (7, 8, 10). This increase in plasma vitamin E provides the evidence of compliance with the supplement. The supplement provided close to five times the RDA for vitamin C and vitamin E. It is difficult to determine which antioxidant might be more effective in protecting against oxidative insults. Vitamin C and vitamin E have been shown to affect arachidonic acid metabolism, but the role of antioxidants in this mechanism is not fully understood. Ascorbate can regenerate vitamin E from the tocopheroxyl radical (14) and act synergistically with vitamin E to inhibit lipid peroxidation (47); therefore, both nutrients appear to be important.

Our data suggest that vitamin C and vitamin E supplementation above the minimum dietary requirement in children with asthma with low intake of vitamin E provides some protection against the acute effects of ozone on their lungs. It remains to be determined what is the optimal dose of antioxidant for adequate protection from ozone-induced lung injury and to what extent such supplementation would provide increased protection over that observed in subjects already considered to have a normal diet.

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